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A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU 5) in commercial fishery areas and the status of bonamia (*Bonamia exitiosa*) in February 2014.

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Michael, K.P.; Forman, J.; Hulston, D.; Fu, D.; Maas, E. (2015) A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU 5) in commercial fishery areas and the status of bonamia (*Bonamia exitiosa*) in February 2014.

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A new design was developed for the February 2014 bonamia survey that reflects the new objectives for annual oyster surveys. This new time-series of surveys has 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 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. Data from future surveys will be comparable with previous surveys and will allow stratum by stratum comparisons to be made. Some limited sampling in background strata was also undertaken to allow these data to be incorporated into stock assessments.

Simulations to determine the optimal number of survey strata and sample stations for sampling the core fishery area used combinations of 1, 4, 7, and 14 strata, and the 2009, 2012 and 2014 survey data. Simulations with 14 strata produced the lowest coefficients of variation (CVs). A target CV of 12% was set to ensure that future surveys were comparable to previous surveys. We used the 2009 and 2012 stock assessment survey data to optimise the numbers of stations for the 2014 survey. A CV of 11.44% was predicted from 55 sample stations. The February 2014 survey achieved a CV for recruit-sized oysters in core strata of 11.2%, and 11.7% for the whole population from an additional 5 stations in the background stratum (10.8% predicted).

This survey was undertaken in collaboration with the Bluff Oyster Management Company who provided information and guidance for the selection of survey strata, provided a vessel, the survey dredge, and vessel crew for the survey. The oyster vessel F.V. *Golden Quest* skippered by Stephen Hawke, sampled 55 first phase and 5 second phase random stations, and 11 fixed stations between the 8th and the 12th of February 2014. Estimates of population sizes are made from randomly allocated stations only. Plots of distribution include fixed stations. We compare survey estimates between the 2012 stock assessment survey and the 2014 bonamia survey.

Pre-survey mortality estimated from recruit-sized new clocks continued to be widespread, as in previous surveys. Recruit-sized new clocks in core strata were higher in 2014, 39.4 million new clocks (6.8% of recruit-sized new clocks and live oysters combined) than in 2012, 22.4 million new clocks (3.2% of recruit-sized new clocks and live oysters combined).

The mean oyster density in core commercial strata has fallen from 1.40 oysters per m^2 to 1.09 oysters per m^2 between 2012 and 2014, and the population size declined 21.8% from 688.1 million oysters in 2012 to 538.0 million oysters in 2014. This decline is mostly attributed to two factors: heightened mortality from bonamia and much lower than average recruitment to the fishery, which we speculate is environmentally driven. Pre-recruit oysters in core strata declined by 50.1% from 297.4 million oysters in 2012 to 148.4 million oysters in 2014, and the numbers of small oysters declined by 65.4% from 451.3 million oysters in 2012 to 156.3 million oysters in 2014.

Pre-sample analysis checks were run to ensure that qPCR assays were producing comparable data to 2013 qPCR data, and to check the 35 Cq cut off level for qPCR positive reactions.

Bonamia infection and mortality have increased since 2012. The estimated number of oysters infected with bonamia from heart imprints data in core strata was 89.5 million oysters, higher than for the whole survey area in 2012, when only 83.3 million oysters were infected. The estimate of infected oysters (146.9 million oysters) from qPCR (heart tissues) data was much higher than from heart imprints in 2014. The number of fatally infected oysters in 2014 was projected to reduce the recruit-sized oyster population in core strata from 538.0 million oysters at the time of the survey (February 2014) to 476.3 million oysters by early in the new oyster season (March 2014), a loss of 67.1 million oysters (11.5%). Post-survey mortality of recruit-sized oysters ranged from 4.6% in stratum C1a to 22.1% in C2. Of the core strata, C2, E2, C5, B1, C7, C7a, E4, and C3 had a post survey mortality greater than 10%.

The 2012 stock assessment suggested that recruit-sized stock abundance was about 30% (26–34%) of initial state of B_0. By 2012, the trajectory of the future stock size was already starting to flatten due to the continuing low level of mortality from bonamia and the reduced recruitment since 2009. Bonamia mortality ranged from between 8% and 12% between 2007 and 2012. The population size of recruit-sized oysters in core strata declined by 21.8% over the two years between 2012 and 2014, and fatally infected oysters were expected to further reduce the population by 11.5% within one to two months. This level of bonamia mortality is expected to cause a downward trend in the oyster population. A significant increase in recruitment could have a major restorative effect, but there will be a 4–6 year lag before any heightened recruitment to the population translates to 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 draft National Fisheries Plan for Inshore Shellfish, and is also subject to an approved collaborative fisheries plan, the Foveaux Strait Oyster Fisheries Plan (Ministry of Fisheries 2009).

A Foveaux Strait Oyster Fisheries Plan (Ministry of Fisheries, May 2009) was collaboratively developed by the Foveaux Strait Oyster Fisheries Plan Management Committee 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 long-term goal of the Fisheries Plan is to maximise oyster production in the oyster fishery. That is, to manage the fishery so that the oyster population can rebuild to levels where it will support near historical levels of catch.

In 2010, a strategic research plan (SRP) for OYU 5 was developed for five years from 2010 to 2015 (Michael 2010). The 2010 SRP provides a broad range of research programmes aimed at maximising production from the oyster fishery and meeting the Fisheries Plan goals and objectives. The SRP identified research that could better inform strategies to minimise oyster losses from the oyster disease *Bonamia exitiosa* (bonamia); to provide information and technologies for the acquisition of better data from the commercial fishery that could be used for monitoring between assessments; to develop a better understanding of the drivers of oyster production and how they vary in different parts of the fishery; to collect information that could be used to optimise fishing strategies; and to increase the efficiency of fishing while minimising any negative effects of dredging.

The haplosporidian parasite *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. Declines in localised oyster populations have been reported since 1906. Bonamia was identified in samples taken in during an epizootic in 1964. Early epizootics in the Foveaux Strait oyster population have been followed by long periods when bonamia mortality could not be detected (and when oyster populations have rebuilt). The status of non-fatal infections during periods when there was no heightened oyster mortality is not known. Since 1964, high oyster densities have persisted for long periods in the assumed absence of disease; and the long-term average annual landing from the fishery has been about 80 million oysters.

Two bonamia epizootics in 1985–92 and from 2000 to the present (2014) 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. The first of two recent bonamia epizootics between 1985 and 1992 (Doonan et al. 1994) probably reduced the oyster population to less than 10% of the pre-disease level (Cranfield et al. 2005). In 1993, the fishery was closed to allow the population to rebuild. It was reopened in 1996 after the recruit-sized oyster population increased from 397 million oysters in October 1993 to 782 million oysters in October 1995, and peaked at 1461 million oysters in 1999 (Michael et al. 2001). Between 2000 and 2005, widespread mortality from bonamia again reduced the numbers of oysters to the historically low levels of the early 1990s, 408 million oysters in 2005 (Dunn et al. 2002, Dunn et al. 2003, Michael et al. 2004a, Michael et al. 2004b, Michael et al. 2005, Michael et al. 2006, Michael et al. 2008a, Michael et al. 2008b, Michael et al. 2009). These events suggest that bonamia epizootics can be expected in the future.

Projections from the OYU 5 stock assessment model (Fu & Dunn 2009) indicate that current catch levels of between 7.5 and 15 million oysters are unlikely to have any significant effect on future stock levels. Instead, future disease mortality will determine future stock status. In general, bonamia mortality levels of about 10% of the recruit-sized population have resulted in an increase in oyster abundance, while levels of bonamia mortality of about 20% are expected to reduce the recruit-sized oyster population. The stock has shown an ability to rebuild rapidly in the absence of disease (assuming long-term average recruitment). Since 2006, the mortality of recruit-sized oyster generally range from 8–12%. At this level of disease mortality, the population of recruit-sized oysters increased to 913 million oysters in 2012 (Michael et al. 2013), about 30% of B_0. Oyster density has been increasing across the oyster fishery in recent years, including in eastern fishery areas. Areas with relatively high oyster densities are potentially at the greatest risk of mortality from bonamia. These areas are also of the greatest importance to commercial fishers.

1.1 OYU 5 Research Summary

Biennial and more recently triennial stock assessments estimated the status of the OYU 5 stock and made projections of future stock status based on expected levels of recruitment, harvest, catch rates, population size, and mortality, mostly from bonamia during epizootics (Fu 2013). Surveys of the oyster population are undertaken for each stock assessment to provide estimates of the populations sizes of three size groups of oysters: recruit-sized, pre-recruits, and small oysters. These surveys 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 the 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 2013, 26 strata were sampled (Michael et al. 2014).

In years between stock assessments, smaller focused surveys of bonamia prevalence and intensity (bonamia surveys) were 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. These surveys were not intended to be stock assessment surveys, and did not attempt to sample all survey strata over the whole Foveaux Strait oyster stock assessment area. Bonamia surveys focussed on areas of relatively high oyster density important to the commercial fishery and to the stock, as the effects of bonamia mortality were much higher there. 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).

1.2 Rationale for a new series of bonamia surveys

A recent draft National Fisheries Plan for Inshore Shellfish 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 reportedly best achieved through accurate and frequent monitoring that supports responsive management (see MPI research specifications for OYS2013). It is, however, recognised 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. 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.

MPI and BOMC have monitored the status of bonamia infection and the abundance of oysters within the commercial fishery area biennially from 1990 to 1999, and on an annual basis since 2000 following the outbreak of another bonamia epizootic. These surveys provided a suite of information for management of the OYU 5 fishery and to the oyster industry. The introduction of five-yearly stock assessments 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.

1.2.1 Rationale for research objectives

To be able to monitor trends in oyster densities and population sizes of the three size groups of oysters surveyed, and to determine the effects of bonamia mortality on these populations, a standard bonamia survey area that better defines the core commercial fishery (within the limits of the 2007 stock assessment survey area) needed to be established. Further, annual estimates of oyster densities from the remaining stock assessment area would allow these annual surveys to be included in stock assessments as they would provide estimates of the whole oyster population (objective 1).

In 2012, there were 26 commercial, exploratory, and background strata within the 2007 survey stock assessment area. Objective 2 proposes to undertake simulations to assess the trade-offs in optimising stratification and sampling design on estimates of oyster population size, pre-survey mortality from the numbers of new clocks, and for the population size of oysters with fatal bonamia infections. These simulations aimed to maintain CVs for estimates of population size consistent with those for estimates from the previous surveys.

Given the changes in the information required from these surveys, the survey design will move to a fully stratified random design to better estimate oyster densities (objective 3), mortality (objective 4), and bonamia infection levels (objective 5) within individual strata and for the all survey strata combined. Because of the increase in time between stock assessments for the whole fishery area, the large background stratum will receive five randomly allocated stations that will allow an estimate of oyster population size for the whole fishery area each year that could be incorporated into stock assessments.

Projections of future stock status from the OYU 5 stock assessment model are made using levels of bonamia mortality of 0, 10% and 20% (options a–c in Fu 2013). Percentage mortality estimated from annual surveys will indicate the most likely projection (a, b, or c) of future stock size (objective 6). Increases or decreases in the numbers of pre-recruit and small oysters will also affect the slope of the trajectory of the future stock size.

The development of quantitative polymerase chain reaction (qPCR) method for the detection of bonamia relies on a duplex qPCR assay and a shortened bench top method. The assay was developed to work well with crude extracts. The results of assays are dependent on the integrity of the qPCR reagents and the reactions with oyster tissues occurring without error. All qPCR reagents and procedures will be tested with archived control samples prior to undertaking any analysis of tissue samples from the 2014, 2015 and 2016 surveys (objective 7) so that the values for cycle of quantification (Cq) are consistent between the surveys and the integrity of the time series is maintained.

Research to better understand bonamia has been rated a high priority by stakeholders and MPI, and is the main focus of the SRP. A cost-effective, real-time, sensitive qPCR method to detect low level bonamia infections in oysters has been developed (Maas et al. 2013) to increase the effectiveness of these surveys, and to help advance our knowledge of bonamia and its interaction with the fishery. This method is being used to detect the prevalence of infection, i.e., the number of infected oysters in each sample. The qPCR method needs further development to provide estimates of the intensity of infection (how heavily individual oysters are infected), and principally to estimate the numbers of fatally infected oysters in samples, which are used to make projections of post-survey mortality. To progress the development of this method, research to determine the correlation between the numbers of *Bonamia exitiosa* ITS region copies in infected oysters and the intensity of infection within the oyster needs to be undertaken (objective 8).

Stock assessments of OYU 5 use a length-based model which models changes in population size, growth, mortality, fishing mortality and the effects of bonamia on the oyster population. Data on the size structure of the commercial catch are required to estimate catch-at-length in the fishery and to determine the impacts of mortality from bonamia on the size structure of the population. These data are recorded by a catch sampling programme run by BOMC. This programme also provides information on recruitment to the fishery by estimating the numbers of small spat attached to oysters that cannot be removed by culching (sorting the catch) (Fu et al. 2014). Yearly sampling of the commercial catch for catch-at-length data provides data required for stock assessments. BOMC is undertaking catch sampling during the 2014 oyster season and will continue to do so for at least the next two years. These data will be analysed by NIWA in objective 9.

This report provides a context and rationale for new series of Foveaux Strait oyster surveys, and describes the methods that will be employed. It also gives the results of a survey in February 2014, the first in this new series. This survey estimated the oyster population size and the status of infection by bonamia (*Bonamia exitiosa*), and outlines the implications for the projections of future stock status made in the 2012 stock assessment for OYU5 undertaken under the MPI research programme OYS201301, objectives 1–7.

An MPI Shellfish Working Group meeting in January 2014 convened to review the February 2014 oyster survey design requested that population size frequencies were sampled. Random samples of recruit-sized, pre–recruit, and small oyster were sampled from each survey tow where available. Weighted length frequency distributions for each of these size classes are also presented in this report.

Two of the OYS201301 objectives: objective 8, to determine the correlation between the number of *Bonamia exitiosa* gene copies in infected oysters and the intensity of infection within the oyster; and objective 9, to summarise data on the size structure of the commercial catch, recruitment to the oyster population, and to estimate catch-at-length are not included in this report and will be reported in two separate reports when the research is completed.

2. OVERALL 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.

2.1 Specific objectives

Specific Objective 1

Define the area where the greatest proportion of catch has historically come from over an appropriate number of years within the outer boundary (as defined in 2002) of the fishery.

Specific Objective 2

Undertake simulations to assess the trade off in optimising stratification and sampling design when undertaking objectives 3, 4 and 5.

Specific Objective 3

Using a stratified random sampling design estimate the current recruited abundance and biomass in the area of the commercial Foveaux Strait oyster fishery as defined in objective 1 (for the 2014, 2015 and 2016 fishing years), with a target CV of no more than 20%.

Specific Objective 4

Using a stratified random sampling design estimate the annual mortality from Bonamia in the area of the commercial Foveaux Strait oyster fishery as defined in objective 1 for the 2014, 2015 and 2016 fishing years.

Specific Objective 5

Using a stratified random sampling design estimate the prevalence and intensity of Bonamia in the area covering the commercial Foveaux Strait oyster fishery as defined in objective 1 for the 2014, 2015 and 2016 fishing years.

Specific Objective 6

Evaluate which of the biomass projections, as assessed by the stock assessment model, is the most likely reflection of the current status of the OYU5 fishery, for the 2014, 2015 and 2016 fishing years.

Specific Objective 7

Review all qPCR procedures prior to undertaking any analysis of tissue samples for the 2014, 2015 and 2016 fishing years.

3. METHODS

3.1 Methods for objective 1: Define strata for core commercial fishery area

A new boundary for the annual “bonamia” survey area that better defines the core commercial fishery within the limits of the 2007 stock assessment survey area needs to be established to maintain a constant survey area.

Between 1964 and 1985, the commercial fishery comprised a number of persistent, localised commercial fishery areas (“oyster beds”). The effects of bonamia mortality from two recent bonamia epizootics has changed the distribution of commercial fishery areas and the oyster density within them: oyster density declined during periods of heightened bonamia mortality and increased during periods of low or no apparent disease mortality. Annual patterns of commercial fishing in part reflect these regional changes in oyster density.

There are four sources of data available to map the core commercial fishery areas within Foveaux Strait: catch effort landing returns (CELR data) recorded at spatial scales of oyster fishery statistical areas (Figure 1); oyster fishers’ logbook data recorded at a fine spatial-scale of one nautical mile squares (Figure 2); fishers’ knowledge; and fishery independent survey data from stock assessment and bonamia surveys (Figure 3).

The statistical reporting areas used to record catch and effort on CELR forms are large and often include a number of oyster beds while their boundaries often divide other significant oyster beds. Catch effort data in fishers’ logbooks are recorded at finer spatial-scales (one nautical mile square grids). A number of factors have influenced the spatial extent of areas fished commercially within Foveaux Strait including reductions in both the numbers of vessels fishing and the total allowable catch, and a focus on fishery areas that provide the best oyster meat quality for market rather than the areas with the highest catch rates. Therefore catch effort data and logbook data tend to reflect patterns of commercial fishing rather than the distribution of oyster density in the fishery.

However, before 1999, fishery independent survey data used different survey designs (stratified random, grid, and targeted sampling) and the extent of survey areas was different. Since 1999, the survey area has been the same and the stratification of surveys has been similar.

Because of the high temporal variation in the distribution of oyster density and patterns of fishing, we investigated three time series of data to define core fishery areas: the period 1976–1985 (pre- recent epizootics), 1993–2000 when the fishery was rebuilding after the 1985–92 epizootic, and 2005–2013 after the second epizootic (1999–2005). We omitted data recorded during epizootics as bonamia mortality rapidly changed the distribution of oyster density during those times.

We ranked the existing strata using catch rate from CELR and logbook data, mean oyster density from fishery independent survey data, and using qualitative data from oyster skippers’ rankings of survey strata. These rankings highlight three categories of strata: those with consistently high catch rates and high mean oyster density, those with variable catch rates and variable oyster density, and importantly those with consistently low catch rates and low oyster density. The three time-series of data should capture the spatial extent of the commercial fishery area and how it has been changed by bonamia mortality.

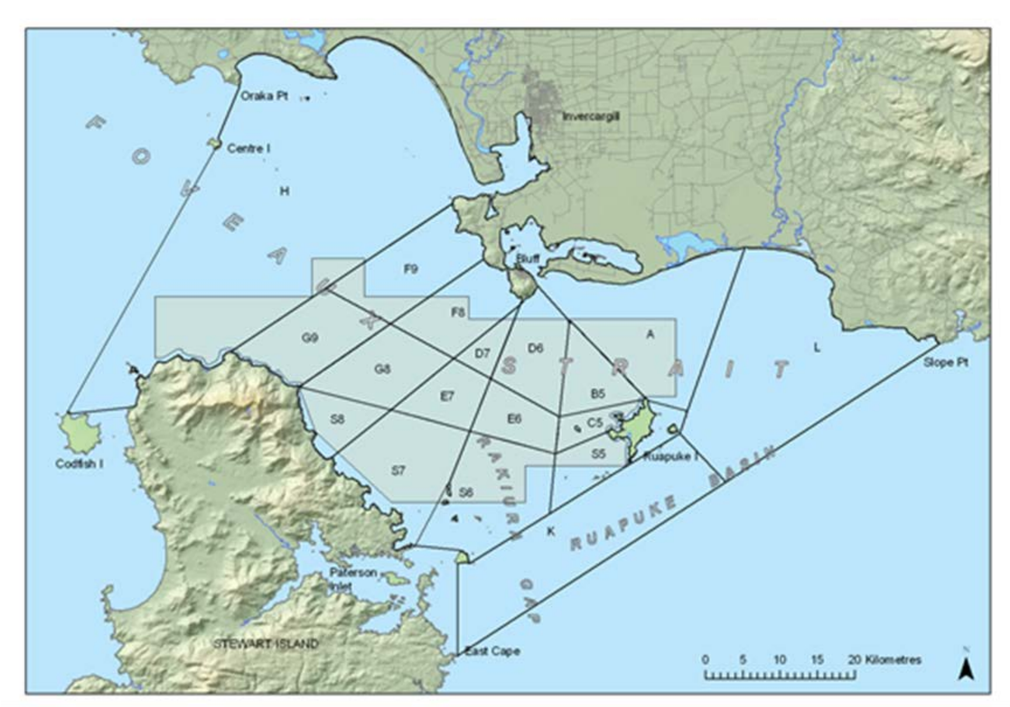

Figure 1: Foveaux Strait (OYU 5) stock boundary and the Foveaux Strait oyster statistical reporting areas (black lines), statistical area labels (black text), and the outer boundary of the 1999 dredge survey area (shaded grey) encompassing almost all the commercial fishery.

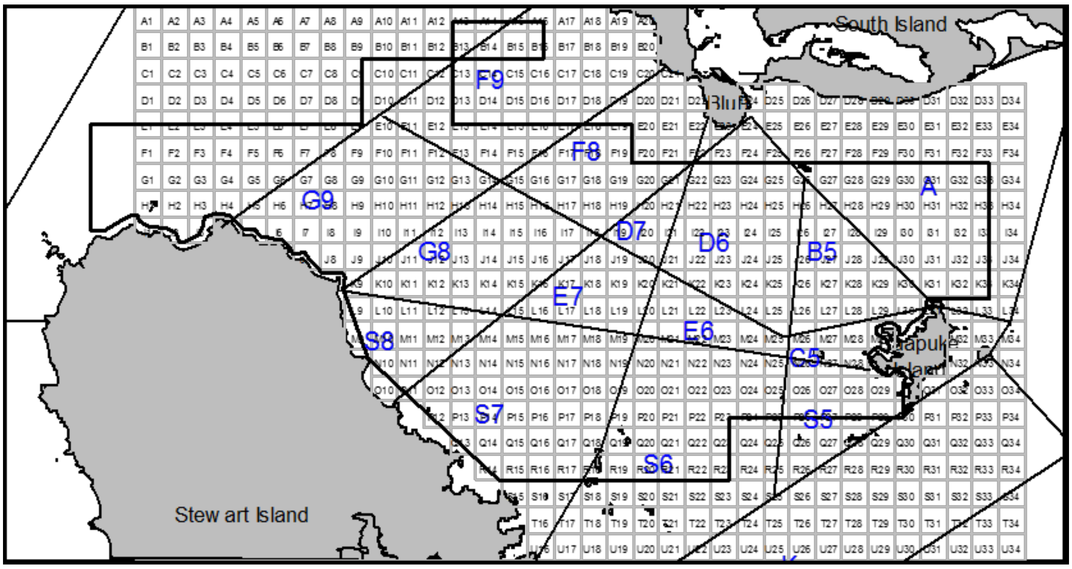

Figure 2: Foveaux Strait oyster logbook grid (one nautical mile squares, grey lines and black labels) established by skippers to cover the commercial fishery area and the boundaries of the Foveaux Strait oyster fishery statistical reporting areas (light, black lines and blue labels). The boundary of the 2007 stock assessment survey area (including stratum B1a) is shown as a heavy black line.

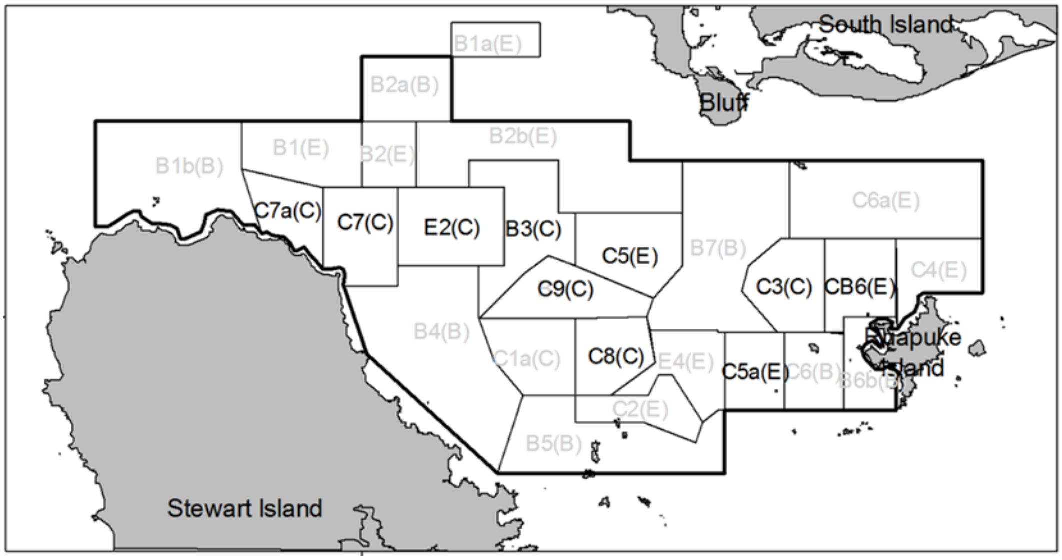

Figure 3: The 2013 survey area with the 2005 survey boundary shown as a heavy, black outer line, and the 2012 survey strata shown as black lines. Strata that were sampled in 2013 labelled with black text and those of the 2012 strata not sampled with grey text. Strata designated commercial in 2013 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B". B1A is a stratum added in the 2007 survey.

3.2 Methods for objective 2: Simulations to optimise stratification and sampling design for objectives 3, 4, and 5.

Because oyster density, bonamia infection, and bonamia mortality vary spatially and temporally, oyster density can be predicted reasonably well from survey and fishers' data, however, the spatial distribution of new clocks and bonamia infection cannot be predicted. There is little relationship between oyster density, the occurrence of new clocks, and patterns of bonamia infection.

During epizootics, especially where there is heightened mortality (40–60%), new clock density may be high in areas of high live oyster density and may represent a relatively large percentage of the total mortality. It is not practical to optimise strata for new clocks alone. Likewise, the prevalence and intensity of bonamia is highly spatially (even at small spatial scales) and temporarily variable, and not dependent on oyster density. Areas of high prevalence and intensity may be separated from stations with no detectable infection by as little as 200 m. It is not practical to optimise strata for bonamia infection.

The survey design was optimised primarily for recruit-sized oysters as the precision of population-scale estimates of oyster mortality (objective 4) and bonamia infection (objective 5) are closely linked to the precision of the survey estimates of recruit-sized oysters (objective 3), as both infection and mortality are scaled to the population size of recruit-sized oysters. The current survey strata delimit areas of different oyster density relatively well, i.e., areas of relatively high, developing, and background oyster densities.

The core strata identified in objective 1 delimiting the bonamia survey area (see Figure 5) comprised fourteen strata. Simulations were used to determine the minimum number of strata and numbers of stations required to achieve a target CV of 10–12% for the estimate of population size. These fourteen strata were merged into seven, four, and two strata, and one stratum representing the commercial fishery area (Figure 4). We used the 2009 and 2012 survey data to run simulations for the 2014 survey as these years represent times for which we have good survey data and the distributions of relatively high oyster density were stable due to relatively low disease mortality. Simulations focused on optimising the stratification and sampling design for recruit-sized oysters. We used the NIWA program Allocate (Francis & Fu 2011) to optimise the number of sample stations in each stratum, and the effects of combining strata on the total number of survey stations required and the estimated coefficients of variation.

We ran Allocate to determine the numbers of stations required to produce coefficients of variation (CVs) ranging from 10% to 20% as this routine is known to be optimistic in its estimation of the numbers of sample stations required to ensure survey CVs of less than 20%. These data were tabulated for oysters and new clock densities, and the prevalence of bonamia infections. We undertook further simulations using the 2014 results to further optimise the survey design and stratification for 2015 and 2016 surveys.

The remaining stock assessment survey strata that do not represent the core commercial fishery area were merged into a single, large background stratum and will be allocated more than 3 sample stations each year.

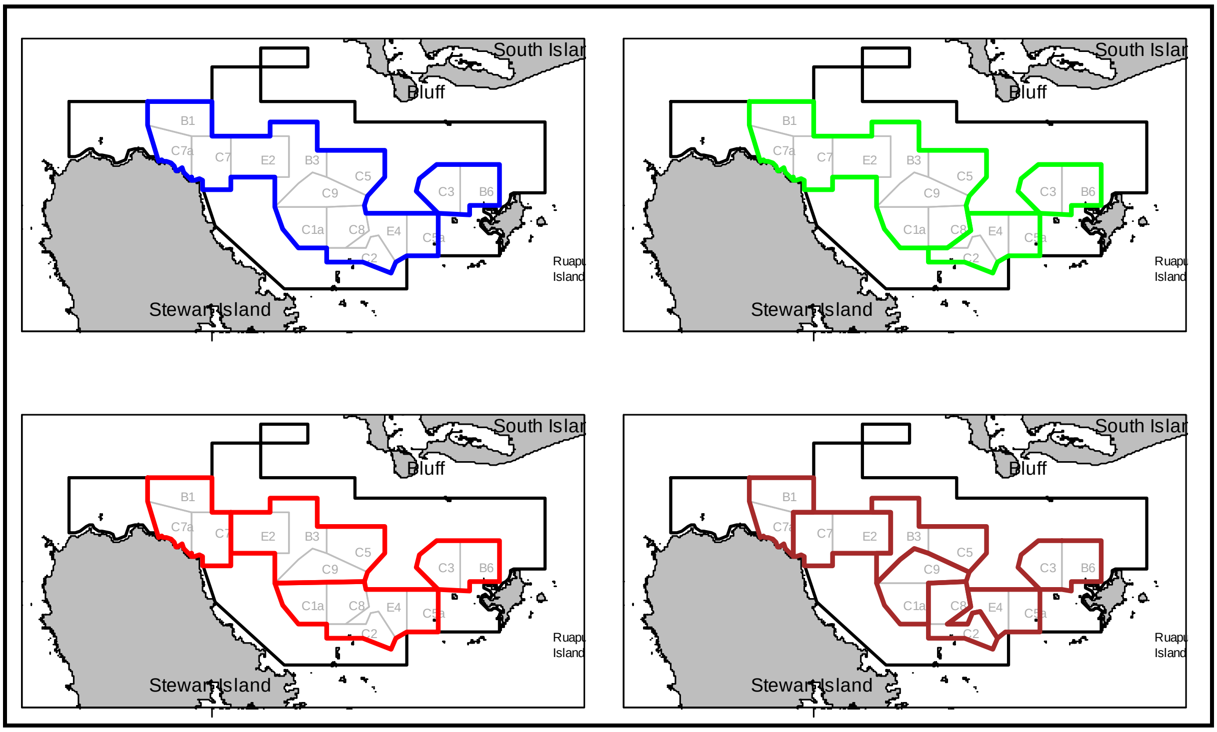

Figure 4: The 2014 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2014 survey strata representing the core commercial fishery area shown as light grey lines. Strata are labelled with grey text. The core strata were merged into a single stratum (blue lines, top left), two strata (green lines, top right), four strata (red lines, bottom left), and seven strata (brown lines, bottom right). The remaining stock assessment survey strata that do not represent the core commercial fishery area were merged into a single, large background stratum.

3.3 Survey sampling procedures and analytical methods for objective 3 to 7

The February 2014 survey is the first in a new series of annual bonamia surveys. These surveys continue to use the 2007 Foveaux Strait oyster survey boundaries covering an area of 1070 km^2 that includes the 2002 survey area (1054 km^2) and stratum B1a. B1a is an additional stratum (16 km^2) that was introduced by oyster skippers for the survey in 2007, but was outside the original (1999) survey area.

The February 2014 survey used a subset of 14 strata (B1, B3, B6, C1a, C2, C3, C5, C5a, C7, C7a, C8, C9, E2, and E4; Figure 5) from the 26 strata sampled in the 2012 stock assessment survey to define the core commercial fishery area (see results for objective 1, Section 4.2). These 14 strata accounted for 46% (491.8 km^2) of the 2007 stock assessment survey area (Table 1) and 74.9% of the population of recruit-sized oysters. Most of these strata had a mean recruit-sized oyster density greater than 1.0 per m^2 in 2012, and the remaining strata had similar densities of oysters before recent bonamia mortality. The remaining 12 strata (B1a, B1b, B2, B2a, B2b, B4, B5, B6b, B7, C4, C6, and C6a) are outside of the core commercial fishery area and were merged into a single background stratum (BK; Figure 5). The inclusion of this large background stratum with minimal sampling allows the data from these annual surveys to cover the entire 2007 stock assessment survey area, and to be included in stock assessments for OYU 5.

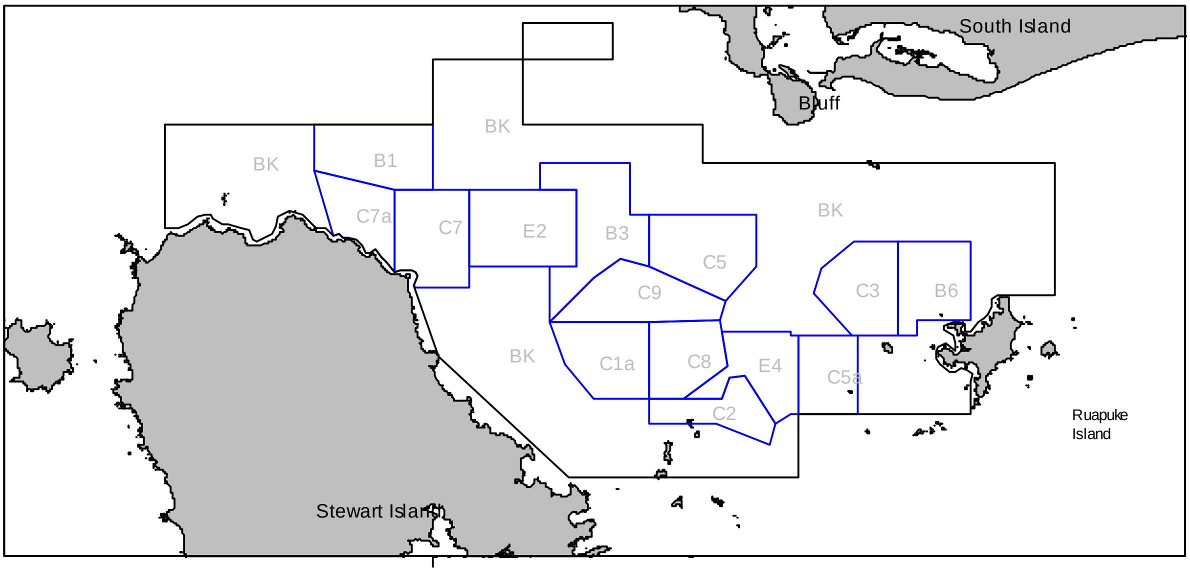

Figure 5: The 2014 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2014 survey strata representing the core commercial fishery area shown as blue lines. Strata are labelled with grey text. The remaining stock assessment survey strata that do not represent the core commercial fishery area were merged into a single, large background stratum (BK).

Simulations in objective 2 using Allocate (NIWA survey software running in R) estimated that 55 stations in the core strata would produce a CV of about 11%. The software also allocated sample stations to strata (Table 1). Rand_Stn (NIWA random station generator) was used to generate 50 first-phase stations (Figure 6) and sufficient stations in each stratum to sample 5 second-phase stations in core strata, and 5 stations (Figure 7) in the background stratum. 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 11 fixed stations were also sampled in February 2014 (Table 1, Figure 7).

Table 1: The allocation of the numbers of randomly selected first and second-phase stations to each stratum in 2014 representing the core commercial fishery area, the numbers of fixed sample 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 core commercial fishery area.

Stratum	First-phase	Second-phase	Fixed	Total	Area (km^2)
B1	3			3	78.2
B3	8			8	44.7
B6	3			3	30.0
B6a			2	2	*
BK	5			5	578.3
C1a	3			3	31.3
C2	3		1	4	21.9
C3	3	1	1	5	32.7
C5	4		1	5	37.7
C5a	4	3	1	8	23.5
C7	3			3	36.1
C7a	3			3	23.6
C8	3		1	4	26.8
C9	3	1		4	34.5
E2	4		2	6	42.8
E4	3		2	5	28.0
Totals	**55**	**5**	**11**	**71**	**1070.3**

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

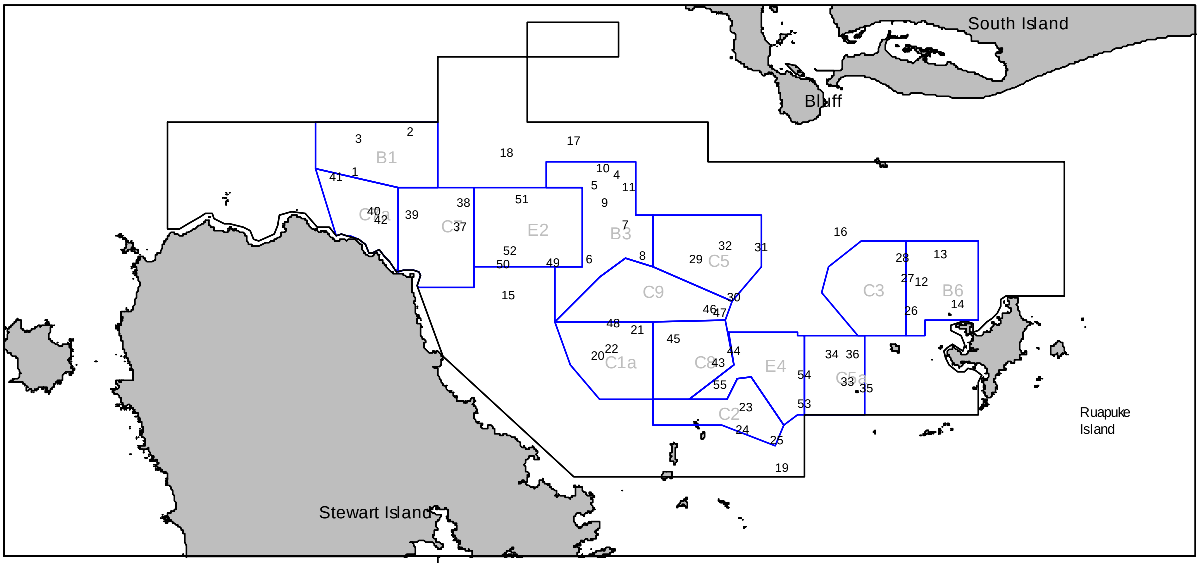

Figure 6: The 2014 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2014 survey strata representing the core commercial fishery area shown as blue lines. Strata are labelled with grey text. The remaining stock assessment survey strata that do not represent the core commercial fishery area were merged into a single, large background stratum (BK). First-phase station numbers are shown in black text.

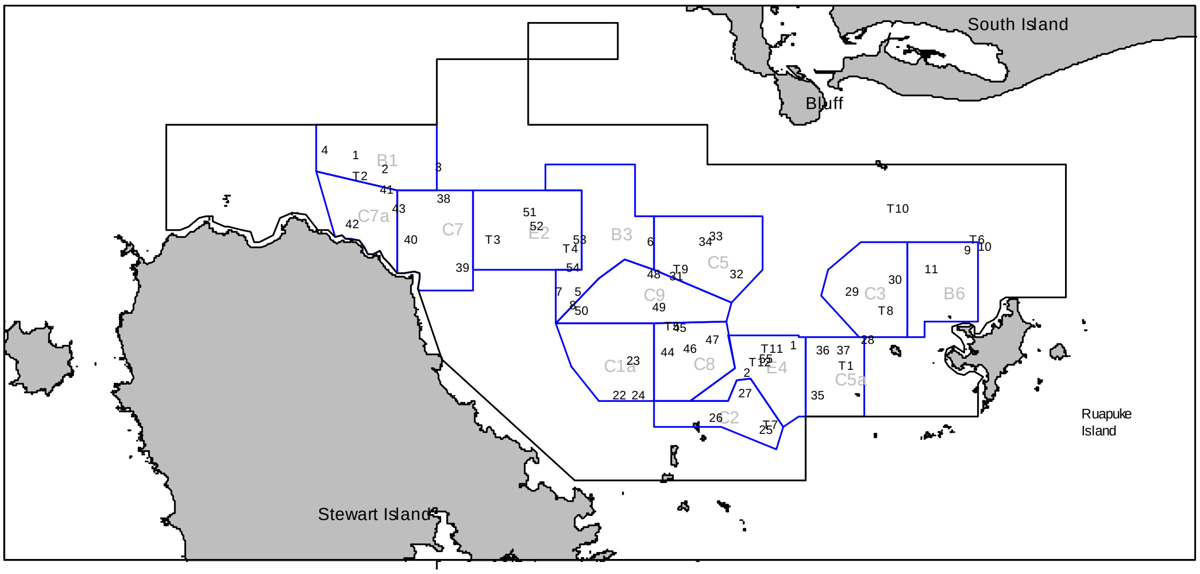

Figure 7: The 2014 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2014 survey strata representing the core commercial fishery area shown as blue lines. Strata are labelled with grey text. The remaining stock assessment survey strata that do not represent the core commercial fishery area were merged into a single, large background stratum (BK). Second-phase and fixed (prefixed "T") station numbers are shown in black text.

Sampling followed similar procedures to surveys between October 2002 and February 2013 (Michael et al. 2004a, Michael et al. 2004b, Michael et al. 2005, Michael et al. 2006, Michael et al. 2008a, Michael et al. 2008b, Michael et al. 2009, Michael et al. 2011, Michael et al. 2012a, Michael et al. 2014). F.V. *Golden Quest*, a commercial oyster vessel skippered by Stephen Hawke, was used for the survey. Survey stations were sampled with a standard survey dredge (commercial dredge 3.35 m wide and weighing 430 kg). The survey dredge was rebuilt prior to the survey: the chainmail ring-bag was replaced and bit bar was rebuilt. These changes to the survey dredge were within specification of the original survey dredge and were not expected to make any difference to sampling performance. NIWA staff ensured consistency of procedures.

Navigation

The survey used standalone high-resolution GPS position fixing (Garmin GPS 17-HVS, position fixing within 5 m, 90% of the time) with positions downloaded to a laptop computer running SEAPLOT navigation software. Start and finish tow positions were recorded both manually and electronically as waypoints (gear up and down), and later saved to file to provide a backup.

Survey tows

Where the start of the tow could not be made on position because of weather, tide, or boundary constraints, the tow direction was reversed and the tow finished on position. Oyster surveys use straight-line tows to enable the area sampled by the dredge to be calculated. This differs to the elliptical tows used by commercial oyster fishers, who fish down tide, then tow back to the start position to enable them to stay on oyster patches. Straight-line tows were made down tide for a distance of 0.2 nautical mile (371 m) at each station. The start of the tow was recorded at the time when the winch brake was applied and tension came onto the warp. The distance towed was monitored against a 0.2 nautical mile range ring on SEAPLOT. Once the dredge had travelled 0.2 nautical miles, the end of tow position was recorded, the winch brake released, and the dredge hauled aboard without washing.

When it was possible in 2014, fixed tows started on station position where possible, were repeated over the same tow line and in the same tow direction as in previous surveys.

Tows that could not be dredged because of foul ground were replaced with random stations selected in the order in which they were generated for that stratum. Tows were repeated with the same station number when the dredge became tangled or did not fish properly. Tows were not repeated when the dredge was landed less than 75% full, but mainly filled with kaeos (*Pyura pachydermatina*) or algae, or when the dredge came fast after 0.1 nautical mile.

All survey data including the presence/absence of bycatch species were recorded on the Foveaux Strait oyster survey form Appendix 1.

Sorting the catch

Only the aft dredge of the two commercial dredges was used for sampling during the survey. Dredge samples were landed onto the aft culching (sorting) bench without washing (i.e., without dipping the dredge) to avoid the loss of small oysters and benthic fauna. The fullness of the dredge was visually estimated while the dredge was suspended above the bench.

The catch of oysters and bycatch from each survey tow were photographed with a digital camera before 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.

In this February survey, new clocks were defined as those that had clean inner valves that had retained their lustre, but may have had some minor speckling of fouling organisms (Figure 8). For this analysis, we assumed that new clocks were only those oysters that had died since summer mortality from bonamia began, and oysters that died before this were categorised as old clocks.

The shells of oysters that are fouled or in which the inner valves have lost their lustre are termed old clocks (Figures 9 and 10). Old clocks can be covered in fouling organisms on both external and internal surfaces, and as the ligaments of oysters are thought to break down over about a three-year period, old clocks represent oysters that died between 1 and 3 years previously (Cranfield et al. 1991). The classification of old clocks may vary depending on habitat. Old clocks from sand habitats may be older as they may be filled with sand preventing the settlement of fouling organisms and reducing physical forces on the hinge and prolonging the time that both valves remain attached beyond three years. Gravel habitats are usually shallower with stronger tidal currents and higher swell energy and the valves of old clocks there may be disconnected much more quickly than three years or the clocks (new and old) may be transported out of the fishery area by the strong tides.

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). Reference rings (58 mm and 50 mm internal diameter) were used to ensure accurate allocation to each size group.

The data recorded at each station included start and finish location of the tow, depth, speed of tow; numbers of oysters, new clocks, and gapers caught; percentage fullness of the dredge; wind force (Beaufort scale); stations where live bryozoans (*Cinctipora elegans*) were observed; and sediment type.

The presence/absence of bycatch species was also recorded directly from the dredge contents. The data form used to record these data is shown in Appendix 1.

Figure 8: New clock (with hinge intact), glossy inner valve with no fouling except a few white coralline specks.

Figure 9: Recent old clock (with hinge intact), glossy inner valve with light fouling.

Figure 10: Old clock with hinge intact. No gloss on inner valve and heavy fouling.

3.3.1 Specific objective 3: Estimates of oyster densities and population size

Objective 3 is the estimation of the recruit-sized oyster abundance and biomass in the area of the commercial Foveaux Strait oyster fishery for the 2014, 2015 and 2016 fishing years with a target CV of no more than 20%. We estimated abundance as the mean density of oysters, but in three size groups (recruit-sized, pre-recruit, and small oysters), and biomass as the population size in millions of oysters for the same size groups by stratum and for all strata combined.

Oyster densities and population sizes for the three groups of live oysters (recruit-sized, pre-recruit, and small oysters) were estimated for strata where three or more randomly selected stations were sampled in February 2014 and these were compared with the estimates from the same strata sampled in the 2012 stock assessment survey. These estimates are presented separately. The absolute population size of each size group of oysters in 2014 was estimated using the combined population sizes in each stratum.

The Shellfish Working Group requested estimates of commercial population size (using the standardised catch of recruit-sized oysters at each station minus 400 oysters) for all strata with three or more randomly selected stations. This estimate of commercial population size was used to estimate yield prior to 2004 and continues this historical time series for comparison of the commercial population size and the distribution of high oyster density.

Estimates of absolute abundance and variance were calculated using standard stratified random sampling theory (Francis 1984, Jolly & Hampton 1990). We use 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 & Cranfield (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*.

Two sets of 95% confidence intervals are presented as request by the Shellfish Working Group (June 2014):

1. The sampling 95% confidence intervals:

$$\bar{x} \pm 1.96 \frac{s}{\sqrt{n}}$$

Where $\bar{x}$ is the estimated mean population size (numbers of oysters), s is the standard deviation for the estimated population size, and n the numbers of stations sampled.

2. 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.

Patterns of recruitment

Small oysters settle and remain attached to settlement surfaces up to a size of about 40 mm in length. The lower valves of oyster spat are fully cemented to settlement surfaces to a size of about 30 mm in length, at which point the shell begins to grows away from the settlement surface and the oyster separated by mechanical disturbance some time thereafter. Most small oysters are found on live oysters, possibly because the survival of juveniles is likely to be better on large, live oysters. Relatively few small oysters are found on other settlement surfaces. Up to seven generations of oysters have been observed in clusters of oysters sampled from areas with relatively little disturbance. We assume the dredge selectivity of recruit, pre-recruit and small oysters to be the same, and in reality, the selectivity is likely to be similar.

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, and the February 2014 surveys. The numbers of small oysters per recruit-sized oyster were estimated to investigate trends in recruitment between 2012 and 2014.

Size frequencies of the population

About twenty oysters of each size group (recruit-sized, pre-recruit, and small oysters) were randomly selected from the dredge catch sampled at each of the first-phase stations. These oysters were confirmed to be in their respective size groups using the reference rings and measured for length (along the anterior-posterior axis) and height (along the dorsal ventral axis), see Figure 11.

Length frequency distributions for recruit-sized, pre-recruit, and small oysters were weighted to the size of the population using the NIWA trawl survey software survCalc (Francis & Fu 2011). Length frequency distributions were scaled by a factor which is the ratio of the stratum area to the average catch rates (density), and are therefore effectively scaled to the population size in that stratum, see Francis & Fu (2011) for details.

3.3.2 Specific objective 2: Estimates of annual mortality from Bonamia

There are a number of potential causes of mortality in Foveaux Strait oysters: predation (Cranfield 1975), incidental mortality from dredging (Cranfield et al. 1997), and disease (Hine et al. 1986, Hine & Jones 1994). Both predation and incidental mortality are thought to be relatively low for recruit-sized oysters. *B. exitiosa*, coccidioses causd by an unidentified apicomplexan, and *Bucephalus longicornutus* can cause mortality in oysters. When two or more of these pathogens occur together as concurrent infections, bonamia is the most likely cause of mortality (Hine et al. 2002). Heavy infestations of Cliona spp, a boring sponge that infects oyster shell, may also be a predisposing factor for bonamia mortality. Survey data since 2000 suggests that trends in recruit-sized oyster population size are driven by bonamia mortality and recruitment.

Most of the recent bonamia mortality has occurred in the summer months, however 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, and
2. Post-survey mortality (within about two months) 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 was estimated as the absolute population size of recruit-sized new clocks and gapers using the same methods as for live oysters (see Section 3.3). In the absence of dredge efficiency data for clocks, we assume that the dredge efficiency is the same for clocks as it is for live oysters. The catchability (dredge efficiency) and persistence of new clocks at the location of death varies spatially for new clocks, and their classification as new (to distinguish them from old clocks with fouling organisms on the inner shells) can be difficult. The eastern fishery area is characterised by strong tidal currents and gravel substrates, and an unknown proportion of the new clocks are probably transported out of the area, therefore underestimating mortality. In western fishery areas, the sand substrate can be mobile and the shells of dead oysters may be buried in sand, initially underestimating mortality, but may eventually be scoured out of the substrate some time later and may be mistaken for new clocks as their burial has preserved the articulation of the hinge and prevented the settlement of the fouling organisms used to distinguish new and old clocks. If new clocks have been buried for some time, the lustre of the inner shell is lost and this is used to distinguish new and old clocks, and reduce misidentification. Dredge efficiency for clocks is likely to be much lower and therefore clock densities are likely to be underestimated.

Post-survey mortality is estimated from the numbers of fatally infected, recruit-sized oysters in the samples. Bonamia studies (Diggles et al. 2003) suggest that category 3 bonamia infections (see Table 2) are elevated and systemic, and are assumed to quickly progress to category 4 and 5 infections, quickly leading to death (soon after the survey). The mean proportion of oysters with category 3−5 infections in each stratum is used as a correction factor for the population estimates, i.e. one minus the mean proportion of category 3−5 infections. Population estimates for each stratum and the total survey area are recalculated to account for the projected mortality. A second estimate of post-survey mortality uses the combined prevalence of oysters with category 3–5 infections, that is the actual numbers of fatally infected oysters in the catch, and stratum and population estimates of fatally infected oysters are made using the same analytical methods as for live oysters, scaled up to the size of the population.

Total post-survey mortality is the difference between the total population size at the time of survey and the population corrected for bonamia mortality (at the end of summer).

3.3.3 Specific objective 5: Methods 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 qPCR assays. The numbers of non-fatally and fatally infected oysters were estimated from bonamia intensity scores derived from heart imprints 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 bagged, labelled with station number, date, and time on waterproof labels and the sacks tied securely. The oysters for bonamia samples were kept cool and damp in oyster sacks, transferred to poly bins, and flown to NIWA, Wellington, for processing. Oyster samples generally arrived in Wellington within 36 hours of capture, and were processed that day. The samples were held in poly bins under cool conditions (about 8–12 °C) in the aquarium. If they could not be processed the day they arrived, they were held in tanks of flowing seawater and processed at the first opportunity.

Heart imprints 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 to estimate the prevalence and intensity of bonamia. Each oyster in the sample was assigned a unique number from 1 to 25, and assigned a size category using oyster size rings, and oysters were measured for length and height (Figure 11) using callipers, and the measurement truncated to the lower whole millimetre. If samples contained insufficient recruit-sized oysters, pre-recruits were used in preference to small oysters. 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. These samples also provide estimates of the numbers of oysters incubating larvae at each station to provide information on reproduction in the fishery. 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 by removing the heart (dark organ adjacent to adductor muscle, see Figure 12) with fine forceps, draining excess water and fluid on filter paper, and lightly dabbing the heart on a slide to deposit a small amount of haemolymph. Three rows of 8 to 10 imprints were made on labelled slides. Slides were placed in slide racks to air dry for at least 5 minutes. The slides were stained with Hemacolor © and oven dried at 60 °C.

Histological samples were taken from the first five oysters processed for heart imprints (these were noted on the bonamia data form as Y). A section was taken through the digestive gland (Figure 12) and fixed in a quantity of 10% formalin in seawater equal to at least five times the tissue volume of the sample.

Sampling methods for qPCR

Oysters sampled for heart imprints were also sampled for qPCR. Remnants of the oyster hearts sampled for heart imprints and samples of gill tissue were placed into separate, uniquely labelled 96 well qPCR plates. Additional samples of gill tissue from the same oysters were placed into new uniquely labelled 96 well qPCR plates as backup tissues should they be required, and a third series of plates containing gill and mantle tissue provided to the Ministry for Primary Industries Biosecurity (Dr Brian Jones). 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.

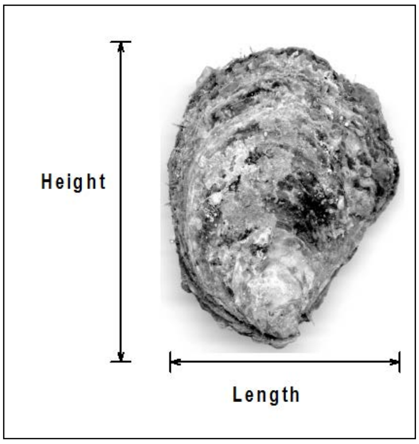

Figure 11: An oyster showing length (anterior-posterior axis) and height (dorsal-ventral axis) dimensions.

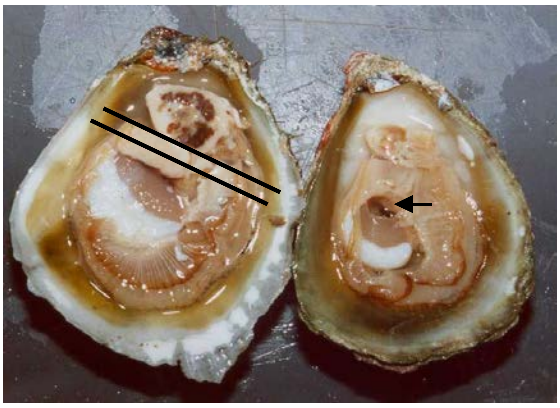

Figure 12: Lines on left oyster show location of 5 mm thick standard section taken for histology. The arrow on the oyster on the right shows the heart, a black organ adjacent to the adductor muscle.

Procedures were implemented to prevent contamination of the qPCR samples. Laboratory staff rinsed utensils for every oyster sample, and replaced gloves and rinse solutions for every station. Pre-labelled 96 well plates covered with plastic film were placed on the chill blocks to keep samples cool. These chill blocks were stored at -20°C between uses. The film was cut and removed to expose a single column of 8 wells on the plate and the wells covered with strip caps after the samples were deposited. The plates were temporarily stored at -20°C then transferred to a -80°C freezer for storage at the end of the day.

Analysis

Analysis of qPCR samples

A detailed account of the qPCR method and testing is given in Maas et al. (2013). This novel qPCR method has been successfully developed to detect and quantify *Bonamia exitiosa* in oysters from Foveaux Strait. This method relies on two key innovations: a duplex qPCR assay and a shortened bench top method. The characteristics of the qPCR assay include the co-amplification of the *Bonamia* target (ITS region of the ribosomal genes) and *Ostrea chilensis* β-actin gene as an internal control. In addition, the assay uses a new master mix containing a robust taq polymerase mix (thermostable DNA polymerase used in polymerase chain reaction (PCR) to amplifying short segments of DNA) that is able to cope with inhibitors often found in crude extracts and extracts from environmental samples. A novel system is also employed 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 has also successfully incorporated a shortened bench top method to minimise handling, and was transferred to a 96 well plate format to allow the simultaneous screening of up to four bonamia stations per hour compared to 3−4 stations per day using histological methods (heart imprints). Oyster heart and gill tissue were analysed using the same method. Tissues were digested and diluted, and aliquots of extract added to qPCR reagents that were then analysed with a BIORAD-CFX96 qPCR (quantitative polymerase chain reaction instrument).

The assay is a chemical reaction that identifies the presence of the target DNA. The sample is subjected to a cyclic process of denaturation, primer annealing, and extension. If the target DNA is present, the annealing process produces fluorescence that is measured by the instrument. At each cycle, the amount of target DNA is doubled, the more target (bonamia) DNA is present, the higher the fluorescence. The instrument records the amount of fluorescence in relative fluorescence units (RFU) at each cycle. This process is generally run over 40 cycles.

The qPCR data were analysed using BioRad CFX Manager™ software (Version 3.0). The cycle of quantification (Cq), the fractional cycle number where fluorescence increases above the baseline was determined by the regression method as implemented in the option using the BioRad software. Samples that produced detectable fluorescence between cycles 10 and 35 were positive.

qPCR data from oyster heart and gill samples were assessed based on the information for each plate contained within the sample sheets to ensure that there were no sampling issues such as tissue size, contamination, or missing samples that may have affected the assay or the interpretation of the results. Plots of relative fluorescence units (RFU) against Cq values were used to look for problems in the qPCR reaction such as very early amplification (less than a Cq of 10) or unusual undulating plots. Cq values for the positive control (Bonamia ITS and β-actin gene) were checked on every plate to ensure that they were within the range (about Cq 26), and the negative internal control was monitored to ensure that there was no reaction occurring that would suggest problems with the assay.

Rules were established for repeating qPCR reactions for each sample or plate, or the rejection of data from the analysis (see Maas et al. 2013 for details). Sample assays were repeated or data were omitted when:

- out of range Cq values for the bonamia positive and negative control wells (at about a Cq of 28) occurred;
- the Bonamia ITS and internal control Cq values were both NA (i.e. there were no values to show that the reaction had worked);
- either the Bonamia ITS or internal control (β- actin) amplified very early in the cycles (Cq less than 10); or
- the internal control (β- actin) Cq values were late (Cq values 40 or more) together with no Bonamia ITS amplification having occurred.

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 is no qPCR data) were also examined. Repeated samples that gave anomalous results such as flatliners where no reaction was detected or early ampers (very low Cq values) were also screened with heart imprints.

Analysis of oyster heart imprint data

Examination of heart imprints is at least as sensitive as histology, but whereas histology is time consuming and expensive, heart imprints can be screened rapidly and are comparatively inexpensive. However correlation studies with in-situ hybridisation have shown that the prevalence of bonamia estimated from heart imprints can underestimate the true infection rate by about 30% (Diggles et al. 2003).

The prevalence and intensity of bonamia infection was determined from heart imprints in all oyster samples that had tested positive by qPCR from all 72 stations, at least 3 randomly selected samples from each station that tested negative with qPCR, and flatliners and early ampers after samples underwent repeat qPCR assays. Oyster heart imprints were examined under a microscope using a times 50 objective lens under oil and scored for intensity of infection using the criteria listed in Table 2. 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. In 2014, heart imprints were examined by a single experienced reader. A review of scoring protocols was undertaken before screening samples.

Table 2: Criteria used to stage intensity of bonamia infection in oysters.

Stage	Criteria
0	No bonamia observed
1	One bonamia cell observed after examining an imprint
2	More than 1, but fewer than 10, bonamia cells observed after examining an imprint
3	More than 10 bonamia present in the imprint, but few in each haemocyte
4	Bonamia present in many haemocytes of each imprint and many in each haemocyte
5	Bonamia present in nearly all haemocytes of each imprint and many in each haemocyte, and extracellularly

In the absence of qPCR assays, it is assumed that category 0 oysters are not infected. Previous studies (Diggles et al. 2003) have suggested that stage 1 and 2 level bonamia infections are relatively light and do not appear to adversely affect the host. Stage 3 infections are much more elevated and systemic, and are associated with minor tissue damage throughout the host. It is likely that stage 3 infections will almost always progress to stage 4 (Diggles et al. 2003). Stage 4 infections are systemic, and all tissues are congested with infected haemocytes; death appears inevitable. Stage 5 infections differ from those of stage 4 in that tissue damage is extreme throughout the animal, tissues have lost their integrity, and the oyster is near death.

Prevalence, the proportion of infected oysters in a sample is estimated from both qPCR and heart imprints. The 96 well plate only allows for heart samples 1–24 to be screened by qPCR, and the 25th sample from each station is screened using heart imprints only. qPCR positives for bonamia infection are determined from those samples where Cq for heart tissues is 35 or less, and for heart imprints where at least one bonamia cell is observed in a haemocyte.

Mean intensity is defined from heart imprints only as the mean category of stages 1–5 oysters (i.e., the mean stage of all oysters examined that had at least one bonamia cell observed). The inclusion of the additional smaller oysters at stations where few recruit-sized oysters were caught is likely to introduce a bias to estimates of prevalence and intensity of infection because oysters are increasingly less vulnerable to infections and mortality as oyster size decreases. 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

Estimates of fatal and non-fatal infections were scaled to the size of the recruit-sized oyster population by scaling mean infected oyster densities to the size of stratum areas, the size of the area for all core strata combined, and to the whole survey area.

Two methods were used. Method 1 used a correction factor based on strata with three or more randomly selected stations only i.e., we did not include fixed 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, scaled to the total catch for each station. The overall intensity was then calculated as the average bonamia level in the population. Variance estimates for prevalence and intensity were estimated using standard methods as for population estimates.

3.3.4 Specific objective 6: Methods to 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 routine 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%, it was predicted that the population size would only increase by 11% over the same period (Fu & Dunn 2009). Bonamia mortality has been 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 has 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 the most appropriate projection for future stock status be selected 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 have been used previously to select the most appropriate projection, the population estimates predicted were similar to the population sizes estimated from subsequent surveys.

3.3.5 Specific objective 7: Methods to 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. New reagents were tested against reagent stock used for the 2013 sample processing to ensure consistency of reaction and Cq values between survey years. 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 Identifying the core commercial fishery area (objective 1)

Fishery data comprising the Ministry for Primary Industries Catch Effort Landing data (CELR) from 1975–2013, fishers' logbook data (2006–2013), oyster skippers' knowledge from fisher interviews; and fishery independent survey data 1993–2012 were accessed to define the area where the greatest proportion of catch has historically come from. The objective required this assessment to be made over an appropriate number of years. Because of the rapid changes in oyster density and population size, rapid changes in the distribution of oyster density during epizootics (periods of high bonamia mortality in the range of 40–80% per year), and changes in fishing patterns as a response to changes in the distribution of commercial fishery areas, we used data from periods when the fishery was either rebuilding or had peaked before the onset on a new epizootic. We used the 1999, 2009, and 2012 survey data and fishery data between 2006 and 2013. To maintain consistency with the time series of OYU 5 stock assessment and bonamia surveys, the outer stock boundary was defined as the 2007 survey area which includes the 1999 survey area and an additional stratum (B1a) included in surveys since 2007.

CELR data 1975–2005 were excluded because they were recorded at a spatial scale that was too large to delineate areas of relatively high oyster density (see Figure 1, Appendix A3.1). Figure A3.1 shows the regions within the fishery that were historically important between 1975 and 2006.

Fisher interview data are summarised in Figure A3.2, showing the survey strata that had been historically important to the fishery, and Figure A3.3, showing the survey strata thought to be important in 2014. Fishers' logbook data (2006–2013) are recorded at a fine spatial-scale of one nautical mile square grid (Figure 2), and these grids are shown with survey strata in Figure A3.4. Figure A3.5 shows the percentage of the annual catch by location in 2009, Figure A3.6 in 2012, and Figure A3.7 in 2013. Although these data give fine-spatial distributions of fishing, these fishing patterns are primarily driven by oyster meat condition (high meat condition receives higher market prices) and not oyster catch rates or oyster density. Oyster meat condition varies spatially and to a lesser extent temporally. Some fishery areas that have high densities of oysters, especially in western fishery areas, have consistently low oyster meat condition and do not receive significant fishing effort. Figures A3.5 to A3.7 show distinct patch fishing with a preference to fish in southern and eastern areas where meat condition is better.

Survey data, from stock assessment surveys, provided the best information on the distribution of oyster density (Appendix 3, Figures A3.8–A3.14, and see Figures 14–16). Bonamia surveys between 2000 and 2013 sampled a subset of the stock assessment survey area that was important to the commercial fishery. Surveys between 2000 and 2006 were aimed at monitoring the spread of bonamia, and subsets of randomly selected stations from stock assessment surveys were sampled to provide station level comparisons of bonamia status and related mortality, and oyster density. From 2007 to 2013, bonamia surveys aimed to include stratum by stratum comparisons of bonamia infection, and estimates of oyster population size for the designated commercial areas. These surveys sampled different combinations of strata and survey areas because of the changing patterns of mortality and rebuilding of oyster densities.

Because of the long time series of stock assessment and bonamia surveys (1990–2013), there is high value in retaining survey stratum boundaries. Fishers target localised oyster populations with the highest meat quality. A move to fine spatial-scale management of fishing to maximise the yield from areas with high oyster meat quality would benefit the industry greatly. Retaining this time series of survey strata will contribute greatly to spatially explicit assessments for these fishery areas.

Data from the 1999, 2009, and 2012 surveys represent peaks in the oyster population size and times when oyster densities had rebuilt over a majority of the fishery area. They are likely to be the most representative of the pre-epizootic distribution of oyster density. The data were plotted over the 2012 survey strata (shown in Figure 13), see Figures 14–16, to identify key strata. The data show consistent patterns in the distribution of oyster densities. Recruited, pre-recruit, and small oyster densities show similar distributions. The fourteen core strata representing commercial fishery areas with relatively high recruit-sized oyster densities (not catch for reasons given above) were B1, B3, B6, C1a, C2, C3, C5, C5a, C7, C7a, C8, C9, E2, and E4. Fishers' logbook data suggest that there were no other significant areas of relatively high oyster density outside of these proposed core strata.

There was one anomaly between the 1999, and the 2009 and 2012 survey data. A relatively high density patch of recruit-sized oysters between Saddle Point and Garden Point in the north-western part of stratum B4 in 1999 was greatly reduced by bonamia mortality shortly thereafter. Fishers consider this patch of oysters largely extinct and of little significance to the fishery now, and this localised patch has received no sampling effort since 2001 (Appendix 3, Figures A3.11–A3.14, Figures 15 and 16). B4 was designated as a background stratum in 2009 and 2012, receiving only three random stations. The area did not feature in subsequent fishers' logbooks. Stratum B4 was not partitioned to incorporate this patch of oysters in the core strata.

The 14 core strata chosen were ranked by oyster density (Table 3) and almost all were above the density considered to provide commercial catch rates of 6–8 sacks per hour (if the oysters were in good condition). Boxplots of the distributions of mean oyster density and the percentage of the total population size by stratum 2009–2013 are shown in Figures 17 and 18. These core strata accounted for 45.9% of the 2012 stock assessment survey area and 74.9% of the recruit-sized oyster population. Core strata and the remaining strata merged into one background stratum are shown in Figure 19.

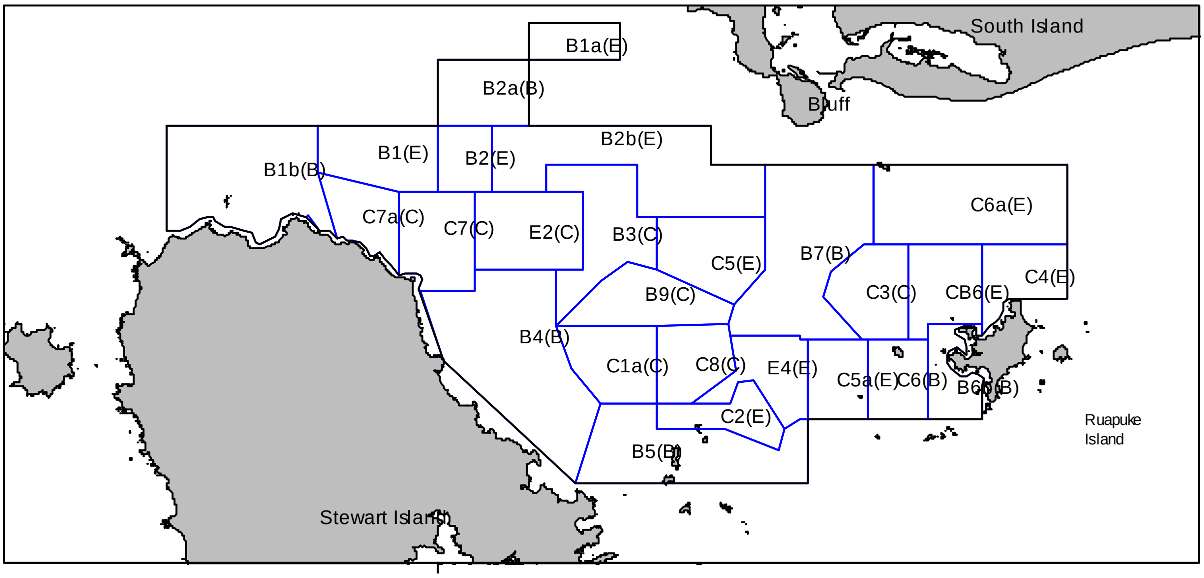

Figure 13: The 2012 stock assessment survey area with the 2007 survey boundary shown as a heavy, black outer line, B1a is a stratum added for the 2007 survey. The 2012 survey strata are shown as blue lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

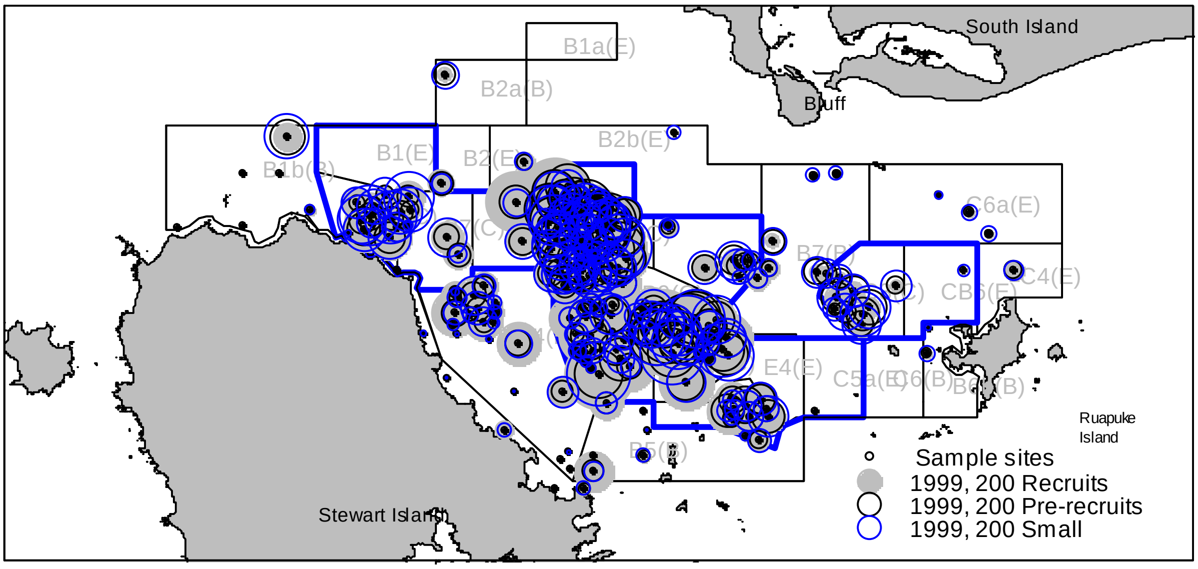

Figure 14: The 1999 survey data for recruit-sized, pre-recruit, and small oysters plotted over the 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata, (heavy blue line) and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

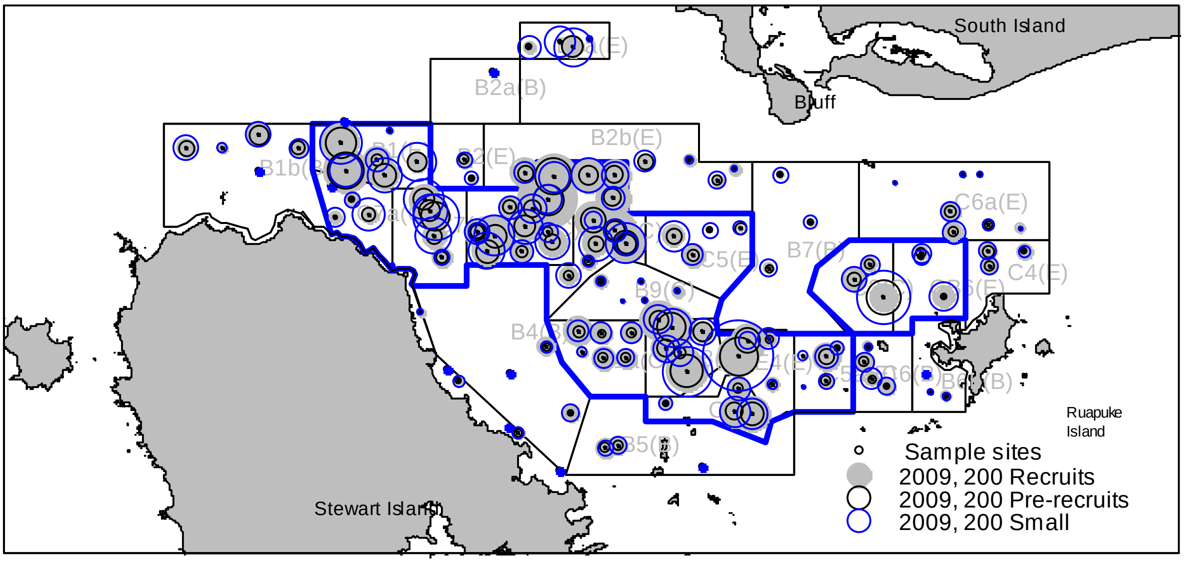

Figure 15: The 2009 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

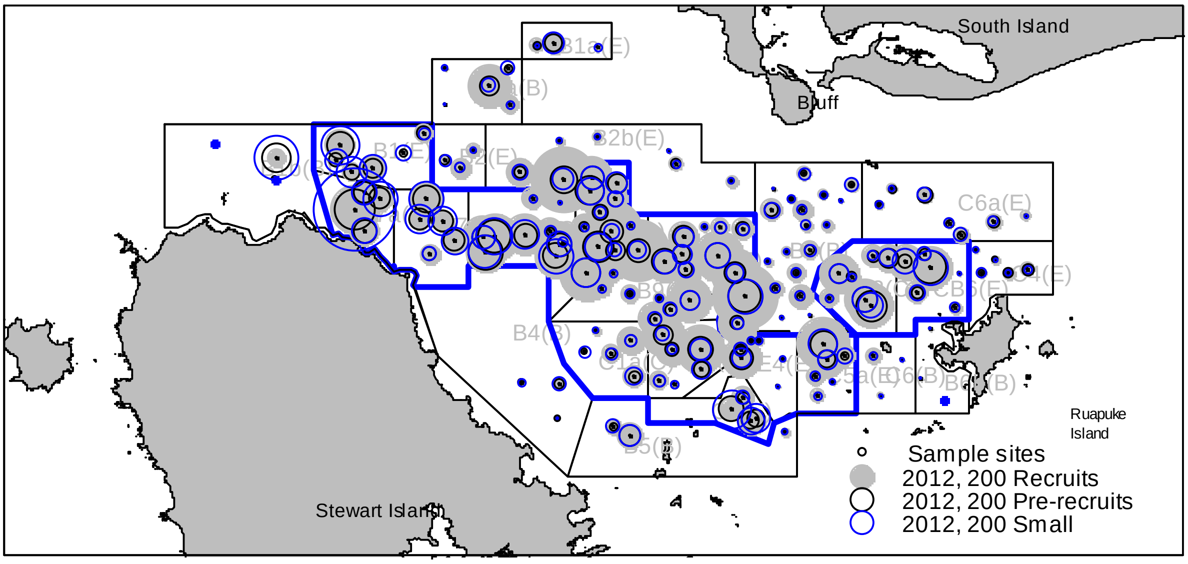

Figure 16: The 2012 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

Table 3: Fourteen core strata identified from the 1999, 2009, and 2012 surveys showing their mean density (density), and the standard deviation (s.d.) and coefficient of variation (CV) of the density estimate, recruited population size (Population) and 95% confidence intervals (95% CI), and the area of each stratum.

Stratum	Stations	Density	s.d.	CV	Population	95% CI	95% CI	Area (km^2)
B3	13	3.56	0.7	0.2	158.9	85.3	263.7	44.7
C5	8	1.98	0.53	0.27	74.5	31.9	131.2	37.7
E2	8	1.87	0.49	0.26	80.3	35.5	142	42.8
C7a	4	1.78	0.76	0.43	42	6.9	86.8	23.6
C8	6	1.65	0.64	0.39	44.3	9.7	88	26.8
C3	6	1.44	0.41	0.29	47.1	19.5	85.3	32.7
C5a	5	1.34	0.86	0.64	31.6	0	79.9	23.5
B6	6	1.15	0.5	0.44	34.4	4.5	71	30
C9	6	1.04	0.49	0.47	35.8	2.1	76.9	34.5
C7	5	1.01	0.18	0.18	36.4	20.2	59.1	36.1
C2	3	0.97	0.16	0.17	21.2	12.3	34.1	21.9
B1	5	0.7	0.24	0.34	54.6	17.3	103.2	78.2
C1a	5	0.58	0.23	0.39	18.2	4	36.6	31.3
E4	4	0.31	0.22	0.7	8.8	0	22.7	28
Sub total	84				688.1			491.8
All survey	146	0.86	0.07	0.08	918.4	600.1	1383.7	1070.3
Percentage	**74.9**				**74.9**			**45.9**

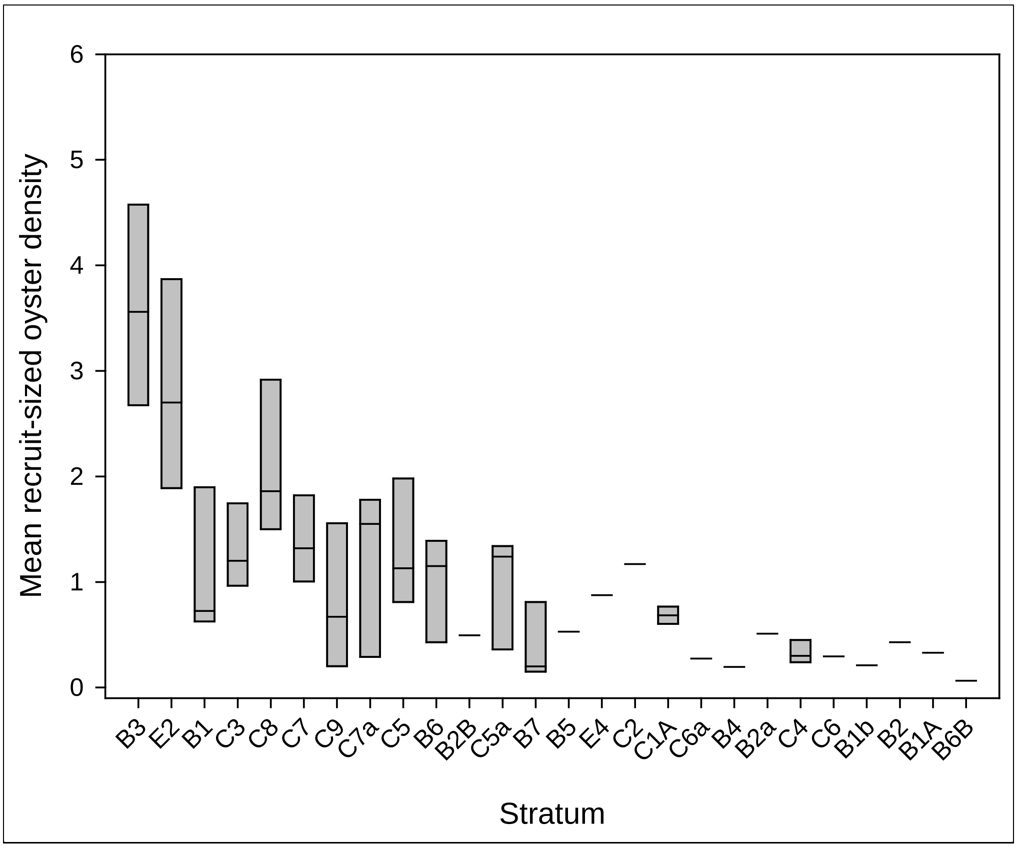

Figure 17: Boxplots of the distributions of mean oyster densities by stratum 2009–2013. Medians shown as solid lines, boxes represent the range of means.

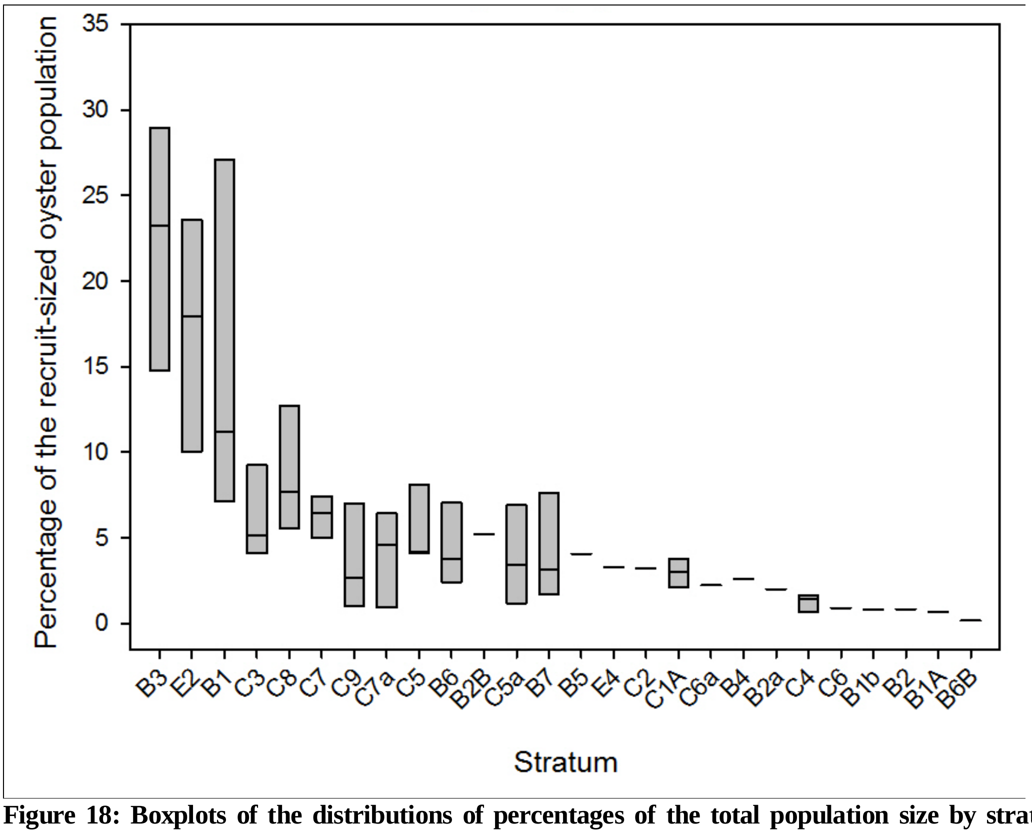

Figure 18: Boxplots of the distributions of percentages of the total population size by stratum 2009–2013. Medians shown as solid lines, boxes represent Medians shown as solid lines, boxes represent the range of means.

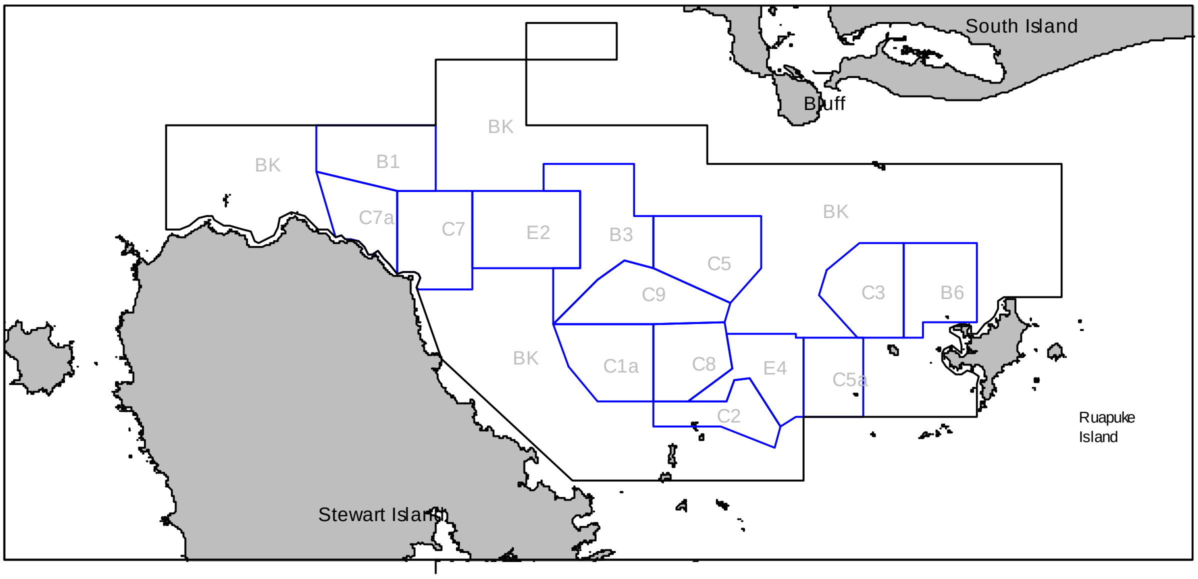

Figure 19: The 2012 stock assessment survey area with the 2014 survey strata shown as blue lines. The remaining 12 strata from the 2012 survey have been merged into a single stratum (BK).

Boxplots of recruit-sized oysters sampled in standardised tows from stock assessment and bonamia surveys 1993–2013 from all core strata selected to represent the new bonamia survey area (core strata) and from all strata outside this area, but within the 2007 stock assessment survey area merged into a single background stratum (Background) are shown in Figure 19. At times, individual tows did record relatively high catches in the background stratum, but these high catches were fewer and lower than in the core strata (Figure 20). A Kruskal-Wallis one way Analysis Of Variance on ranks showed that the difference in the median values between the core and background strata was significantly different (P ≤0.001).

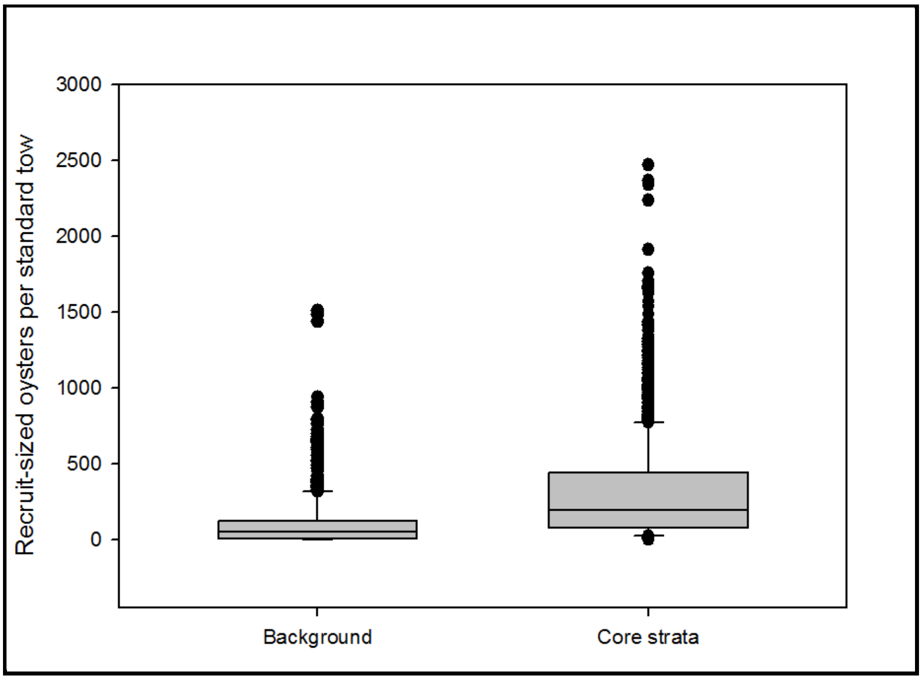

Figure 20: Boxplots of recruit-sized oysters sampled in standardised tows from stock assessment and bonamia surveys 1993–2013 from all core strata selected to represent the new bonamia survey area (Core strata) and all strata outside this area, but within the 2007 stock assessment survey area merged into a single background stratum (Background).

4.2 Simulations to optimise survey design and sample numbers (objective 2)

The core strata identified in objective 1 delimiting the new survey area comprised fourteen strata. As most of the recent surveys have achieved CVs of 8–12% for estimates of oyster population size, we undertook simulations to determine the minimum number of strata and numbers of stations required to achieve a target CV of 10–12% for an estimate of the recruit-sized oyster population.

For the 2014 survey, we used the 2009 and 2012 stock assessment survey data to estimate the numbers of stations as the 1999 survey stratification and sampling distribution differed greatly from the strata used from the 2001 stock assessment surveys onwards. The 2013 survey only surveyed ten of the 14 core strata. Because of the changes in the distribution and density of oyster from heightened bonamia mortality over the summer of 2013–2014, we used only data from the 2012 and 2014 surveys to estimate the numbers of stations required for the 2015 and 2016 surveys. Only randomly allocated sample stations were used to calculate the numbers of sample stations required to give CVs in the range of 10% to 20% for different combinations of merged strata. The results are tabulated in Appendix 4. These data are plotted in Figures 21–23 for recruit-sized oysters, recruit-sized new clocks, and prevalence of infection in recruit-sized oysters. The stratification used for the 2014 survey (14 core strata) produced the lowest CVs in all cases (recruit-sized oysters, new clocks, and prevalence of bonamia, Figures 21–23) and for all data sets (the 2009, 2012, and 2014 survey data). Simulations with the 2009 and 2012 data predicted that 55 sample stations in the 14 core strata would give a CV of 11.03% and 11.44% respectively.

Simulations made with the 2009 and 2012 survey data, and with the 2014 data post survey predicted different numbers of stations required to produce CVs of around 12% for recruit-sized oysters (Appendix 4). The 2009 and 2012 surveys of the oyster population were undertaken at a time when the oyster population was increasing and oyster density was more variable, mostly because of the numbers of localised patches of relatively high oyster density. The higher variance in estimates resulted in higher numbers of samples being required to achieve a CV of 12%. Heightened bonamia mortality over the summers of 2012–2013 and 2013–2014 reduced the numbers of high density patches and overall recruit-sized oyster density, reducing variability. Simulations made with the 2014 survey data predicted fewer stations required to achieve the target CV of 12%.

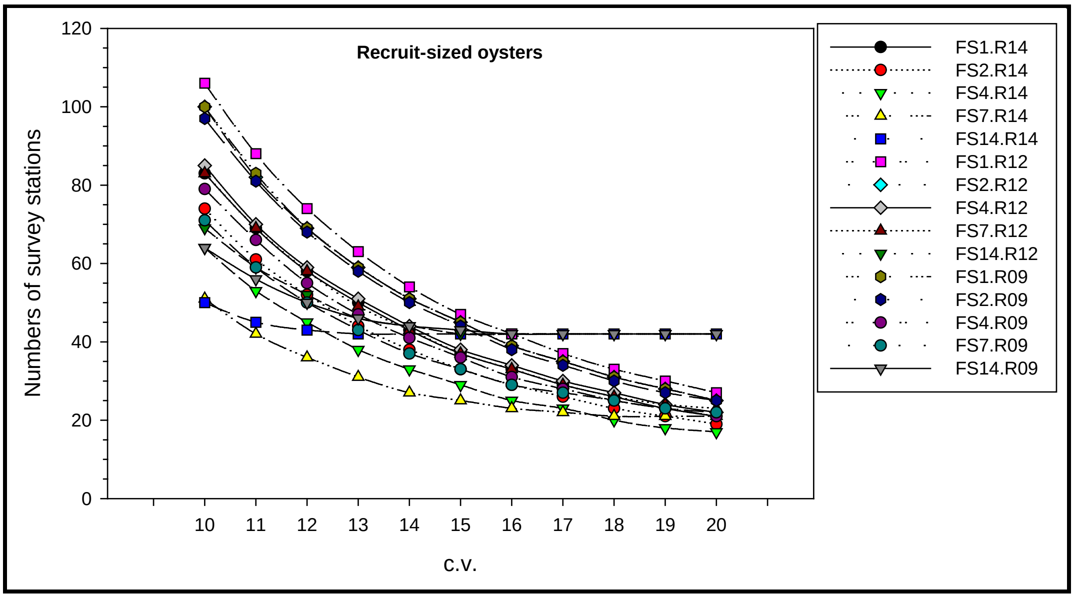

Figure 21: The numbers of sample stations required to give CVs in the range of 10% to 20% for estimates of recruit-sized oysters (.R) calculated using data from randomly allocated stations from the 2012 and 2014 surveys (suffix 12 and 14 respectively) with the NIWA software Allocate. The fourteen 2014 survey core strata (prefixed FS14) are merged into 1, 2, 4, and 7 strata, FS1, FS2, FS4, and FS7 respectively.

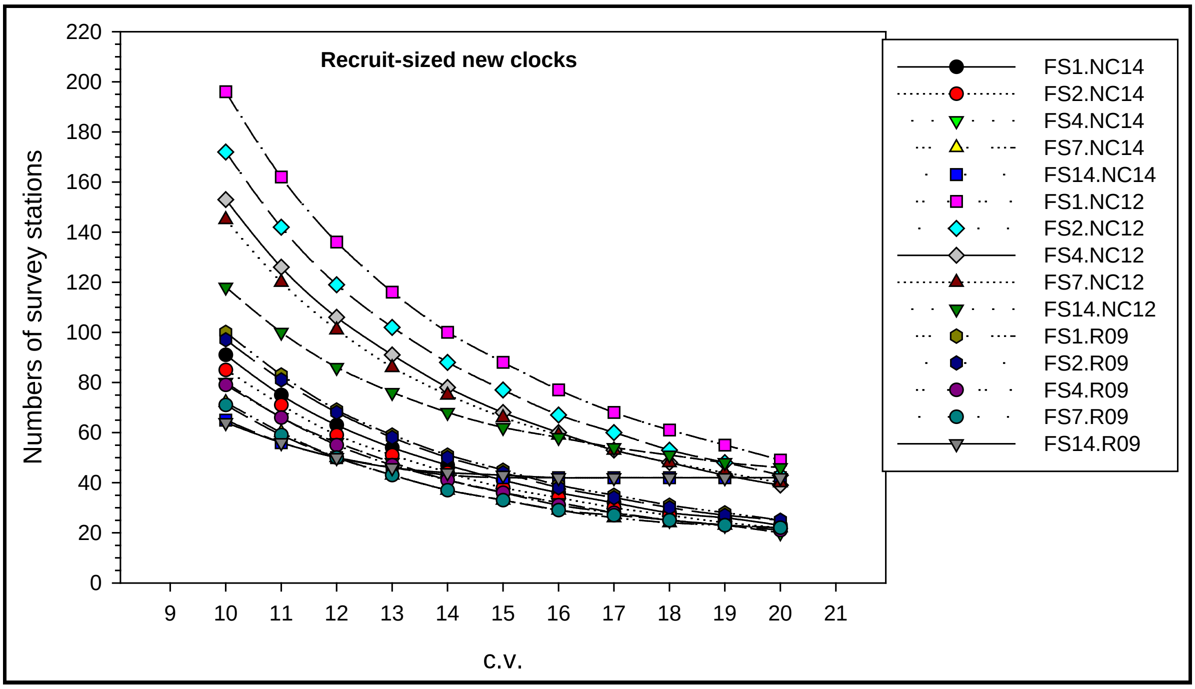

Figure 22: The numbers of sample stations required to give CVs in the range of 10% to 20% for estimates of recruit-sized new clocks (.NC) calculated using data from randomly allocated stations from the 2012 and 2014 surveys (suffix 12 and 14 respectively) with the NIWA software Allocate. The fourteen 2014 survey core strata (prefixed FS14) are merged into 1, 2, 4, and 7 strata, FS1, FS2, FS4, and FS7 respectively.

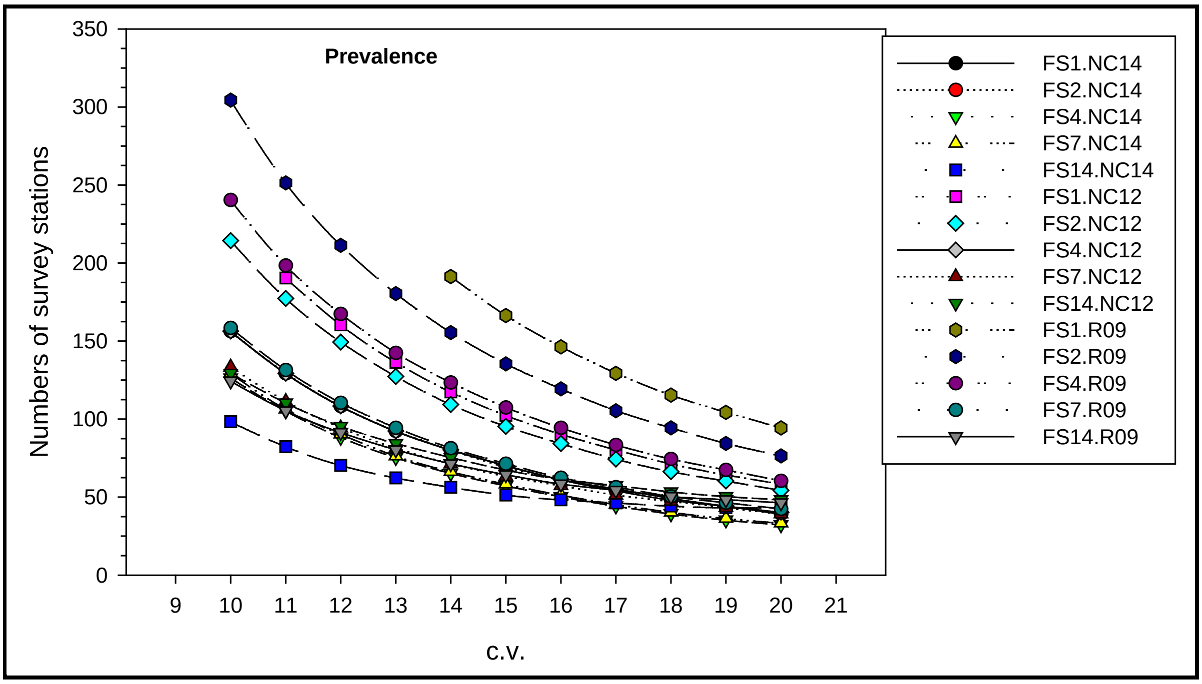

Figure 23: The numbers of sample stations required to give CVs in the range of 10% to 20% for estimates of recruit-sized oysters infected with bonamia (.Prev) calculated using data from randomly allocated stations from the 2012 and 2014 surveys (suffix 12 and 14 respectively) with the NIWA software Allocate. The fourteen 2014 survey core strata (prefixed FS14) are merged into 1, 2, 4, and 7 strata, FS1, FS2, FS4, and FS7 respectively.

4.3 Oyster abundance (objective 3)

4.3.1 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 11 fixed stations (T2 was not sampled because it was at the same location as station 1). Several allocated stations and some of their replacement stations couldn't be sampled because of rough ground, stations 19, 24, 25, and 35 were replaced by stations 67, 82, 80, and 92 respectively. Replacement stations 81, 90, and 91 also couldn't be sampled because of rough ground. Station 1 was allocated very close to station T2, and only station 1 (one of only three random stations in stratum B1) was sampled. Sampling began on the 8th of February 2014 and was completed on the 12th of February, sampling on all four days over this period. Survey tows completed are shown in Figure 24, and the numbers of repeat and fixed stations sampled in each stratum are shown in Table 4.

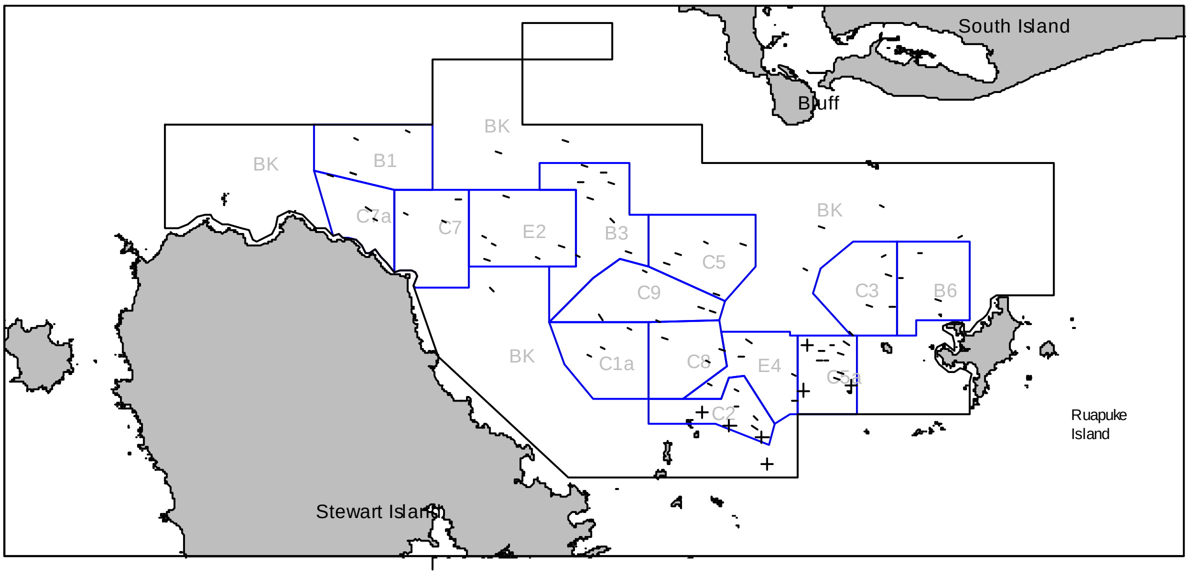

Figure 24: The survey tows (black lines) sampled in February 2014 to determine the status of bonamia infection and oyster density. The stations that could not be dredged because of foul ground are shown as crosses. The 2007 survey area is bound by the outer black line and the February 2014 survey strata are bound by the blue lines. The 2014 survey stratum designations are shown in brackets. Commercial strata designated by oyster skippers in 2014 have a "C" suffix and the background stratum shown as "BK".

Dredge tow lengths were almost all about 0.2 nautical miles (371 m) in length (Figure 25). One of the tows, station 48, had a tow length of 0.3 nautical miles and was 20% full suggesting that it had not become full over the extra distance towed. 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 light wind conditions (Figure 26). The median wind force was 2 on the Beaufort scale (3−6 knots), with 5 and 95 percentiles of Beaufort scale 0 (less than 1 knot) and 3 (7−10 knots) respectively, and the maximum wind during sampling was about 20 knots. These wind and resulting sea conditions were similar to sampling conditions on previous surveys and were mostly below the level likely to affect dredge efficiency.

Table 4: **The numbers of first-phase, second-phase, and fixed stations sampled in February 2014 by stratum.**

Stratum	First-phase	Second-phase	Fixed	Total
B1	3			3
B3	8			8
B6	3			3
BK	5		2	7
C1a	3			3
C2	3		1	4
C3	3	1	1	5
C5	4		1	5
C5a	4	3	1	8
C7	3			3
C7a	3			3
C8	3		1	4
C9	3	1		4
E2	4		2	6
E4	3		2	5
Totals	**55**	**5**	**11**	**71**

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 50%, higher than in 2013 when median dredge fullness was 30% (range 1–70%). Dredge saturation is not likely to have had a large effect on sampling effectiveness in 2014 and on the survey (Figure 27). 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 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. A few dredges tows were observed with dredge contents fitting this description in 2014, but these data are not routinely recorded in station records and it would be difficult to apply a correction factor for dredge saturation as it is likely to vary over space, time and habitat.

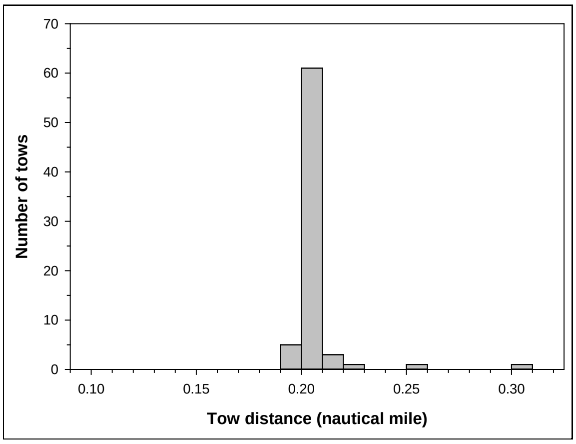

Figure 25: Distribution of dredge sample tow lengths from the February 2014 survey. The standard tow length was 0.2 nautical mile (371 m).

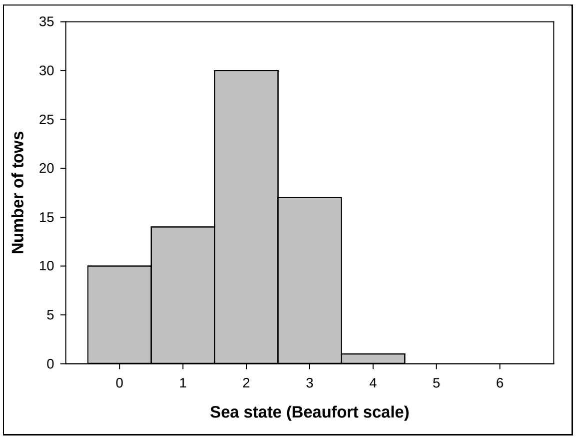

Figure 26: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2014. Beaufort scale: 0, under 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

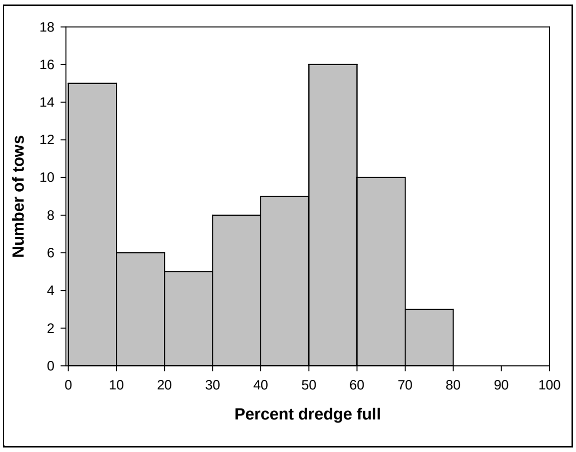

Figure 27: Distribution of dredge fullness recorded for survey tows in February 2014. No tows were landed with a dredge fullness of greater than 80%, suggesting that it may have saturated before the end of the tow leading to an underestimate of oyster density. Unpublished video data suggests that dredge saturation may occur below 80% full.

4.3.2 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 higher than in recent years and indicative of disease mortality at the time of sampling. These observations suggest detectable levels of bonamia mortality before and during the February 2014 survey.

Recruitment to the commercial fishery areas surveyed appears to be relatively low in February 2014. Most of the catches had relatively low numbers of pre-recruit and small oysters relative to the numbers of recruit-sized oyster in the catch, and spat and wings were notably absent from the catch.

4.3.3 Survey estimates of population size

Estimates of absolute population size, for recruit-sized, pre-recruit, and small oysters from the February 2014 survey are shown in Tables 5–7. 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.

Tables 8–10 show the population estimates for the three size groups of oyster from the 2012 survey 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 stations sampled in total in 2014. Table 11 compares survey estimates for recruit-sized, pre-recruit, and small oysters in 2012 and 2014, and give the percentage change in mean density and therefore population size.

The density and population size of recruit-sized oysters in core strata (commercial fishery areas) declined between 2012 and 2014 (Tables 5 and 8). The mean density in core strata declined from 1.40 to 1.09 oysters/m^2 and the population size from 688.1 million oysters to 538.0 million oysters respectively, a decrease of 21.8%. The coefficients of variation for all core strata combined were similar, 9.2% CV (number of stations sampled, N = 84) in 2012 and 11.2% CV (N = 55) in 2014. The predicted CV before the survey for all core strata combined in 2014 was 11.4%. The density and population size for all background strata combined doubled between 2012 and 2014 (Tables 5 and 8), 230.3 million oysters in 2012 to 482.9 million oysters in 2014, and the CVs were similar 19.7% in 2012 and 21.3% in 2014. It is not known to what degree the differences in the population size are driven by the small numbers of stations sampled in 2014 (62 in 2012 and 5 in 2014). The estimate of population size for all the background strata combined clearly contributed to the increase in total population size for the 2007 stock assessment survey area, 918.4 million oysters in 2012 to 1020.9 million oysters in 2014. The coefficients of variation for these estimates were relatively low and reflect differences in sampling effort, 8.5% in 2012 (N = 146) and 11.7% in 2014 (N = 60). Mean densities at eight of the fourteen core strata declined by more the 25%; strata ranked from the greatest decline were B3, C7a, C5a, C7, E4, C5, E2, and B6 (ranging from 63% to 28%), four strata remained similar (C3, C1a, C8, and B1), and two strata (C2 and C9) increased by 24% and 172% respectively. Strata C9, C8, E2, B3, C3, C5, and C2 (ranked highest down) had mean oyster densities higher than 1.0 oyster/m^2 (Table 5).

Pre-recruit oyster densities in core strata halved between 2012 and 2014 (Tables 6 and 9), from 0.60 to 0.30 oysters/m^2 and the population size has declined from 297.4 million oysters to 148.4 million oysters respectively. The population size for all the background strata combined and for the 2007 stock assessment survey area showed a similar downward trend (Tables 6 and 9) with the whole population of pre-recruits declining from 414.3 million oysters to 226.2 million oysters. Mean oyster density for pre-recruits decreased in most of the strata, ranging from a 27% to an 86% decrease. Mean oyster density in three strata remained similar, and in one stratum (E4) mean oyster density almost doubled.

The mean densities and population sizes of small oysters have decreased markedly between 2012 and 2014 (Tables 7 and 10). The size of the population in the core strata declined from 451.3 million oysters to 156.3 million oysters, a reduction of 65%. The population for all the background strata combined remained similar at 160.9 million oysters (2012) and 156.3 million oysters (2014). Overall, the population size of small oysters declined by about 50% from 612.2 million oysters to 302.6 million oysters (Tables 7 and 10). Mean small oyster densities were low across the core strata, all the background strata combined, and averaged for the fishery, and ranged between 0.72 oysters/m^2 and 0.14 oysters/m^2 (Tables 7 and 10).

Table 5: **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), 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 km^2, 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	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 6: 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), 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 km^2, 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 7: 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), 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 km^2, 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 8: 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), 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 km^2, 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 9: 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), 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 km^2, 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 10: 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), 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 km^2, 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

A summary of mean densities and population sizes for all common strata combined and the 2007 stock assessment survey area by size class is shown in Table 11. Only the recruit-sized oyster population over the whole survey area shows an increase in 2014, all size groups in the core strata have declined markedly (21.8% to 65.4%, Table 11).

Table 11: Percentage changes in the absolute population estimates from randomly allocated stations only for recruit-sized, pre-recruit, and small oysters in the 2014 core strata (N = 14), and for the whole 2007 stock assessment survey area (26 strata) sampled in 2012 and in 2014. 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), 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 percentage change in mean oyster density and therefore population size.

	2012							2014							
Core Strata	**Mean density**	**CV**	**Pop.n**	**S.lower 95%CI**	**S.upper 95%CI**	**B.lower 95%CI**	**B.upper 95%CI**	**Mean density**	**CV**	**Pop.n**	**S.lower 95%CI**	**S.upper 95%CI**	**B.lower 95%CI**	**B.upper 95%CI**	**Percentage change**
Recruit	1.4	0.09	688.1	674.4	701.8	449.2	1046.7	1.09	0.11	538	522.3	553.7	343.6	832.2	-21.8
Pre-recruit	0.6	0.1	297.4	291.0	303.8	192.6	454.4	0.3	0.12	148.4	143.2	153.6	93.7	230.7	-50.1
Small	0.92	0.16	451.3	435.6	467.0	261.5	731.7	0.32	0.1	156.3	152.4	160.2	101.1	239.4	-65.4
Survey total															
Recruit	0.86	0.08	918.4	906.3	930.5	600.1	1383.7	0.95	0.12	1020.9	991.0	1050.8	635.1	1554.2	11.2
Pre-recruit	0.39	0.1	414.3	407.4	421.2	267.8	629	0.21	0.14	226.2	218.0	234.4	135.1	352.1	-45.4
Small	0.57	0.14	612.2	598.3	626.1	370.3	967.9	0.28	0.11	302.6	294.4	310.8	189.2	459.2	-50.6

4.3.4 Estimates of commercial population size

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), all core strata combined, all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014 are shown in Table 12 and in 2012 in Table 13. Ten of these strata supported commercial densities in 2012, and six in 2014. The mean density in the combined background strata had commercial densities in 2012, but not in 2014 (Tables 12 and 13). The commercial population size declined from 473.9 million oysters in 2012 to 211.3 million oysters in 2014.

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 12: 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. stn), 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), 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 km^2, 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 13: 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. stn), 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), 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 km^2, 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

4.3.5 The distribution of oyster densities

Stratified random surveys are generally not as good as grid design surveys at estimating the distribution of oysters in Foveaux Strait, especially given their patchy distribution. The February 2014 survey sampled 60 first and second-phase random stations generated with a 0.75 nautical mile exclusion that spread sampling effort, and 11 fixed stations. All 71 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 2014, 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.

The distributions of oyster densities for recruit-sized, pre-recruit, and small oysters in 2012 and 2014 are shown in Figures 28–30. The distribution of oyster densities is widespread, covering most of the fishery area with the highest densities in core fishery strata. Densities of all three size groups of oysters are lower in 2014 than in 2012. The numbers and sizes of localised areas of relatively high recruit-sized oyster densities have decreased between 2012 and 2014, most likely the result of ongoing, low to moderate level bonamia mortality and reduced recruitment to the fishery (Figure 28). Recruit-sized oyster densities are generally decreasing in western areas where there has been virtually no fishing. They are decreasing in central areas, and generally static or increasing in southern and eastern areas (Figure 28).

The densities of pre-recruit oysters are low and very patchy in 2014 compared to 2012 (Figure 29). 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 with the exception of central fishery areas where there are very few (Figure 29).

The distribution of small oyster densities shows a similar pattern to pre-recruit sized oyster densities (Figure 30), they are low and very patchy in 2014 compared to 2012. Small oysters are not vulnerable to bonamia mortality. The low densities reflect the ongoing patterns of low settlement and spat survival observed since 2009.

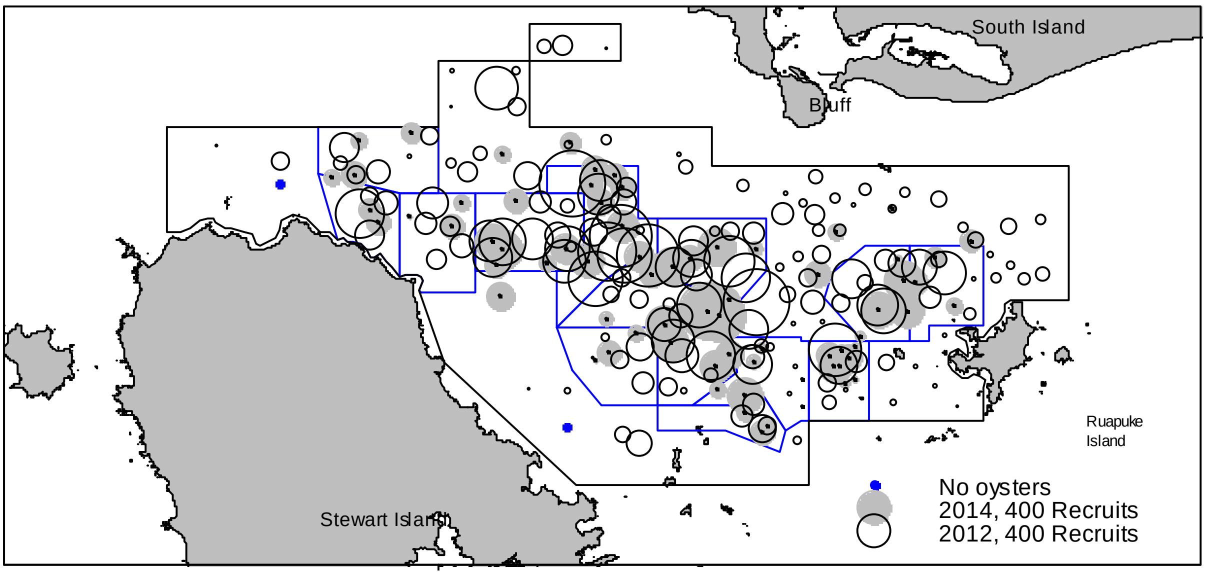

Figure 28: The densities (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled during the February surveys in 2014 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

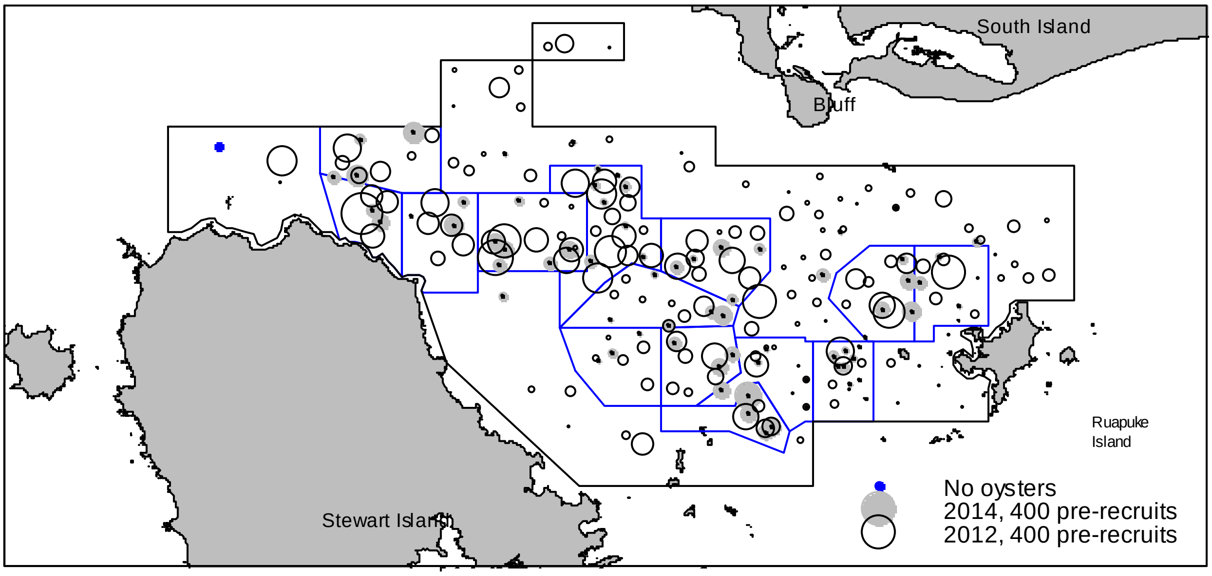

Figure 29: The densities (numbers of oysters per standard tow, 1221 m^2) of pre-recruit oysters sampled during February surveys in 2014 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

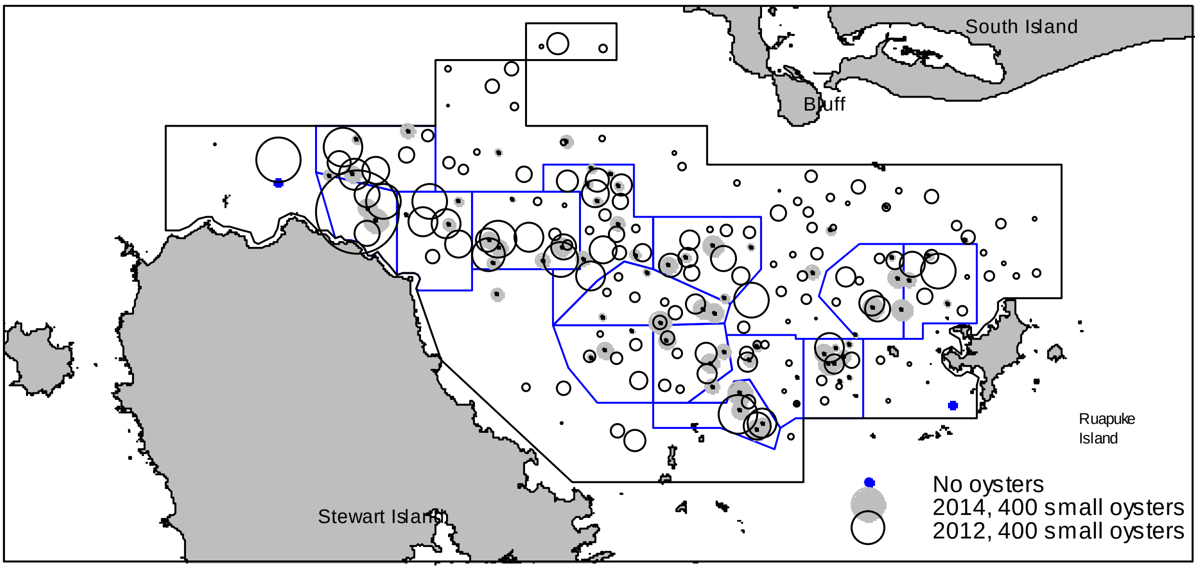

Figure 30: The densities (numbers of oysters per standard tow, 1221 m^2) of small oysters sampled during February surveys in 2014 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

4.3.6 Changes in oyster densities between 2012 and 2014

The last stock assessment survey in February 2012 provided reference points for the sizes of the oyster population in three oyster size groups. The 2012 survey sampled 146 random stations in all, 84 in the core strata sampled in February 2014. Fifty five random stations were sampled in core strata in 2014. Boxplots of standardised catches of oysters for all stations sampled 2012 and 2014 (including the one large background stratum (BK), N=60), and for only the core strata (core) sampled in 2012 and in 2014 for the three size groups of oysters are shown in Figure 31.

The median catches of recruit-sized oysters from the whole of the survey area are higher in February 2014 than in 2012, however, there were fewer large catches in 2014 compared to 2012. The median catches of recruit-sized oysters from only the core strata are similar in February 2014 to 2012, but there were also fewer large catches in 2014 compared to 2012.

The median catches of pre-recruit oysters from the whole of the survey area are similar, but lower in core strata in February 2014 compared to 2012. There were fewer large catches of pre-recruits both from the whole survey area and core strata in 2014 compared to 2012. Trends in small oysters are similar to those for pre-recruits (Figure 31).

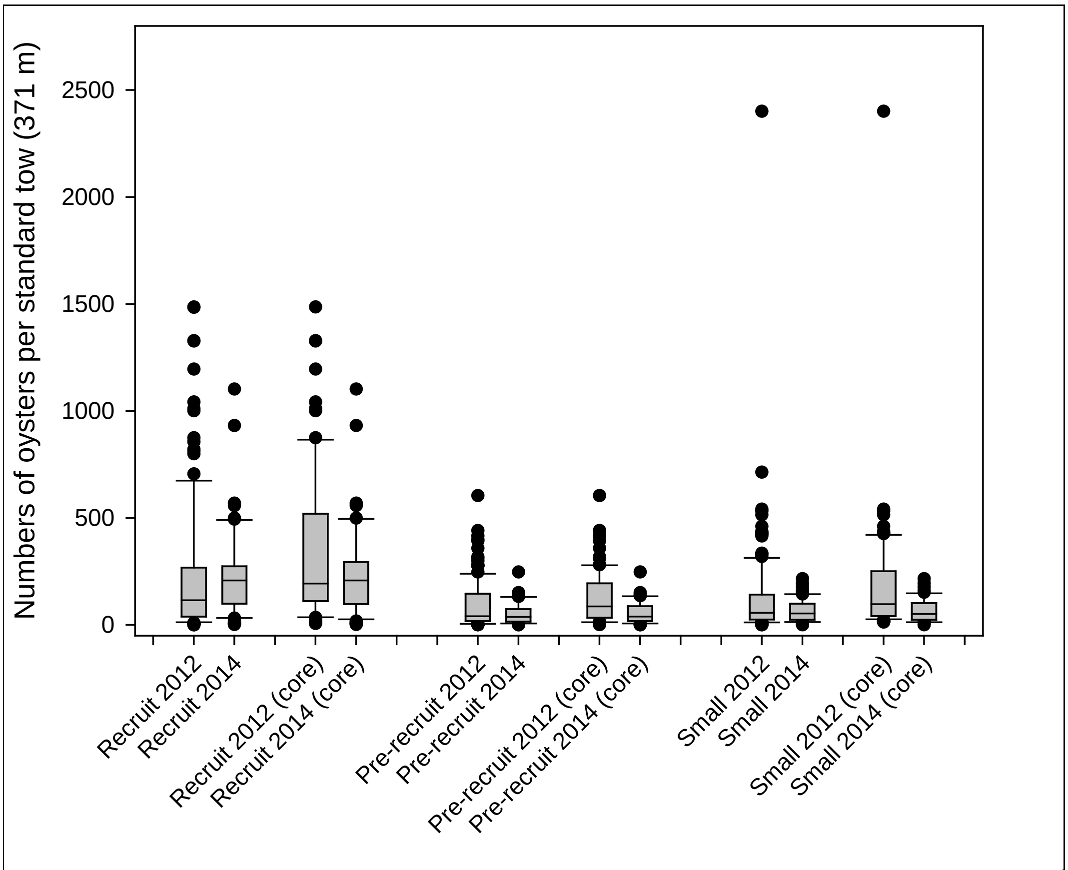

Figure 31: Boxplots of standardised numbers of oysters per tows for recruit, pre-recruit, and small sized oysters, from all strata sampled in 2012 (N = 146) and in 2014 (N = 60), and from core strata sampled in 2012 survey (N=84) and 2014 survey (N=55). Medians shown as solid lines, boxes represent 50 percentiles (25–75%) and whiskers 90 percentiles (5–95%), and outliers smaller than 5% and greater than 95% as filled circles.

4.3.7 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 relative proportions of small to recruit-sized oyster densities declined markedly between 1999, 2009 and 2012 suggesting reduced recruitment even in times when the numbers of recruit-sized oysters were relatively high and increasing (see Figures 28–30).

The numbers of small oysters per recruit show high fluctuations in a broadly cyclic trend between 1993 and 2014. The numbers of small oysters per recruit are markedly lower in 2014 than in any other survey 1993–2012 (Figure 32), suggesting that recruitment to the population is low and that this will have an effect on the growth of the stock. 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).

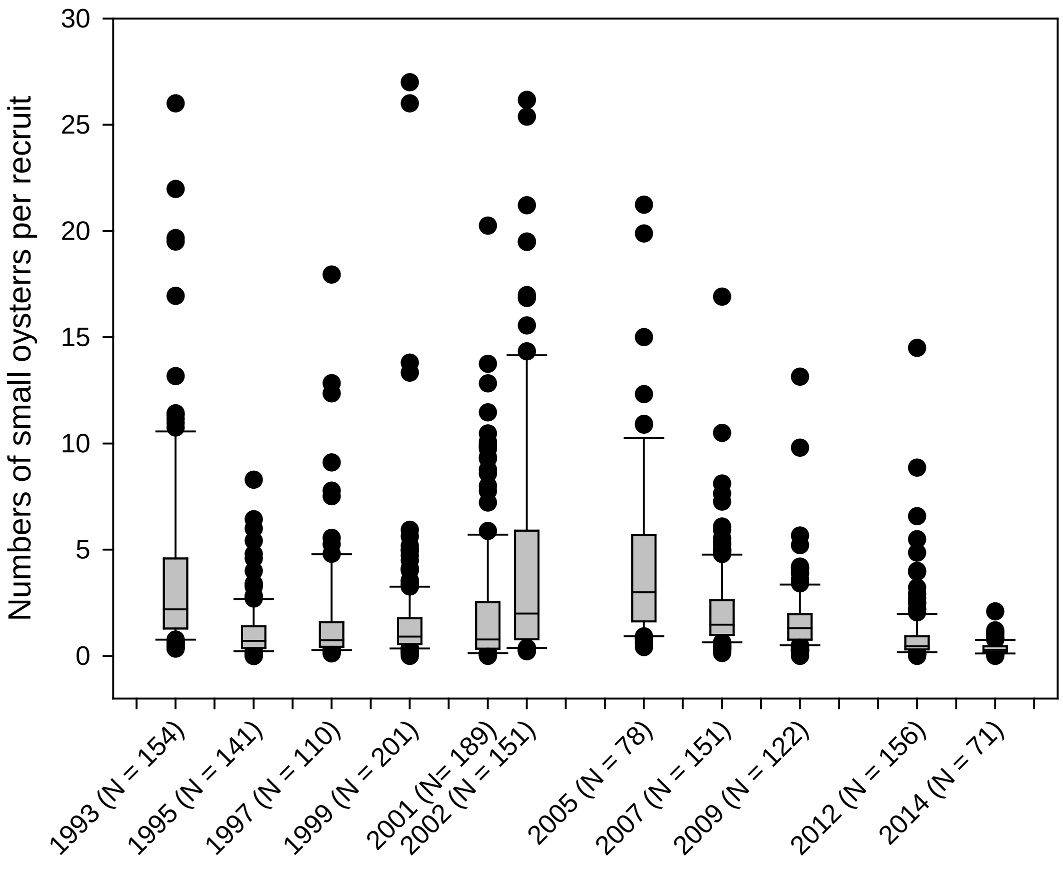

Figure 32: The numbers of small oysters per recruited oyster sampled at common stations between 1993 and 2014. The numbers of stations sampled each year varies. Medians shown as solid lines, boxes represent 50 percentiles (25–75%) and whiskers 90 percentiles (5–95%), and outliers smaller than 5% and greater than 95% as filled circles. Graph truncated at 30 small oysters per recruit, maximum is 80 per recruit.

4.3.8 Length frequency distributions

Length frequency distributions for recruit-sized, pre-recruit, and small oysters sampled in February 2014 were scaled to the size of their respective populations (Figure 33). In all, 11241 recruit-sized oysters were sampled from a total catch of 13777, 1034 pre-recruits were sampled from a catch of 3123, and 3270 small oysters were sampled from a catch of 20864, representing 81.6%, 33.1%, and 15.7% respectively, and 41.2% of the whole catch.

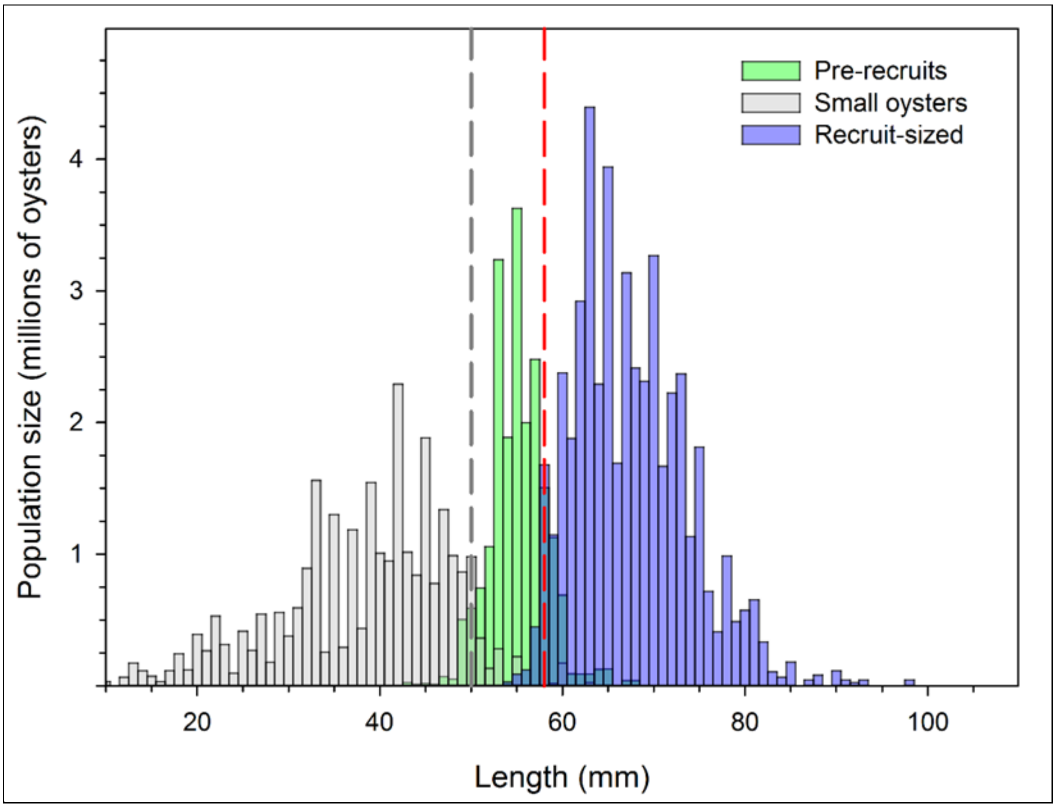

Figure 33: Length frequencies for recruit-sized, pre-recruit, and small oysters from the February 2014 bonamia survey, scaled to the size of their respective populations. Grey dashed line set at 50 mm and red dashed line at 58 mm to delineate the three size groups.

4.4 Estimates of oyster mortality before and during the February 2014 survey (objective 4)

Descriptive statistics for the percentages of recruit-sized and pre-recruit new clocks and gapers sampled in 2014 are given in Table 14 along with statistics for the 2012 and 2013 surveys for comparison (Table 14). Increases in many of these statistics for recruit sized new clocks and gapers suggest that per-survey mortality was higher in 2014 that in the previous two years. Per-survey mortality for pre-recruits peaked in 2013.

Table 14: Descriptive statistics for the percentages of new clocks and gapers sampled from survey tows with more than 50 live oysters 2012–2014, by size (Recruit-sized and pre-recruits).

Percentage new clocks and gapers	Recruit sized			Pre-recruits		
Year	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
No. stations	112	49	61	78	36	31
Median	2.5	3.4	6.4	1. 9	3.2	1.7
Minimum	0	0	0	0	0	0
Maximum	28.9	15.0	15.0	12.5	11.61	8.1
Lower 5th percentile	0.3	0.3	1.7	0	0	0
Upper 95th percentile	7.2	8. 7	13.5	10.1	7.8	7.8
No. stations with no new clocks	5	1	1	11	3	8

The number of stations sampled in 2012 (159) was much higher than in 2013 (58) and 2014 (71) as the 2012 survey was a stock assessment survey and the subsequent bonamia surveys sampled smaller numbers of stations in mainly commercial fishery areas.

New clock and gaper numbers sampled from survey tows with more than 50 live oysters 2012–2014 showed increasing pre-survey mortality for recruits, but an increase then decrease for pre-recruits. (Figure 34). The percentages of recruit-sized new clocks and gapers were significantly higher in February 2014 than in February 2012 and 2013 (Kruskal-Wallis one way analysis of variance on ranks, $P = <0.001$). An all pairwise multiple comparison using Dunn's method showed significant differences between 2014 and 2012, and 2014 and 2013. There were no significant differences in the same comparisons between years for pre-recruits, possibly driven by the falling numbers of pre-recruits in the fishery.

There were few gapers observed during the February 2014 survey, 14% of stations had one recruit-sized gaper, and there were no pre-recruit-sized gapers sampled. Fewer gapers were observed in 2014 than in 2012 and 2013, 26% and 25% of the stations sampled recorded up to 5 recruit-sized gapers per station and 4% and 5% of stations had pre-recruit gapers in 2012 and 2013 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 35). The densities of new clocks and gapers were generally low with a couple of high density stations in the west. The 2013 survey shows a similar pattern of distribution (Figure 36), however, many of the stations show higher numbers of recruit-sized new clocks and gapers. Many of these stations are located close to where the 1986 and 2000 bonamia epizootics are thought to have begun. The densities of new clocks and gapers were low in southern fishery areas. The 2014 survey found a similar pattern in the distribution of new clocks and gapers densities to the 2012 and 2013 surveys, but the densities of new clocks and gapers were generally higher, while the densities of recruit-sized oysters were lower (Figure 37).

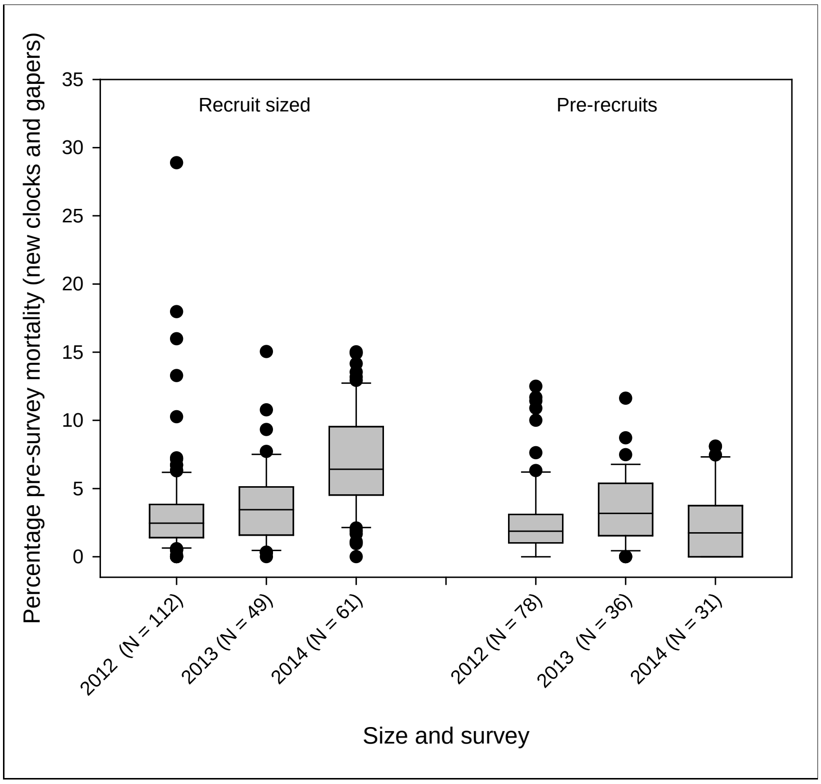

Figure 34: The percentages of new clocks and gapers for survey tows with more than 50 live oysters 2012–2014, by size.

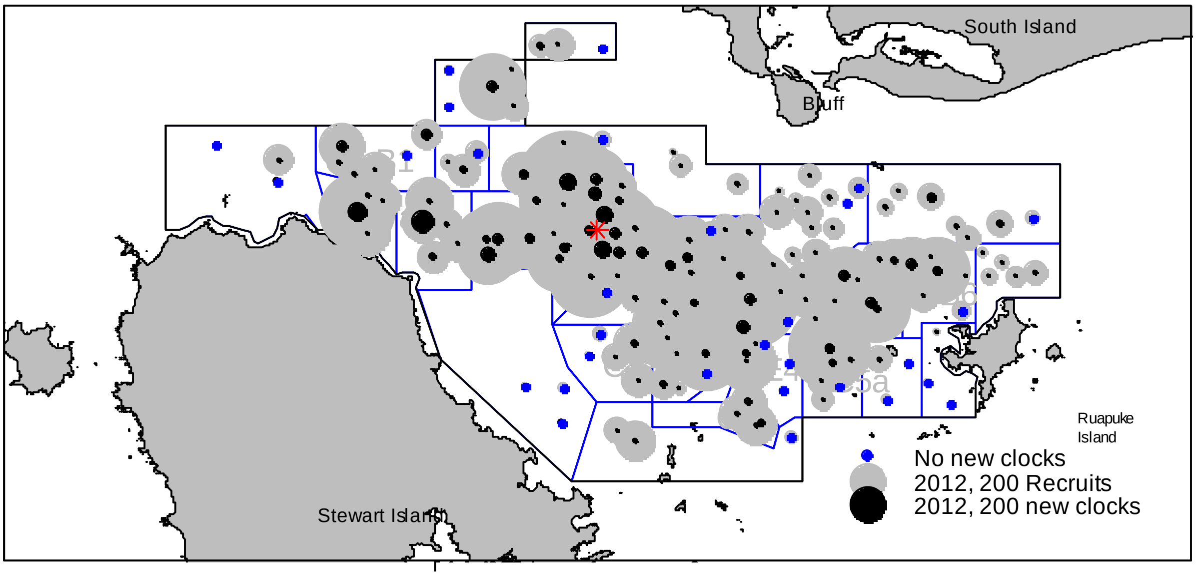

Figure 35: The distribution of recruit-sized oysters, new clocks, and gaper densities combined (filled grey circles) and the densities of recruit-sized new clocks and gapers combined (black circles) showing the pre-survey mortality in February 2012. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles. The red asterisk denotes the locations where the 1986 and 2000 bonamia epizootics are thought to have begun.

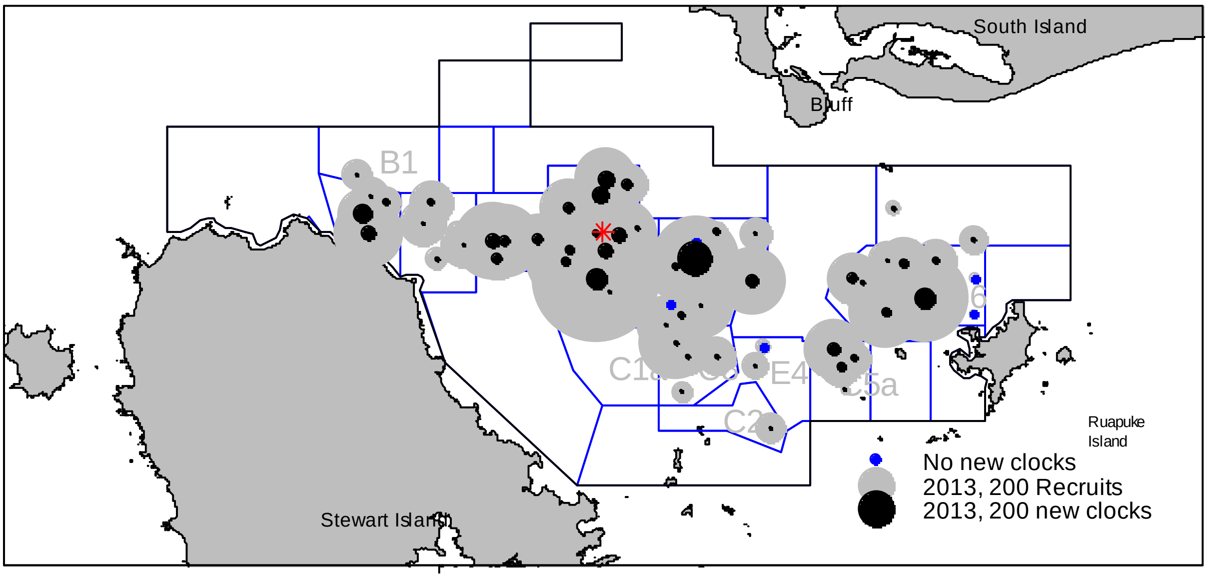

Figure 36: The distribution of recruit-sized oysters, new clocks, and gaper densities combined (filled grey circles) and the densities of recruit-sized new clocks and gapers combined (black circles) showing the pre-survey mortality in February 2013. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles. The red asterisk denotes the locations where the 1986 and 2000 bonamia epizootics are thought to have begun.

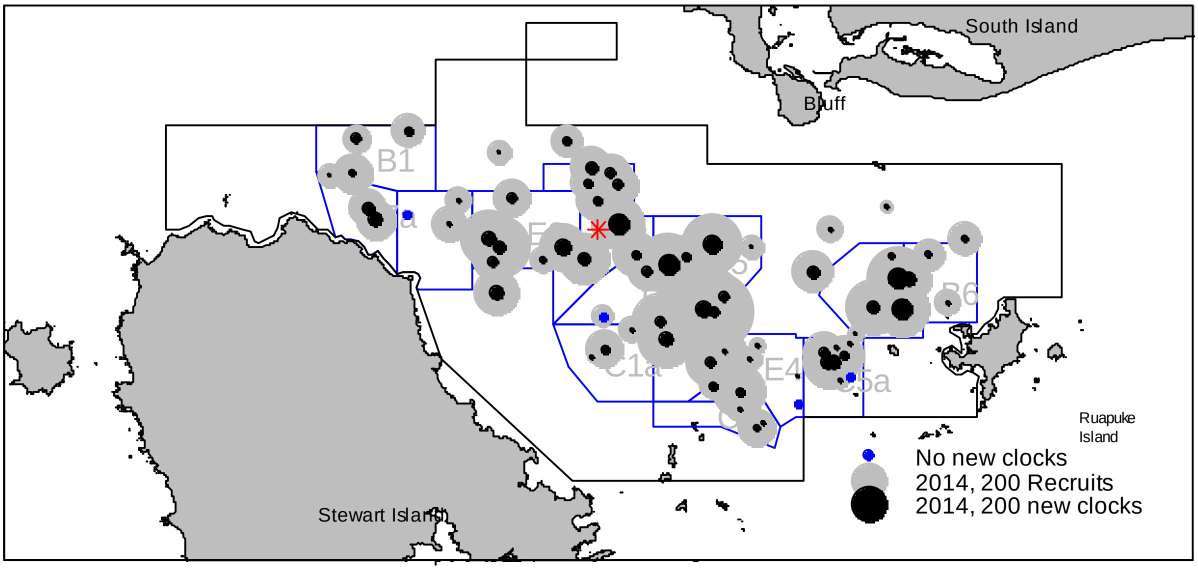

Figure 37: The distribution of recruit-sized oysters, new clocks, and gaper densities combined (filled grey circles) and the densities of recruit-sized new clocks and gapers combined (black circles) showing the pre-survey mortality in February 2014. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles. The red asterisk denotes the locations where the 1986 and 2000 bonamia epizootics are thought to have begun.

Estimates of recruit-sized new clocks sampled at randomly selected stations in 2014 and 2012 are shown in Table 15 and Table 16 respectively. Pre-survey mortality in core strata is estimated to be higher in 2014, with 39.4 million new clocks (CV of 12%, 95% CI 24.7–61.4) than in 2012, with 22.4 million new clocks (CV of 17%, 95% CI 12.8–36.6). Post survey mortality in these core strata was also higher in 2014 (6.8%) than in 2012 (3.2%).

Differences in the population sizes of recruit-sized new clocks in the background strata between 2014 and 2012 are less comparable because of the differences in sampling effort (Tables 15 and 16). These data suggest an increase in pre-survey mortality from 3.2% in 2012 to 8.5% in 2014. This high estimate of pre-survey mortality in 2014 contributes to the high estimate for the entire survey area, 7.6% in 2014 and 3.2% in 2012.

There were fewer strata with recruit-sized new clock densities above 0.1/m^2 in 2012 (B3 and C7) than in 2014 (B3, C3, C5, C7a, C8, and C9) suggesting a westward spread of heightened pre-survey mortality. 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.

Estimates of pre-recruit new clocks sampled in 2014 are shown in Table 17 and for 2012 in Table 18. Pre-survey mortality in core strata was similar in 2012 (2.9%) and 2014 (2.3%), and similar over the whole survey area between these years. The numbers of pre-recruit new clocks scaled to the size of core strata area are relatively small (3.6 million pre-recruit new clocks, 95% CI 2.2–5.7) compared with the numbers of recruit-sized new clocks.

Table 15: **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), 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 km^2, 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 16: 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 m² (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), 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. km²) in km², 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.km²
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 17: 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), 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 km^2, 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 18: 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), 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 km^2, 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

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

Pre-test runs with old and new reagents showed some differences that were resolved. Thirty one tests of positive controls for both bonamia and β-actin, and corresponding negative controls were undertaken. 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 the qPCR reaction occurred by detecting DNA from oyster tissue in the sample, were tightly distributed around the expected means (Figure 38). 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 38).

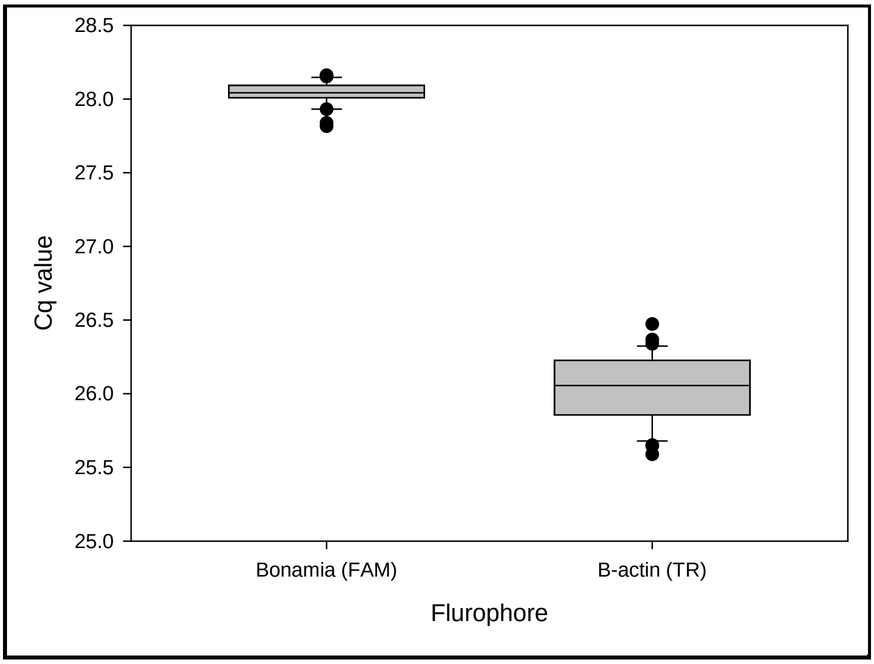

Figure 38: The range of Cq values for positive controls fort bonamia FAM (6-carboxyfluorescein) fluorophore used to detect bonamia and TR (Texas-red, sulforhodamine 101 acid chloride) fluorophore used as a cross check to ensure the qPCR reaction occurred by detecting DNA from oyster tissue in the sample. Differences in CQ values for FAM and TR are caused by an internal control block that allows the FAM to fluoresce before the TR.

Replicate, serial dilutions of positive controls, diluted with water or with water and β-actin (oyster tissue diluted at 1:500 in water), were run to check the Cq cut off level. Both FAM (Figure 39) and TR (Figure 40) fluorophores were plotted against dilution. There was some variation between dilutions with water and those with β-actin. The cut off level was established at Cq 35 as for previous years.

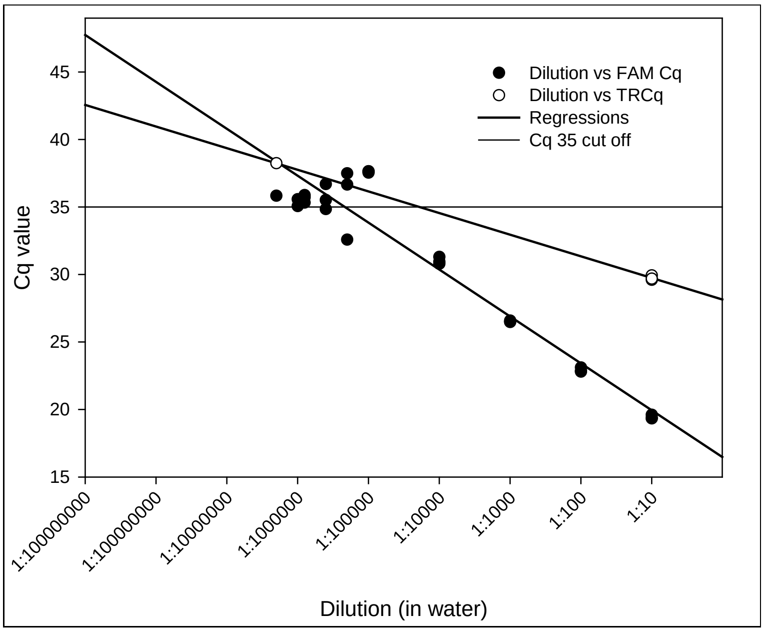

Figure 39: Replicate serial dilutions of positive controls, diluted with water, plotted against Cq value. Both FAM and TR fluorophores were plotted. The Cq 35 cut off is shown as the horizontal grey line.

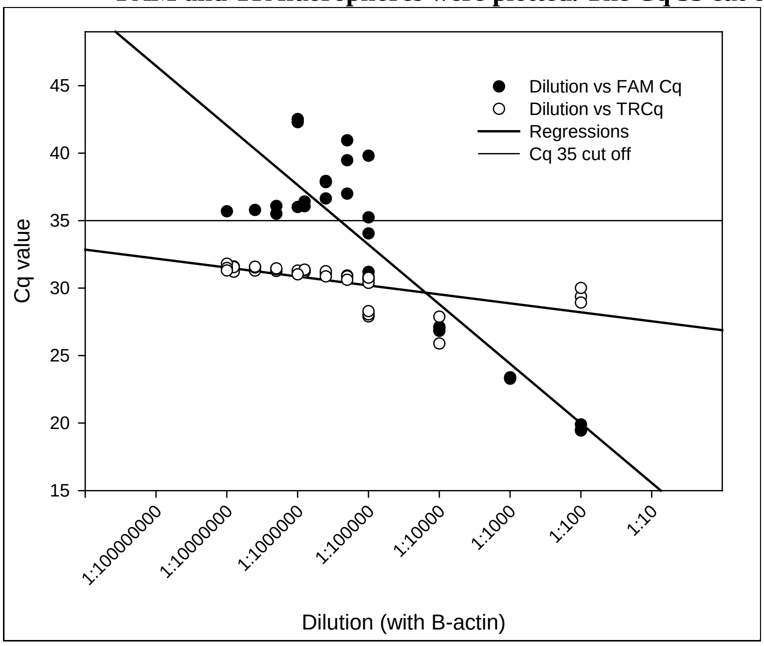

Figure 40: Replicate serial dilutions of positive controls, diluted with water and β-actin, plotted against Cq value. Both FAM and TR fluorophores were plotted. The Cq 35 cut off is shown as the horizontal grey line.

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

4.6.1 Sampling effectiveness for the prevalence and intensity of infection by bonamia

Samples of 25 oysters were collected from all but three stations (53, N = 2; 54, N = 6; and 39, N = 23) totalling 1731 samples of heart imprint slides and heart and gill tissue samples for qPCR. Stations 53 and 54 were not used in the analysis. This sample comprised 1683 recruit-sized oysters, 28 pre-recruits, and 20 small oysters. Almost all of the samples (97.2%) were of recruit sized oysters, similar to previous surveys.

Heart imprints were made from all individual oysters sampled and matching heart and gill tissue samples were taken for qPCR. All heart imprint slides have been archived and replicate gill tissue samples have also been taken and archived for future reference.

4.6.2 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 19). 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 19) 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 samples for the 25th heart imprint slide from each station (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 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” (the samples where either or both fluorophores amplified very early in the cycling (Cq less than 10 cycles)).

Samples omitted from the qPCR data (Table 19) and examined by heart imprints included 94 (5.7%) heart and 102 (6.4%) gill tissue “flatliners”, and 6 (0.4%) heart and 66 (4.1%) gill “early ampers”. Gill tissues were more prone to early amplification than heart tissue, which may be related to external contamination by substance on the gill tissue.

There was no bonamia infection detected by heart imprints in the 207 random samples selected from qPCR negative samples, i.e. there were no false negatives.

Table 19: The numbers of samples screened for bonamia using qPCR (heart and gill tissues) and heart imprints. 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. Note that there are no qPCR false negatives.

qPCR samples					
Bonamia infection	**Sample (N)**	**Omitted**	**qPCR.N**	**Positive (<35Cq)**	**Negative (>35 Cq)**
Heart	1 652	100	1 552	691	861
Gill	1 598	168	1 430	574	856
Both Heart and Gill				286	
Heart imprint samples					
Number of slides	1 119				
qPCR infection	**Sample (N)**	**Histo+ve**			
Heart & gill qPCR +ve	286	219			
Heart qPCR +ve	574	388			
Gill qPCR +ve	691	432			
Selection qPCR -ve	207	0			
all qPCR	1 472	820			

4.6.3 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). More than 50% of histological samples in which bonamia could not be detected score positive by qPCR. A larger number of gill tissue samples tested positive for bonamia than heart tissues i.e., at a cut-off of 35 Cq, gill tissues generally produced lower Cq values than heart tissues (Figure 41), which may either mean they are more sensitive (provide for better amplification) or that 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 heart imprints as the qPCR Cq values estimate numbers of Bonamia ITS region copies and heart imprint scores categorise the average number of bonamia cells in oyster haemocytes. Of the heart imprint samples that were positive for bonamia infection, all but three of the Cq values from matched qPCR gill samples were positive (below the 35 Cq cut-off); two of these gill tissues had matching heart tissues that were positive and one heart that didn't amplify. There may have been either some inhibition of the qPCR reaction in these samples, possibly because of the small tissue samples sizes (from small oysters), incomplete digestion of tissue, or significant loss of blood during the heart imprint process (Figure 41).

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 (Figure 41).

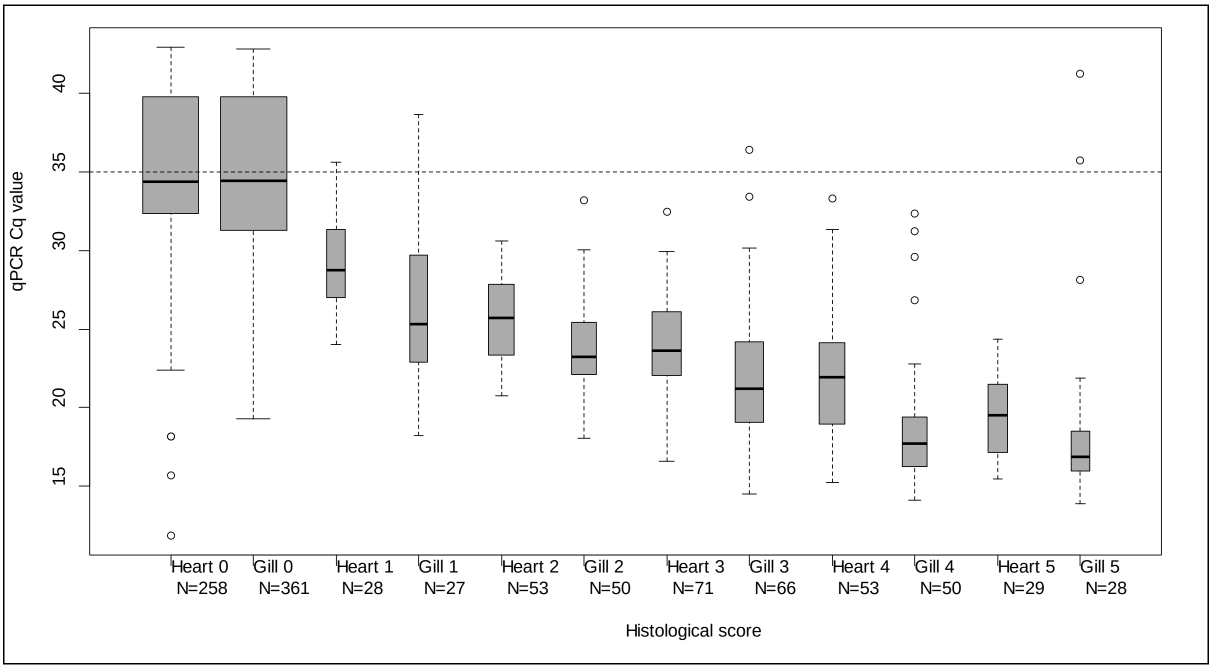

Figure 41: Boxplots of Cq values from qPCR analysis of the for bonamia ITS region for paired samples of heart and gill tissues by histological score from the February 2014 survey. Cut-off levels set at 35 Cq (dashed line). Matching heart tissue sample for the outlier for gill 4 was strongly positive suggesting a reaction problem with that sample. 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.

4.6.4 Prevalence and intensity of infection in oysters by bonamia

Estimates of prevalence at each station differed with the sampling method (heart imprints and qPCR) and between heart and gill tissues (Table 20). Infection intensity was estimated from heart imprints.

Table 20: Mean and median prevalence Prev (%) and intensity at each station estimated by heart imprints (heart imprints), and prevalence from heart (qPCR.heart) and gill (qPCR.gill) tissues using qPCR.

	Heart imprints		qPCR.heart	qPCR.gill
	Prev (%)	Intensity	Prev.H (%)	Prev.G (%)
N	58	58	58	58
Mean	15.2	3.0	25.0	28.9
Median	12.0	3.0	21.3	26.1
s.d.	12.15	0.65	17.45	18.41
L95%CI	12.1	2.8	20.5	24.2
U95%CI	18.3	3.2	29.5	33.6

Heart imprints underestimate true prevalence. The mean prevalence from heart imprints was lower than from the qPCR estimates, but higher in 2014 (15.2%) than for previous February surveys (8–12%). qPCR analysis of heart tissues was more sensitive than heart imprints, but less sensitive than qPCR analysis of gill tissues. Mean prevalence from qPCR analysis of heart tissues was 25%, higher than in 2013 (19.6%), and mean prevalence from qPCR analysis of gill tissues was 28.9%, similar to 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 heart imprints and heart and gill tissue qPCR samples are shown in Table 21.

The percentage of infected oysters determined from heart imprints for all the oysters that were sampled combined (random stations only) was slightly higher in 2014 than in 2010–2013. Of the 1724 oysters examined for bonamia in 2014, only 85.8% had no detectable infection, compared with 90%, 88%, 89%, and 88% for 2010 to 2013 respectively. Of the remaining 14.2% of oysters with detectable infections in 2014, 4.9% had light category 1 and 2 infections (3–4% in 2010–2013), and 9.3% had category 3 and higher infections (7–8% in 2010–2013) which are normally fatal. The prevalence of infection ranged from 0% to 44% in 2014, similar to 2012 and 2013. Peak prevalence was lower than the recent high in 2011 (52%). The median prevalence of 12% in 2014 (Table 21) was higher than for 2011–2013 (5–8%).

Intensity of infection has been determined from heart imprints only to maintain the time series of bonamia survey data. The median infection for stations was category 3.0 (Table 21). Infection levels were generally high with 50% or more of infected oysters expected to die within a few weeks of sampling. The mean intensity of infection at stations (3.0) was similar for the years 2009–2013. The percentage of stations in 2014 with category 3 and higher infections (81%) was the same as in 2013, and ranged from 67%–94% between 2009 and 2012 (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 (top panel, Figure 42). In 2014, the upper 50% percentiles have a higher spread of infection, with a median prevalence at about 13%, and 25% of the stations had a prevalence of infection above 24%. The prevalence of infection estimated from qPCR shows that 75% of stations had a prevalence of infection higher than 12% (top panel, Figure 42).The range of mean intensity of infection (stations with bonamia infection only) in 2014 is similar to 2013, and to the longer inter-annual trend (bottom panel, Figure 42).

The percentage of stations with no detectable infection decreased between 2009 and 2010, and slightly increased in 2011, and remained at a similar level until 2012, but has dropped to a five year low in 2013 and remained low in 2014 (Figure 43). The percentage prevalence of infection by station was generally low between 2009 and 2013, but increased markedly in 2014. Figure 43 shows a departure in the distribution of prevalence from the last five years with a marked shift to the right showing more stations with higher prevalence.

Mean intensity of infection at stations with infection has been generally high since February 2009 (Figure 43), and in 2014 there has also been a marked shift in the distribution of mean intensity of infection to the right from where it has been over the last five years – stations generally had higher mean intensity of infection (Figure 44). The differences in mean intensity between February 2007 and 2014 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 reabsorption of ova post spawning.

The increased prevalence and high intensity of infection is likely to lead to higher bonamia mortality over the summer of 2013 and 2014.

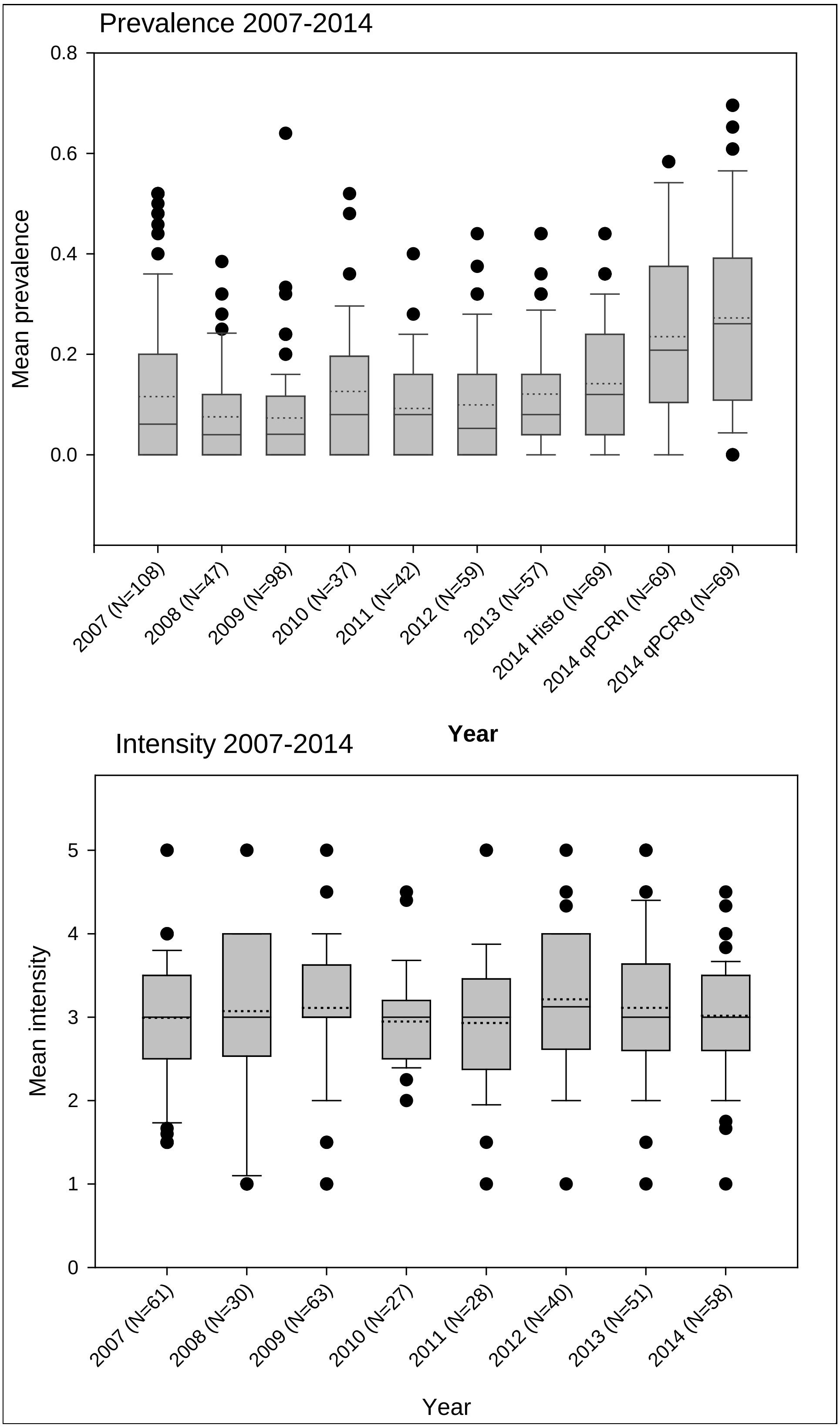

Figure 42: Boxplots of infection status by bonamia 2007–2014. The mean prevalence of infection at all stations determined from heart imprints and from qPCR heart tissues (2014 qPCRh) and gill tissues (2014 qPCRg) in 2014 (top), and the mean intensity of infection from those stations that had some infection from heart imprints alone (bottom). Medians shown as solid lines, means as dotted lines, boxes represent 50 percentiles and whiskers 95 percentiles, and outliers are shown as filled circles.

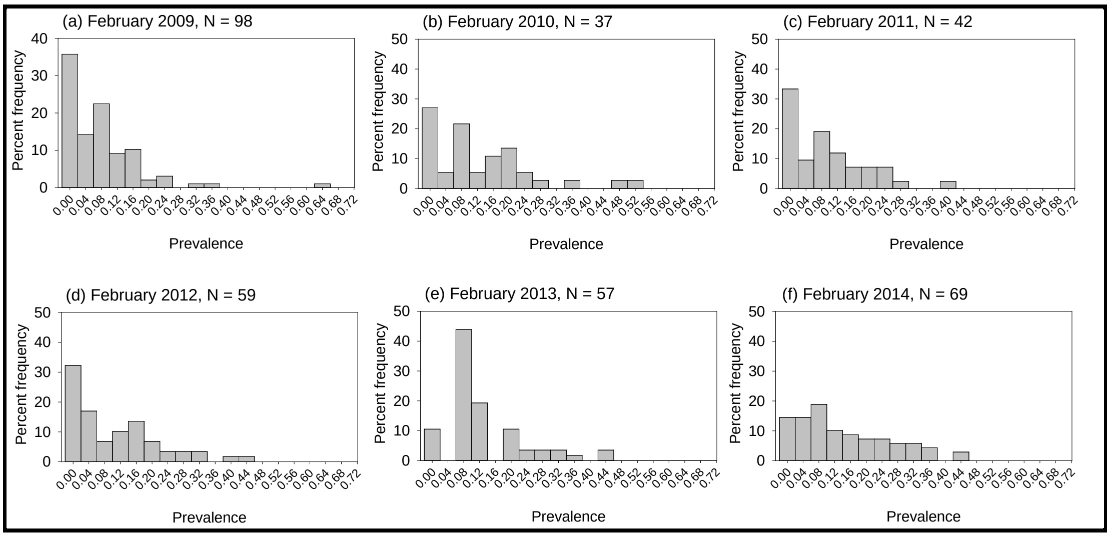

Figure 43: Percentage prevalence of bonamia infection at stations sampled in (a) February 2009, (b) February 2010, (c) February 2011, (d) February 2012, (e) February 2013 (f) February 2014.

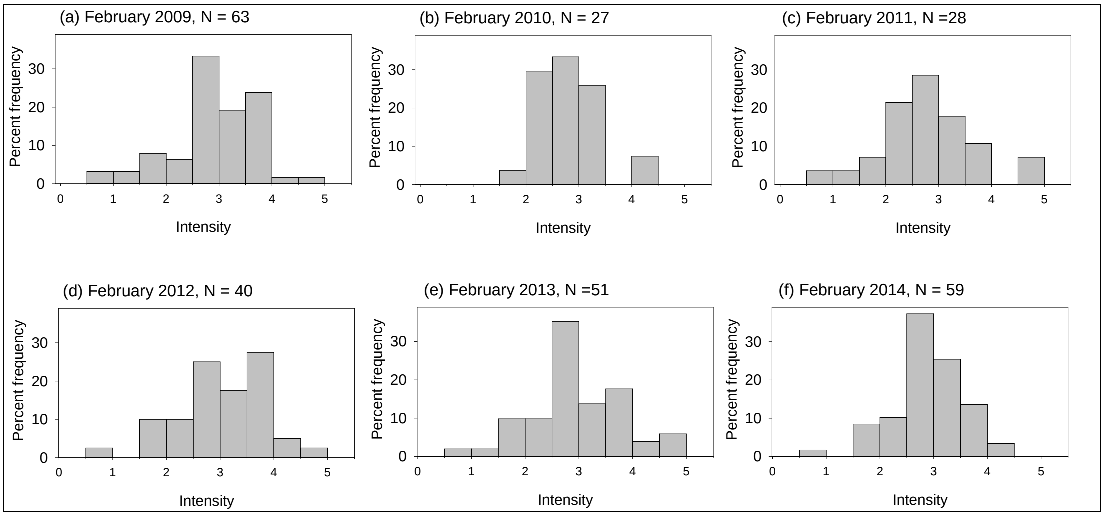

Figure 44: Percentage mean intensity of bonamia infection at stations sampled in (a) February 2009, (b) February 2010, (c) February 2011, (d) February 2012, (e) February 2013 (f) February 2014.

Table 21: Details of numbers 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 based on heart imprints. The numbers of heart (Heart.No.) and gill (Gill.No.) tissues where qPCR assays met criteria for data inclusion and the prevalence of bonamia infection detected in heart (Prev.H (%)) and gill (Prev.G (%)) tissues from the February 2014 survey.

			Heart imprint slides						qPCR assays			
Station	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	Prev (%)	Intensity	Heart.No.	Prev.H (%)	Gill.No	Prev.G (%)
1	226	0.18	25	22	0	3	12.0	4.3	24	8.33	23	13.04
2	163	0.13	25	23	0	2	8.0	4.5	24	45.83	23	21.74
3	111	0.09	25	18	1	6	28.0	3.4	24	37.50	24	45.83
4	216	0.18	25	21	1	3	16.0	3.3	24	29.17	24	25.00
5	172	0.14	25	20	2	3	20.0	2.6	24	20.83	23	30.43
6	383	0.31	25	24	0	1	4.0	3.0	24	20.83	24	25.00
7	367	0.30	25	23	1	1	8.0	3.0	24	50.00	24	37.50
8	300	0.25	25	22	1	2	12.0	3.7	24	45.83	23	34.78
9	243	0.20	25	17	2	6	32.0	3.4	24	45.83	23	56.52
10	234	0.19	25	25	0	0	0.0	0.0	24	4.17	24	8.33
11	223	0.18	25	18	4	3	28.0	2.6	24	45.83	23	56.52
12	258	0.21	25	20	1	4	20.0	3.6	24	37.50	23	34.78
13	145	0.12	25	23	1	1	8.0	2.5	24	12.50	23	13.04
14	97	0.08	25	24	1	0	4.0	1.0	24	8.33	23	39.13
15	274	0.22	25	22	3	0	12.0	1.7	24	16.67	23	21.74
16	95	0.08	25	25	0	0	0.0	0.0	24	4.17	24	8.33
17	152	0.12	25	23	1	1	8.0	2.5	24	20.83	23	13.04
18	95	0.08	25	23	0	2	8.0	3.0	24	12.50	23	13.04
20	10	0.01	25	21	1	3	16.0	3.5	24	25.00	23	47.83
21	104	0.09	25	24	0	1	4.0	3.0	24	12.50	23	4.35
22	207	0.17	25	24	0	1	4.0	3.0	24	8.33	24	16.67
23	107	0.09	25	18	5	2	28.0	2.0	24	33.33	23	52.17

Station	Recruits	Density		Heart imprint slides						qPCR assays			
				Total	Un.inf	NF.inf	Fatal.inf	Prev (%)	Intensity	Heart.No.	Prev.H (%)	Gill.No	Prev.G (%)
26	392	0.32		25	17	3	5	32.0	3.3	24	54.17	23	60.87
27	549	0.45		25	23	1	1	8.0	2.0	24	8.33	23	26.09
28	35	0.03		25	24	0	1	4.0	3.0	24	4.17	23	8.70
29	251	0.20		25	18	4	3	28.0	2.6	24	33.33	23	43.48
30	183	0.15		25	22	0	3	12.0	3.3	24	16.67	23	26.09
31	88	0.07		25	20	1	4	20.0	3.6	24	25.00	24	33.33
32	492	0.40		25	16	4	5	36.0	2.7	24	37.50	23	47.83
33	43	0.03		25	25	0	0	0.0	0.0	24	0.00	23	8.70
34	250	0.20		25	23	1	1	8.0	3.0	24	33.33	23	30.43
36	228	0.19		25	21	2	2	16.0	3.0	24	20.83	23	26.09
37	202	0.17		25	19	2	4	24.0	2.7	24	25.00	23	30.43
38	109	0.09		25	20	2	3	20.0	3.4	24	37.50	23	39.13
39	10	0.01		23	19	0	4	17.4	3.5	23	21.74	23	26.09
40	188	0.15		25	22	1	2	12.0	3.3	24	54.17	23	39.13
41	93	0.08		25	25	0	0	0.0	0.0	24	0.00	23	8.70
42	197	0.16		25	17	1	7	32.0	3.6	24	58.33	23	65.22
43	337	0.28		25	22	0	3	12.0	3.3	24	20.83	23	30.43
44	288	0.24		26	24	0	2	7.7	3.0	24	25.00	24	20.83
45	456	0.37		25	25	0	0	0.0	0.0	24	37.50	23	21.74
46	1097	0.90		25	23	0	2	8.0	3.0	24	12.50	23	8.70
47	928	0.76		25	23	1	1	8.0	3.0	24	12.50	23	26.09
48	78	0.06		25	25	0	0	0.0	0.0	24	0.00	23	4.35
49	104	0.08		25	23	0	2	8.0	3.5	24	16.67	23	13.04
50	209	0.17		25	19	3	3	24.0	2.3	24	25.00	23	39.13
51	204	0.17		25	19	3	3	24.0	2.8	24	20.83	24	25.00
52	562	0.46		25	14	5	6	44.0	3.1	24	54.17	23	60.87
53	2	0.00		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
54	4	0.00		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
55	95	0.08		25	16	6	3	36.0	2.2	24	54.17	23	47.83
67	233	0.19		25	14	1	10	44.0	3.5	24	58.33	23	69.57

Station	Recruits	Density	Heart imprints Total	Un.inf	NF.inf	Fatal.inf	Prev (%)	Intensity	qPCR assays Heart.No.	Prev.H (%)	Gill.No	Prev.G (%)
80	198	0.16	25	16	1	8	36.0	3.4	24	37.50	23	34.78
82	428	0.35	25	17	3	5	32.0	2.9	24	50.00	23	69.57
83	41	0.03	25	24	0	1	4.0	4.0	24	4.17	23	4.35
92	62	0.05	25	25	0	0	0.0	0.0	24	0.00	23	4.35
103	197	0.16	25	22	1	2	12.0	3.0	24	16.67	23	8.70
113	362	0.30	25	19	3	3	24.0	2.3	24	25.00	23	39.13
114	32	0.03	25	24	1	0	4.0	2.0	24	0.00	23	0.00
115	18	0.01	25	24	0	1	4.0	3.0	24	4.17	24	8.33
T1	308	0.25	25	19	1	5	24.0	3.8	24	54.17	23	34.78
T3	446	0.36	25	25	0	0	0.0	0.0	24	20.83	24	37.50
T4	307	0.25	25	23	0	2	8.0	3.5	24	12.50	24	29.17
T5	482	0.39	25	21	3	1	16.0	1.8	24	12.50	23	17.39
T6	166	0.14	25	24	0	1	4.0	3.0	24	0.00	23	0.00
T7	78	0.06	25	25	0	0	0.0	0.0	12	16.67	11	27.27
T8	423	0.35	25	24	0	1	4.0	3.0	24	20.83	23	30.43
T9	484	0.40	25	25	0	0	0.0	0.0	24	12.50	23	13.04
T10	25	0.02	25	21	2	2	16.0	3.3	24	0.00	23	0.00
T11	38	0.03	25	21	2	2	16.0	2.3	24	16.67	23	4.35
T12	82	0.07	25	23	1	1	8.0	3.5	24	4.17	23	8.70

4.6.5 Changes in the distribution of prevalence and intensity of bonamia infection

The distribution of the prevalence of bonamia estimated from heart imprints and from qPCR analysis of heart and gill tissues (Figure 45) shows similar patterns of distribution but with the qPCR tissues showing higher sensitivity than heart imprints. qPCR is detecting bonamia at stations where there was no bonamia detected by heart imprints. (Figure 45).

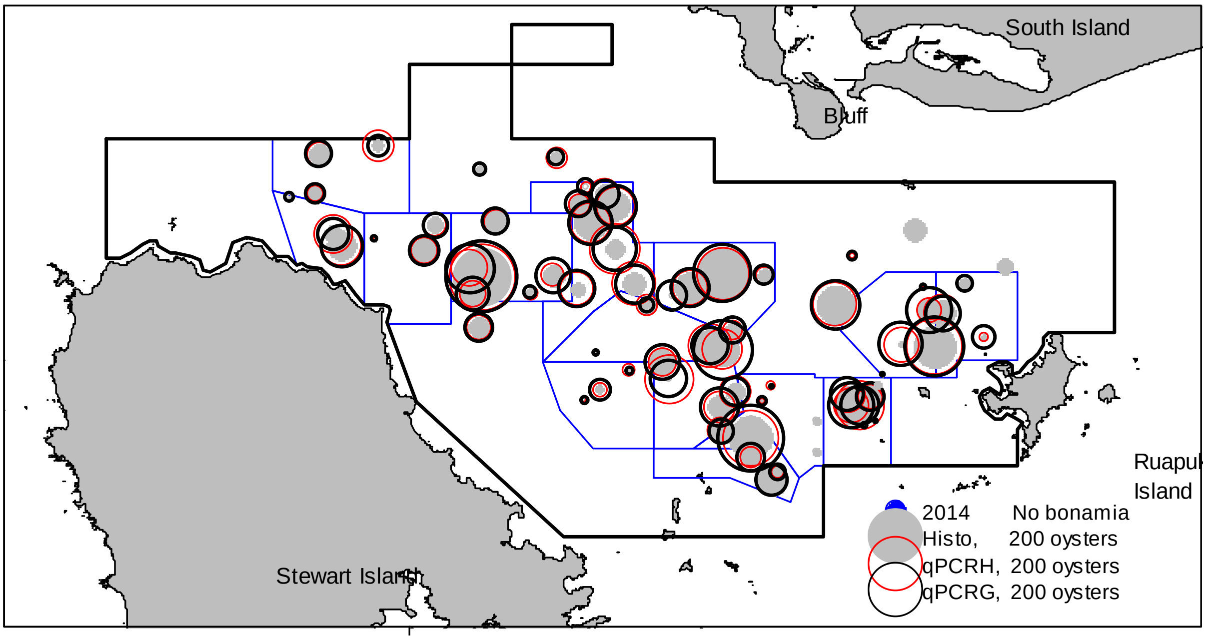

Figure 45: The distribution of bonamia infection in February 2014 estimated from heart imprints, and qPCR analysis of heart and gill tissues. Numbers of oysters with bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles), qPCR heart tissues (qPCRH, open red circles), and qPCR gill tissues (qPCRG, open black circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line) and the February 2014 survey strata (blue lines) are shown.

The designs and distribution of sampling effort for the 2012, 2013, and 2014 surveys differed and the distributions of bonamia infection across years must be interpreted with some caution. However, these surveys all sampled the commercial fishery areas and these data describe general patterns of non-fatal and fatal infections in relation to oyster density.

The prevalence of infection was highest in eastern, southern, and western fishery areas in February 2012, at which time there was little infection in the central fishery areas where oyster density was high (Figure 46). 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 2013 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 47). The distribution of recruit-sized oyster density and infection in February 2014 (Figure 48) showed the marked effects of bonamia mortality between the 2013 and 2014 surveys. Oyster density had decreased in most areas (see Figures 47 and 48). 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 48).

Patterns in the distribution of prevalence and intensity of infection between 2012 and 2013 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 have not been fished since 2008 because of the low meat quality there.

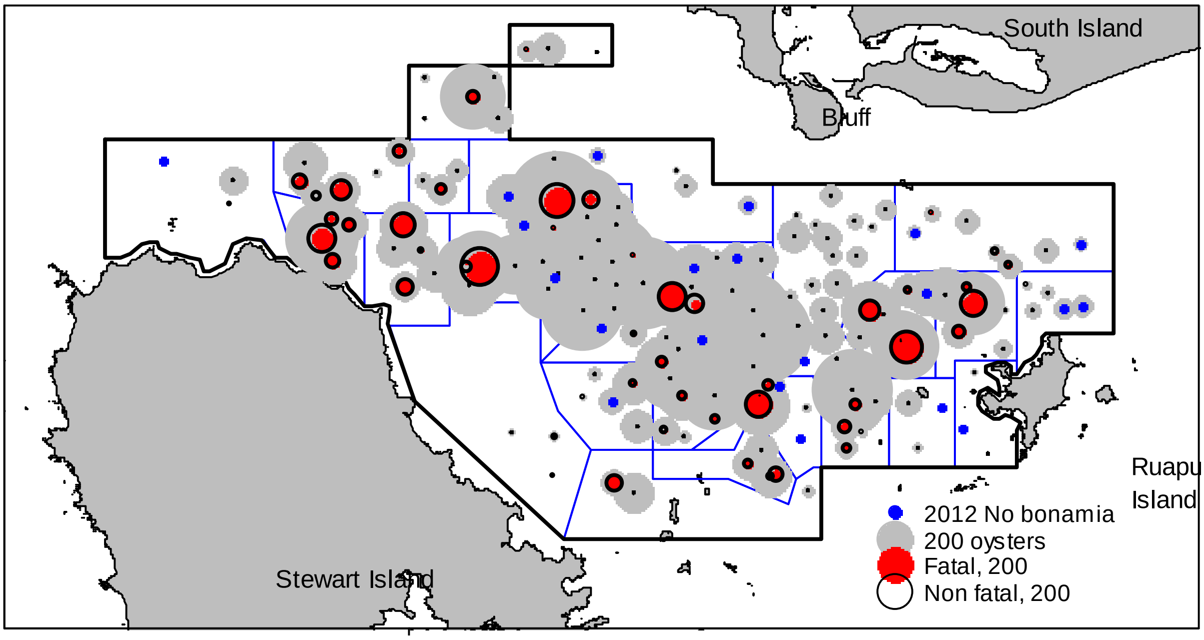

Figure 46: The distributions of oysters and bonamia infection in February 2012. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line) and the February 2012 survey strata (blue lines) are shown.

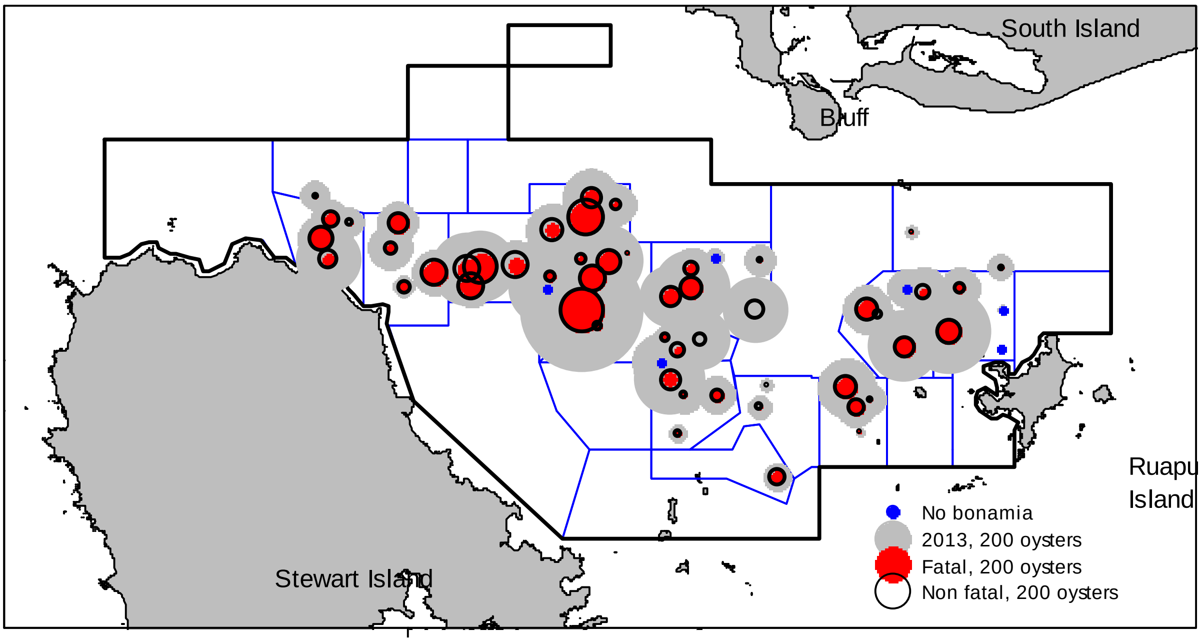

Figure 47: The distributions of oysters and bonamia infection in February 2013. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line) and the February 2013 survey strata (blue lines) are shown.

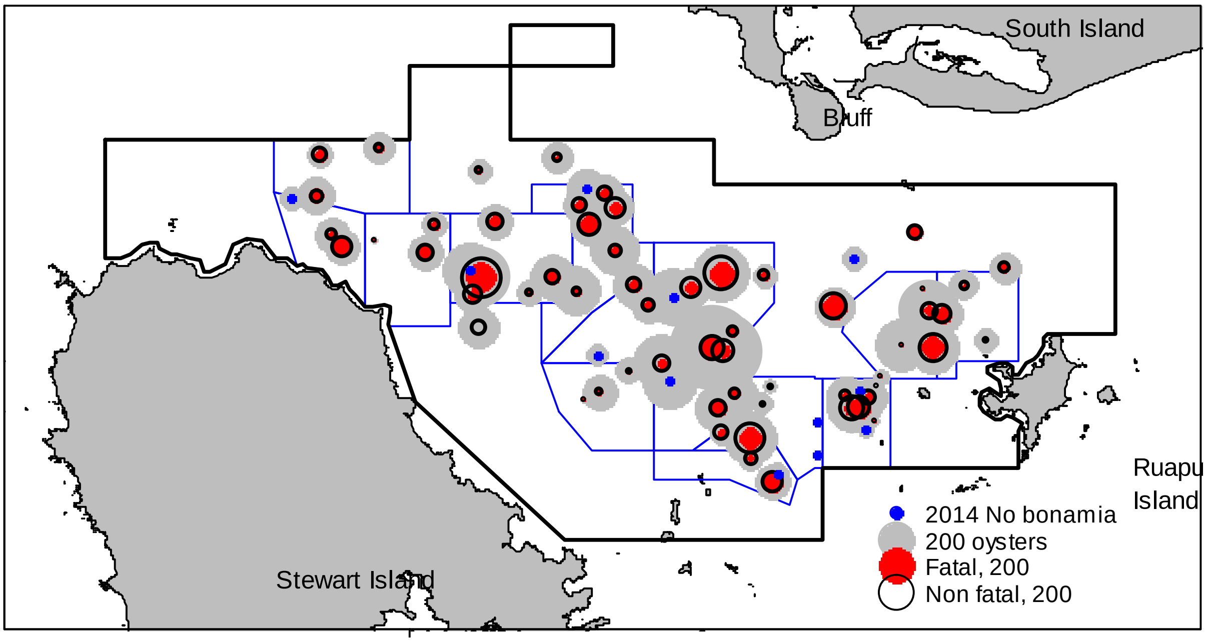

Figure 48: The distributions of oysters and bonamia infection in February 2014. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line) and the February 2014 survey strata (blue lines) are shown.

4.6.7 The total numbers of recruit-sized oysters infected with bonamia

The estimates of the total numbers of recruit-sized oysters infected with bonamia from heart imprint data scaled up from the catches at randomly selected stations in 2014 are given in Table 22. The total estimate for core strata was 89.5 million (95% CI 50.8–146.1, CV 0.17) in 2014. The estimate of infected oysters for the whole 2007 survey area was 176.1 million (95% CI 63.9–325.3, CV 0.31), but this figure should be viewed with caution as there were only five stations sampled in the background stratum which accounted for 54.0% of the survey area.

The 2012 stock assessment survey did not sample for bonamia infection from all strata, all core strata, or the whole survey area. The strata sampled for bonamia in 2012 accounted for 628.6 km^2 (58.7%) of the survey area (Table 23). Only stratum C6a of the strata sampled in the background strata in 2014 was sampled in 2012.

The number of infected oysters in core strata in 2014 was higher than for a similar area surveyed in 2012, 83.2 million oysters (CV 0.26). In a stratum by stratum comparison between 2012 and 2014 (Tables 22 and 23), the numbers of infected oysters increased markedly in strata C5, C9, E2, and C3, and decreased markedly in strata B6, C7, B1, and B3.

The scaled up estimates of infected oysters from heart tissues using qPCR are given in Table 24. The qPCR estimate for core strata was much higher than that estimated from heart imprints, 146.9 million (95%CI 88.2–234.5, CV 0.15) for qPCR heart tissues, compared with 89.5 million (95%CI 50.8–146.1, CV 0.17) for heart imprints. Prevalence in the background stratum in 2014 are difficult to compare because prevalence wasn't well estimated, 127.8 million (95%CI 0–287.6, CV 0.52) from qPCR compared with 86.6 million (95%CI 0–209.9, CV 0.60) from heart imprints.

Table 22: Scaled up estimates of the population size of recruit-sized oysters with fatal bonamia infection estimated by heart imprints from 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. 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 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 km^2, 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	0.13	0.03	0.26	10.2	7.2	13.2	4.5	17.9	78.2
B3	8	0.18	0.04	0.24	7.8	6.5	9.1	3.8	13.3	44.7
B6	3	0.11	0.07	0.67	3.3	0.8	5.8	0	8.2	30
C1a	3	0.02	0.01	0.42	0.7	0.4	1.0	0.1	1.5	31.3
C2	3	0.38	0.15	0.4	8.4	4.6	12.2	1.8	16.9	21.9
C3	4	0.18	0.11	0.64	5.9	2.2	9.6	0	14.4	32.7
C5	4	0.35	0.18	0.52	13.1	6.4	19.8	0	29.4	37.7
C5a	7	0.1	0.06	0.59	2.4	1.4	3.4	0	5.6	23.5
C7	3	0.12	0.06	0.56	4.2	1.5	6.9	0	9.6	36.1
C7a	3	0.14	0.09	0.67	3.2	0.8	5.6	0	8.1	23.6
C8	3	0.1	0.06	0.56	2.7	1.0	4.4	0	6.2	26.8
C9	4	0.23	0.1	0.44	7.8	4.4	11.2	1	16.4	34.5
E2	4	0.43	0.26	0.61	18.4	7.4	29.4	0	45	42.8
E4	3	0.05	0.05	1	1.5	0	3.2	0	5	28
Core total	55	0.18	0.03	0.17	89.5	85.5	93.5	50.8	146.1	491.8
Background strata	5	0.15	0.09	0.60	86.5	41.0	132.0	0	209.9	578.4
Survey total	60	0.16	0.05	0.31	176.1	162.3	189.9	63.9	325.3	1070.2

Table 23: Scaled up estimates of the population size of recruit-sized oysters with fatal bonamia infection in 2012 estimated by heart imprints for, all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2012. The 2012 stock assessment survey did not sample for bonamia infection from all the 2014 core strata (N = 14) 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. 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 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 km^2, 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	3	0.21	0.06	0.28	16.1	11.0	21.2	6.6	28.8	78.2
B3	3	0.37	0.26	0.7	16.4	3.4	29.4	0	42.1	44.7
B6	3	0.24	0.13	0.57	7.1	2.5	11.7	0	16.4	30
C1a	3	0.02	0.02	0.88	0.6	0	1.2	0	1.8	31.3
C2	NA	NA	NA	NA	NA	NA	NA	NA	NA	21.9
C3	3	0.11	0.1	0.85	3.7	0.1	7.3	0	10.5	32.7
C5	3	0.1	0.1	1	3.7	0	7.9	0	11.7	37.7
C5a	3	0.06	0.03	0.53	1.5	0.6	2.4	0	3.3	23.5
C7	3	0.24	0.13	0.53	8.8	3.5	14.1	0	19.7	36.1
C7a	4	0.27	0.12	0.45	6.4	3.6	9.2	0.7	13.5	23.6
C8	3	0.07	0.01	0.13	1.8	1.5	2.1	1.1	2.8	26.8
C9	3	0.01	0.01	1	0.2	0	0.4	0	0.7	34.5
E2	3	0.37	0.36	0.98	15.8	0	33.3	0	50.3	42.8
E4	NA	NA	NA	NA	NA	NA	NA	NA	NA	28
Core total	37	0.19	0.05	0.26	*82.0	75.1	88.9	36.6	142.3	491.8
Background strata	9	0.01	0	0.69	**1.3	0.7	1.9	0	3.3	578.4
Survey total	46	0.13	0.03	0.26	***83.3	77.0	89.6	37.5	147.6	1070.2

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

Table 24: Scaled up estimates of the population size of recruit-sized oysters with bonamia infection estimated by heart tissues using qPCR from 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. 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 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 km^2, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 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
B1	3	0.24	0.07	0.29	18.4	12.4	24.4	7.4	33.1	78.2
B3	8	0.44	0.1	0.22	19.5	16.5	22.5	10.1	32.5	44.7
B6	3	0.2	0.14	0.69	6	1.3	10.7	0	15.4	30
C1a	3	0.05	0.02	0.41	1.6	0.9	2.3	0.3	3.4	31.3
C2	3	0.52	0.27	0.51	11.5	4.9	18.1	0.2	25.6	21.9
C3	4	0.27	0.19	0.73	8.7	2.5	14.9	0	23.1	32.7
C5	4	0.39	0.18	0.47	14.6	7.9	21.3	1	31.4	37.7
C5a	7	0.15	0.08	0.49	3.6	2.3	4.9	0.1	7.9	23.5
C7	3	0.15	0.07	0.47	5.4	2.5	8.3	0.4	11.6	36.1
C7a	3	0.34	0.17	0.51	7.9	3.3	12.5	0	17.5	23.6
C8	3	0.5	0.16	0.33	13.5	8.5	18.5	4.6	25.3	26.8
C9	4	0.35	0.16	0.46	12.1	6.6	17.6	1.2	25.6	34.5
E2	4	0.5	0.33	0.65	21.6	7.8	35.4	0	54.4	42.8
E4	3	0.08	0.08	1	2.3	0	4.9	0	7.5	28
Core total	55	0.3	0.04	0.15	146.9	141.1	152.7	88.2	234.5	491.8
Background strata	5	0.22	0.11	0.52	127.8	69.5	186.1	0	287.6	578.4
Survey total	60	0.26	0.07	0.25	274.8	257.4	292.2	124.2	480.4	1070.2

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

The distributions of non-fatal bonamia infections across years must be interpreted with some caution (see Section 4.6.5 for details), however, these surveys did all sample the commercial fishery areas and these data should describe general patterns of non-fatal infection in relation to oyster density.

The distribution of non-fatal infections in February 2014 was widespread and variable across the fishery (Figure 49). The prevalence of non-fatal, category 1 and 2 infection 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.

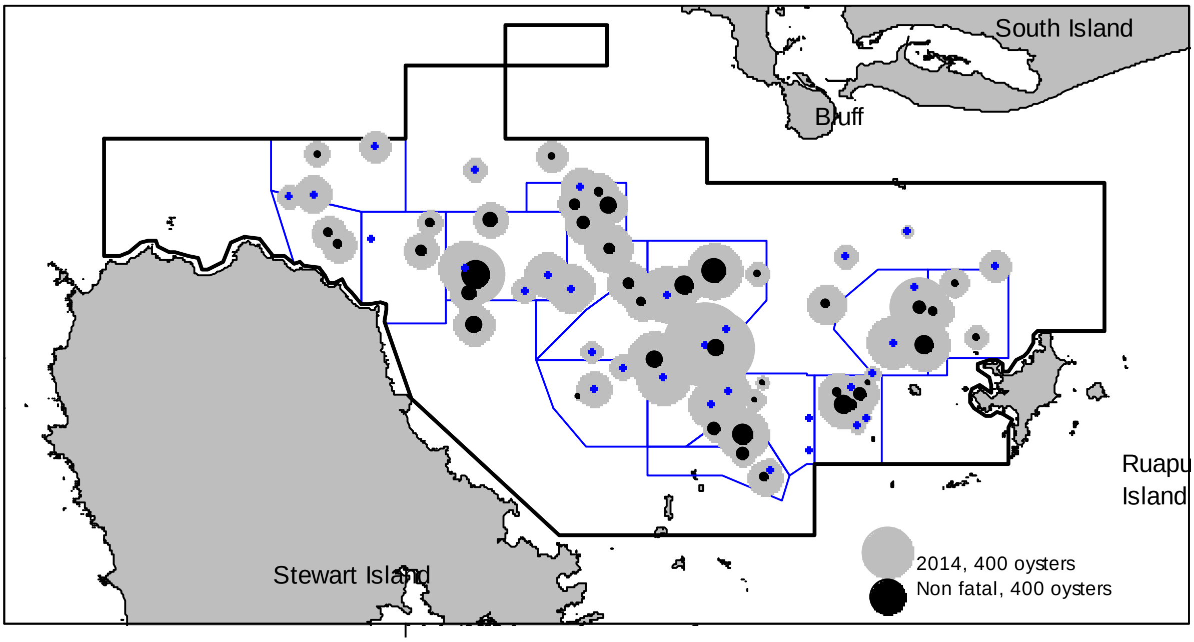

Figure 49: The distribution of recruit-sized oysters (filled grey circles showing numbers per standard tow) and oysters with category 1 and 2 infections (open black circles, the numbers of oysters scaled to the size of the catch with intensity of infection category 1 and 2) in February 2014. Stations with no bonamia infection are shown by open blue circles.

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

Pre-survey mortality in 2014 was estimated from the population size of recruit-sized new clocks and gapers in Section 4.4. The pre-survey mortality in all core strata combined was estimated to be 39.4 million oysters (6.8%, CV 0.12). 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 use 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 is estimated from the numbers of infected oysters in each sample scaled to the catch, then to stratum, and to the survey area levels.

4.7.1 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. 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 25) 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 538.0 million oysters at the time of the survey (February 2014) to 476.3 million oysters by early in the new oyster season (March 2014), a loss of 67.1 million oysters (11.5%), (Table 25). Post-survey mortality of recruit-sized oysters by stratum (Table 25) ranged from 4.6% in stratum C1a to 22.1% in C2. Of the core strata, C2, E2, C5, B1, C7, C7a, E4, and C3 had a post survey mortality greater than 10%.

The estimates of post-survey mortality from individual catches of fatally infected oysters in 2014 within the core strata with three or more randomly selected stations were similar to that using averaged correction factors for strata, 59.9 million oysters (11.1%) (Table 26). Strata E2, B1, and C5 had the highest mortality (Table 26). The estimates of post-survey mortality from individual catches of fatally infected oysters in 2012 within the commercial fishery area, with three or more randomly selected stations are shown in Table 27.

The percentage of oysters with fatal infections can give an overall standardised estimate of potential mortality. Although the commercial strata sampled in 2012 and core strata sampled in 2014 differed in the sizes of their survey areas, the increase in the percentage of fatally infected oysters from 7.7% in 2012 to 11.5% in 2014 suggests that bonamia mortality is likely to have increased. The percentages of infected recruit-sized oysters each year with fatal infections are similar, 68.8% of infected oysters were fatally infected in 2012, 72.9% in 2013, and 70% in 2014.

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 is high, and occurs in areas of relatively high oyster densities, it is assumed that these areas may eventually be subjected to heightened mortality.

Table 25: Absolute population estimates for recruit-sized oysters after projected mortality from bonamia based on category 3 and higher infections: The correction factor used for the mean stratum estimate of density (Factor), the number of randomly selected stations sampled (No. stns), the mean oyster density per m^2 (Mean density), standard deviation of the density estimate (Density s.d.), coefficient of variation (CV) of the estimate of oyster density, mean post-mortality population size (Post-mort pop.n) in millions of oysters, upper and lower 95% confidence intervals (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, the area of each stratum (Area.km^2), by stratum for the February 2014 survey. The population size at the time of the survey (Pop.n at survey), mortality in millions of oysters (Mortality), and the percentage mortality are also shown.

Stratum	Factor	No. stns	Mean density	Density s.d.	CV	Post-mort pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.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	41.9	66.5	29.2	89.3	78.2	63.9	9.8	15.3
B3	0.91	8	1.20	0.12	0.10	53.6	49.9	57.3	34.5	80.5	44.7	58.7	5.1	8.7
B6	0.91	3	0.74	0.21	0.29	22.3	15.0	29.6	8.9	39.8	30.0	24.6	2.3	9.4
C1a	0.95	3	0.50	0.27	0.53	15.7	6.3	25.1	0.0	35.5	31.3	16.5	0.8	4.6
C2	0.78	3	0.94	0.37	0.39	20.5	11.5	29.5	4.6	40.7	21.9	26.4	5.8	22.1
C3	0.9	4	1.12	0.57	0.51	36.7	18.4	55.0	0.0	81.8	32.7	40.9	4.2	10.2
C5	0.84	4	1.04	0.35	0.34	39.3	26.2	52.4	12.5	74.1	37.7	46.9	7.6	16.2
C5a	0.93	7	0.65	0.23	0.36	15.2	11.1	19.3	4.3	29.5	23.5	16.4	1.2	7.3
C7	0.85	3	0.45	0.23	0.52	16.1	6.6	25.6	0.0	35.9	36.1	19.0	2.8	14.9
C7a	0.85	3	0.67	0.14	0.21	15.8	12.0	19.6	8.2	26.3	23.6	18.5	2.7	14.6
C8	0.94	3	1.66	0.23	0.14	44.7	37.6	51.8	27.1	70.1	26.8	47.6	2.9	6.0
C9	0.94	4	2.65	1.18	0.45	91.4	51.1	131.7	11.4	193.5	34.5	97.4	6.0	6.1
E2	0.82	4	1.09	0.41	0.37	46.6	29.7	63.5	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	8.3	0.0	12.6	28.0	4.6	0.5	11.3
Core total	0.88	55	0.95	0.11	0.12	476.3	461.6	491.0	295.4	723.5	491.8	538.0	61.7	11.5
Background strata	0.87	5	0.73	0.16	0.21	422.2	344.5	499.9	219.2	704.9	578.4	482.9	60.7	12.6
Survey total		60	0.84	0.1	0.12	898.5	871.2	925.8	558.9	1367.7	1070.2	1020.9	122.4	12

Table 26: 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 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 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 km^2, 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	0.12	0.03	0.27	9.7	6.7	12.7	4.2	17.0	78.2
B3	8	0.11	0.03	0.25	5.0	4.1	5.9	2.4	8.6	44.7
B6	3	0.08	0.06	0.83	2.3	0.1	4.5	0	6.6	30.0
C1a	3	0.02	0.01	0.46	0.7	0.3	1.1	0.1	1.5	31.3
C2	3	0.25	0.11	0.44	5.5	2.8	8.2	0.8	11.5	21.9
C3	4	0.11	0.07	0.66	3.5	1.2	5.8	0	8.7	32.7
C5	4	0.20	0.09	0.47	7.5	4.0	11.0	0.5	16.2	37.7
C5a	7	0.05	0.03	0.59	1.2	0.7	1.7	0	2.8	23.5
C7	3	0.08	0.04	0.56	2.7	1.0	4.4	0	6.3	36.1
C7a	3	0.11	0.08	0.73	2.6	0.5	4.7	0	6.9	23.6
C8	3	0.10	0.06	0.56	2.7	1.0	4.4	0	6.2	26.8
C9	4	0.17	0.09	0.54	5.9	2.8	9.0	0	13.6	34.5
E2	4	0.23	0.14	0.61	10.0	4.0	16.0	0	24.4	42.8
E4	3	0.02	0.02	1.00	0.5	0	1.1	0	1.7	28.0
Core total	55	0.12	0.02	0.16	59.9	57.4	62.4	34.9	96.7	491.8
Background strata	5	0.10	0.09	0.85	59.6	15.2	104.0	0	173.2	578.4
Survey total	60	0.11	0.05	0.43	119.4	106.4	132.4	19.4	247.3	1070.2

Table 27: 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 of the 2007 stock assessment survey area sampled in 2012. 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 km2 (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 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 km^2, 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	3	0.14	0.06	0.41	10.6	5.7	15.5	2.0	21.3	78.2
B3	3	0.23	0.17	0.75	10.4	1.6	19.2	0.0	27.8	44.7
B6	3	0.19	0.09	0.48	5.8	2.6	9.0	0.3	12.4	30.0
C1a	3	0.02	0.02	1.00	0.6	0.0	1.3	0.0	1.8	31.3
C2	NA	NA	NA	NA	NA	NA	NA	NA	NA	21.9
C3	3	0.11	0.10	0.85	3.7	0.1	7.3	0.0	10.5	32.7
C5	3	0.02	0.02	1.00	0.9	0.0	1.9	0.0	2.9	37.7
C5a	3	0.04	0.02	0.53	1.0	0.4	1.6	0.0	2.2	23.5
C7	3	0.18	0.09	0.50	6.5	2.8	10.2	0.2	14.3	36.1
C7a	4	0.16	0.06	0.38	3.7	2.3	5.1	0.9	7.3	23.6
C8	3	0.05	0.01	0.29	1.3	0.9	1.7	0.5	2.3	26.8
C9	3	0.01	0.01	1.00	0.2	0.0	0.4	0.0	0.7	34.5
E2	3	0.27	0.26	0.97	11.4	0.0	23.9	0.0	36.2	42.8
E4	NA	NA	NA	NA	NA	NA	NA	NA	NA	28.0
Core total	NA	NA	NA	NA	NA	NA	NA	NA	NA	491.8
Background strata	NA	NA	NA	NA	NA	NA	NA	NA	NA	578.4
Survey total	46	0.09	0.02	0.27	*56.9	52.5	61.3	24.4	102.2	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.**

4.8 Projection of future stock size (objective 6)

Given the low likelihood of any significant recruitment to the fishery in the next two to three years and the high level of bonamia mortality over the summer of 2013/2014, the current status of the fishery is likely to be best represented by series “c” in Figure 50 which assumes a bonamia mortality of 20% and incorporates the decreased recruitment between the 2009 and 2012 stock assessments. Any short-term decline in the fishery will be mostly determined by the level of bonamia mortality in the fishery. Survey data suggest that levels of bonamia mortality have increased in 2013 and 2014 compared to previous surveys.

Between 2005 and 2008, the fishery was rebuilding rapidly with good spatfall and juvenile survival driving recruitment, and 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 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 50). Significant summer mortality from bonamia (15.9%) in 2013 along with the low recruitment to the fishery has led to the first decline in the recruit-sized population since 2005. The 2014 survey accounts for bonamia mortality that occurred before the survey. A further decline of 11.5% in recruit-sized oysters is expect from the estimate of fatally infected oysters that were likely to die soon after the survey.

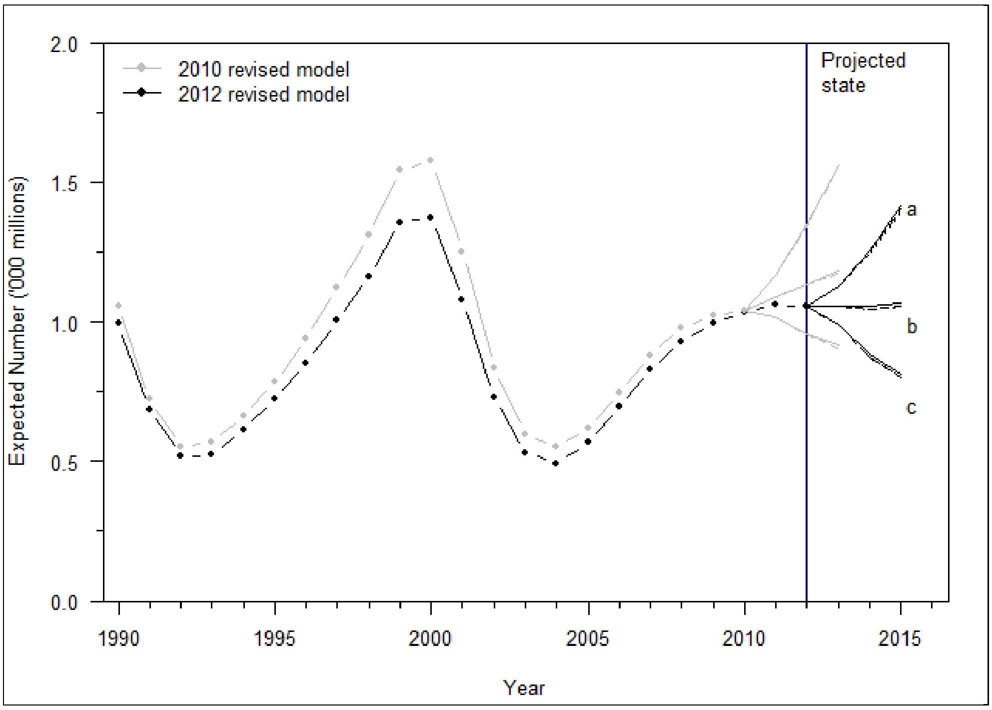

Figure 50: Model estimates of recent recruit-sized stock abundance (2012) and projected recruit-sized stock abundance for 2013–15 with catch of 7.5 (solid line), 15 (dash dot), and 20 million oysters (dash line) under assumptions of (a) no disease mortality, (b) disease mortality of 0.10 y^{-1}, and (c) disease mortality of 0.20 y^{-1}, for the 2010 and 2012 revised models (Figure reproduced from Fu 2013).

5. DISCUSSION

The introduction of five-yearly stock assessments 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 motivate a new time series of bonamia surveys with two specific design features: a standard bonamia survey area that better defines the core commercial fishery within the limits of the 2007 stock assessment survey area and a fully randomised, two-phase sampling design aimed at better estimating oyster density and population size, especially for recruit-sized oysters on which the second-phase allocation of stations is based. Because estimates of bonamia infections (fatal and non-fatal) are scaled to the size of the recruited oyster population, better estimates of oyster density are likely to give more precise estimates of total summer mortality.

The core commercial fishery area delimited in Objective 1 is likely to represent almost all of the fishery area with relatively high recruit-sized oyster density. Fishery independent survey data, especially those data used for assessments that sampled the whole stock area provide the best estimates of the distributions of oyster density. Stock assessment surveys 1990–93 (Cranfield et al. 1991, Doonan et al. 1992, Cranfield et al. 1993) used a grid design that is better suited to estimating the distribution of oyster density, but these surveys were undertaken at a time when the population size of oysters was at its lowest and the distribution of oysters greatly reduced by bonamia mortality (Doonan et al. 1994, Cranfield et al. 2005). Stock assessment surveys in 1999 and 2012 when the population size of oysters peaked and the distribution of relatively high recruit-sized oyster density was the most widespread (Michael et al. 2001, Michael et al. 2013) used a two-phase, random stratified design that does not estimate distribution well. However, these surveys used large numbers of strata and the stratification of the survey area effectively delineated areas of relatively high, medium and low oyster density and were able to effectively map the commercial fishery area.

The fourteen of the twenty six stock assessment survey strata identified as core commercial strata represented 75% of the recruit-sized oyster population in 2012 and 46% of the survey area. Many of these strata have consistently defined areas of relatively high oyster density, and relatively high commercial catch rates and landings over long periods of time. The stratum boundaries have remained largely unchanged since 1999, some of the original strata were partitioned to better describe the distribution of oyster density. Therefore estimates of the oyster population within the core strata combined are comparable to those from previous stock assessment surveys, and estimates from individual strata are comparable with previous stock assessment and bonamia surveys. The sampling methods are the same for these two time series of surveys increasing their respective value.

The MPI Shellfish Working Group (28 January 2014) agreed that there is a lot of value in maintaining a time series of data from the whole stock assessment area. It was decided that all the 2007 stock assessment survey strata that were outside the core commercial area would be merged into a single background stratum and sampled with five first-phase stations, allowing data from this and future bonamia surveys to be incorporated into stock assessments. Further, because localised areas with oysters of high meat quality are targeted by commercial fishers, it may be advantageous to use spatial management strategies to maintain production in these areas. Maintaining a consistent set of survey strata will contribute to spatially explicit stock assessments should they be required.

Stock assessment and bonamia surveys typically achieve CVs between 8% and 14% for estimates of oyster population size; with bonamia surveys sampling as few as 30 stations. To be able to better compare estimates of oyster population size and determine trends in the fishery, the new series of surveys should aim to achieve similar CVs. Simulations using the 2012 and 2014 survey data estimated lower CVs from the highest numbers of strata (N = 14), and a CV of 11.44% was predicted from 55 sample stations. The CV achieved for recruit-sized oysters in core strata was 11.2% in 2014, and 11.7% for the whole population from an additional 5 stations in the background stratum (10.8% predicted). These CVs are consistent with those of estimates from previous surveys and in part confirms that the survey stratification represents the distribution of oyster density relatively well.

5.1 2014 survey results and implications for the OYU 5 fishery

Oyster density was increasing across the Foveaux Strait oyster fishery, including the eastern fishery areas, until 2012. Since then, between 2012 and 2014, both the mean oyster density in core commercial strata has fallen (from 1.40 oysters per m^2 to 1.09 oysters per $m^{2)}$, and the population size has fallen (from 688.1 million oysters to 538.0 million oysters, a drop of 21.8%). Pre-recruit sized oysters and small oysters also declined in core strata by 50.1% and 65.4% respectively over the same time. Although pre-recruit sized oysters are thought to be as vulnerable to bonamia mortality as recruit-sized oysters, the cause of the decline in small oysters is unknown. The size of the recruit-sized oyster population over the whole survey area increased from 918.4 million oysters in 2012 (CV 8%) to 1020.9 million oysters in 2014 (CV 12%). Despite the low CVs, this result is likely to be an artefact of sampling as there were 62 random stations sampled in background strata in 2012 and only 5 random stations in the single background stratum in 2014. The decline in recruit-sized oysters in core strata is mostly attributed to two factors: heightened mortality from bonamia and much lower than average recruitment to the fishery.

In February 2009, bonamia infection was widespread throughout the fishery, but highly variable at small spatial scales, stations with no detectable infections were interspersed with stations with low and high prevalence of infection. Infected stations generally had mostly fatal infections. Bonamia infection remained widespread in the core commercial fishery areas between 2010 and 2013, but the prevalence 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 bonamia infection was higher again in 2014 as were the numbers of fatally infected oysters.

In the current epizootic, bonamia mortality is thought to mainly occur over the summer, after spawning. Summer mortality is represented by the combined tally of two different estimates: pre-survey estimates of mortality from new clocks and gapers and post-survey mortality projected from the numbers of oysters with fatal infections. It is acknowledged that these two estimates represent different estimates of mortality that are not directly comparable. Estimates of the population sizes of new clocks and gapers use the same dredge efficiency as for live recruit sized oysters, but it is not known how well this represents the real dredge efficiency for new clocks and gapers. Further, new clocks may be carried away from the location of death by tidal currents and may be accumulated at other sites. Projections of post survey mortality are closely linked to the precision of the estimates of population size as fatal infections are scaled by catch and stratum area. However the combined totals of these two estimates provide the only estimate available of total summer mortality which is important in determining which of the projections from the previous stock assessment is best able to inform future stock status. Mortality from bonamia was higher over the summers of 2012/2013 and 2013/2014 than in previous summers. Summer mortality was estimated to be 15.9% in 2013 and 18.3% in 2014.

The numbers of pre-recruit and small oysters are at historically low levels. We can only speculate that this lower than long-term recruitment to the oyster population is climatically driven as spawning stock size and the settlement surfaces that these oysters provide have been relatively high in recent years, but settlement of oyster larvae and the survival of spat has been low.

The 2012 stock assessment showed that the stock continued to rebuild after the outbreak of the recent bonamia epizootic 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. However, the population size of recruit-sized oysters in core strata declined 21.8% over two years, and fatally infected oysters were expected to further reduce the population by 11.5%. This level of bonamia mortality is expected to cause a downward trend in the oyster population.

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.

5.2 The development of the qPCR method to detect and quantify bonamia

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, and that bonamia is likely to continue to cause heightened mortality in the future. Determining the status of bonamia is a key input into the management of the fishery (Ministry for Primary Industries 2013).

Until recently, heart imprint methods were the most cost effective for fishery-scale monitoring of bonamia. However correlation studies with in-situ hybridisation have shown that the prevalence of bonamia estimated from heart imprints can underestimate the true infection rate by about 30% (Diggles et al. 2003). Recent developments in molecular methods have provided the opportunity to develop a quantitative polymerase chain reaction (qPCR) method that is cost-effective, provides results in real-time, and has high sensitivity to detect low level bonamia infections, and other bonamia species and haplosporid infections. A high sensitivity and specificity tool will allow us to better determine infection rates, especially in individual oysters with low and currently undetectable infections. This capability also is critical if we are to investigate the temporal course of infection and drivers of epizootics.

Molecular methods have been used to detect bonamia infections in dredge oysters and have been shown to be more sensitive and less ambiguous than standard histological and heart imprint techniques (Carnegie et al. 2000, Carnegie et al. 2003, Balseiro et al. 2006, Marty et al. 2006), and in New Zealand, qPCR is much more sensitive than heart imprints in detecting *Bonamia exitiosa* in *Ostrea chilensis* (Michael et al. 2012b). In 2014, estimates of the size of the oyster population infected with bonamia was 64% higher using qPCR than using heart imprints. To maintain consistency with the current time series of bonamia infection data, only the estimate of infected population size from heart imprints was compared to previous surveys.

Currently, only the preliminary screening of samples is carried out by qPCR. We used the presence/absence data from the qPCR analysis of heart tissues only to select a subset of the heart imprint slides to be scored for bonamia infection. Estimates of fatal and non-fatal infections were derived from heart imprints alone. This combination of qPCR and heart imprint methods has the advantages of maintaining a historical time series of bonamia infection data, while reducing the cost and time required to provide survey results. We aim to fully transition bonamia screening to qPCR by developing the ability to quantify bonamia infection, and therefore remove the necessity for heart imprints, and further reduce costs. To do this, cycles of quantification (Cq) from qPCR results will need to be correlated with the heart imprint infection categories (1 to 5). Heart imprints score infection based on numbers of bonamia cells while Cq values are determined by the amount of bonamia DNA (amplicons) in the sample. The objective is to establish Cq values indicating non- fatal and fatal infections as well as presence/absence of bonamia. If achieved, this will enable the historical time series of bonamia data from heart imprints to be comparable to data from the new qPCR method. The increased sensitivity of the qPCR method will provide the opportunity to investigate the temporal course of bonamia infection, the time between initial infection or detection of infection and the intensification to fatal infections, and opportunities to study the epidemiology of bonamia. The specificity of the qPCR method will increase the ability to determine concurrent infections.

We will also investigate differences in Cq values between paired heart and gill tissues; gills tend to have lower Cq values than hearts suggesting greater infection. This difference may stem from differences in the digestion of the two types of tissues or it may be because the gill samples are externally contaminated with water-borne bonamia and therefore overestimate prevalence. Both these possibilities will be investigated as part of the qPCR method development.

Improving the ability to estimate bonamia mortality will allow us to better select which of the future stock projections from the assessment model reflects the population trajectory.

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APPENDIX 1: SURVEY STATION FORM

FOVEAUX STRAIT OYSTER SURVEY, STATION DATA RECORD

Vessel name

Recorder ....

Date: Day | Month | Year | Time NZST | Station no. | Stratum

Start position: Latitude | S | Longitude | E | Depth (m) | Speed (knots)

Finish position: Latitude | S | Longitude | E

Number of Oysters ≥58 mm: Live | Gapers | New clocks* | Old clocks**

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

APPENDIX 2: SURVEY BONAMIA FORM

FOVEAUX STRAIT OYSTER BONAMIA DATA RECORD

Page ___ of ___

Recorder

Comments

Station no.	Date: Day	Date: Month	Date: Year	Time NZST

Oyster no	Length (mm)	Height (mm)	Size category [3]	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'.

APPENDIX 3: SURVEY AND FISHERY DATA USED TO DEFINE THE NEW SURVEY STRATA.

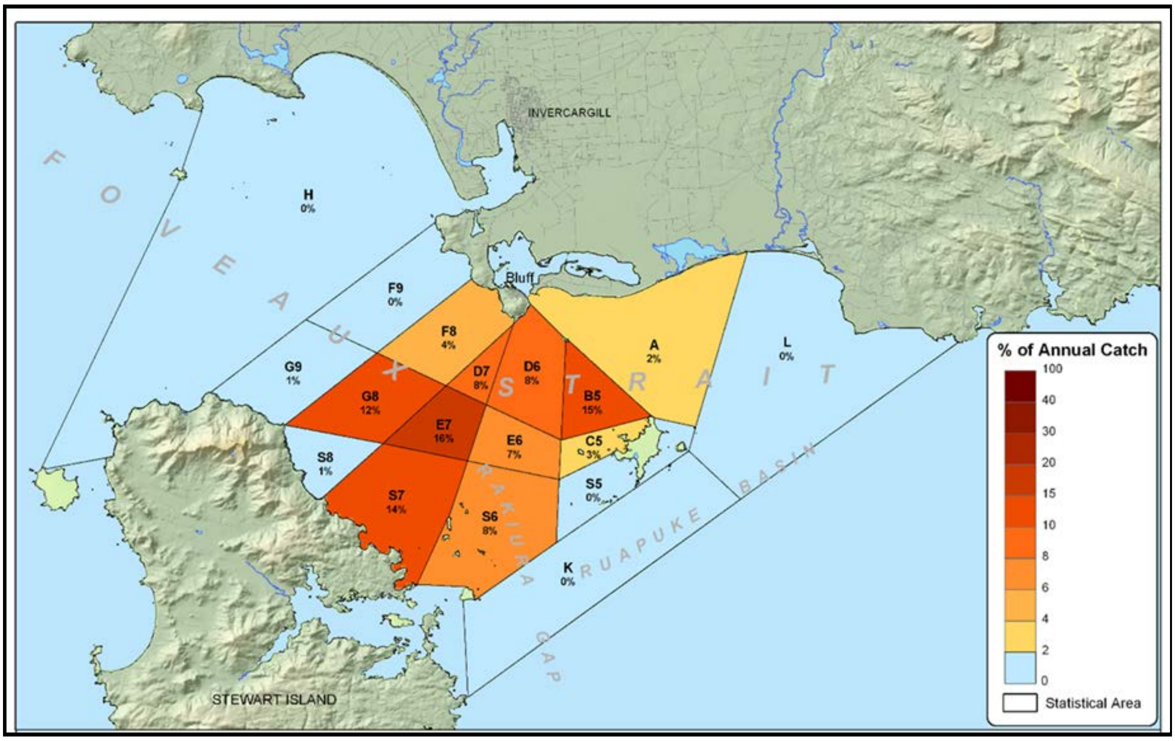

Figure A3.1: The percentage of the annual catch by Foveaux Strait statistical reporting areas for the years 1975 to 2006 combined.

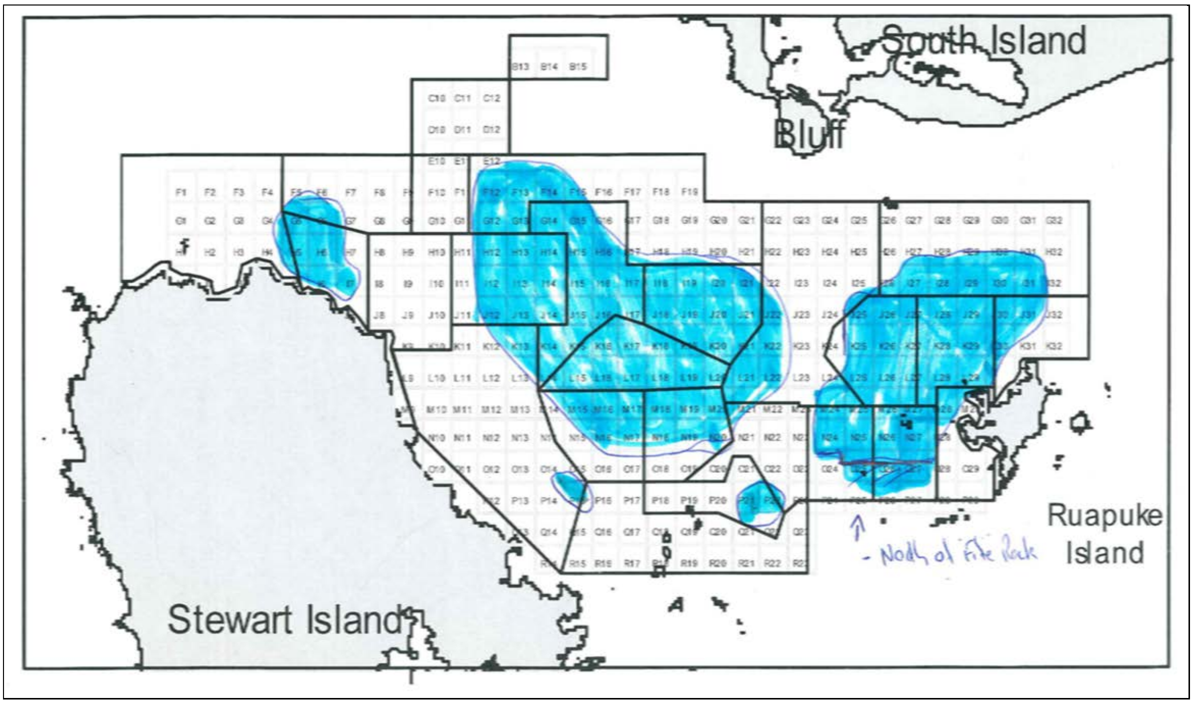

Figure A3.2: The historical extent of the commercial oyster fishery, 1990–2013 from fisher interviews to record skippers' knowledge.

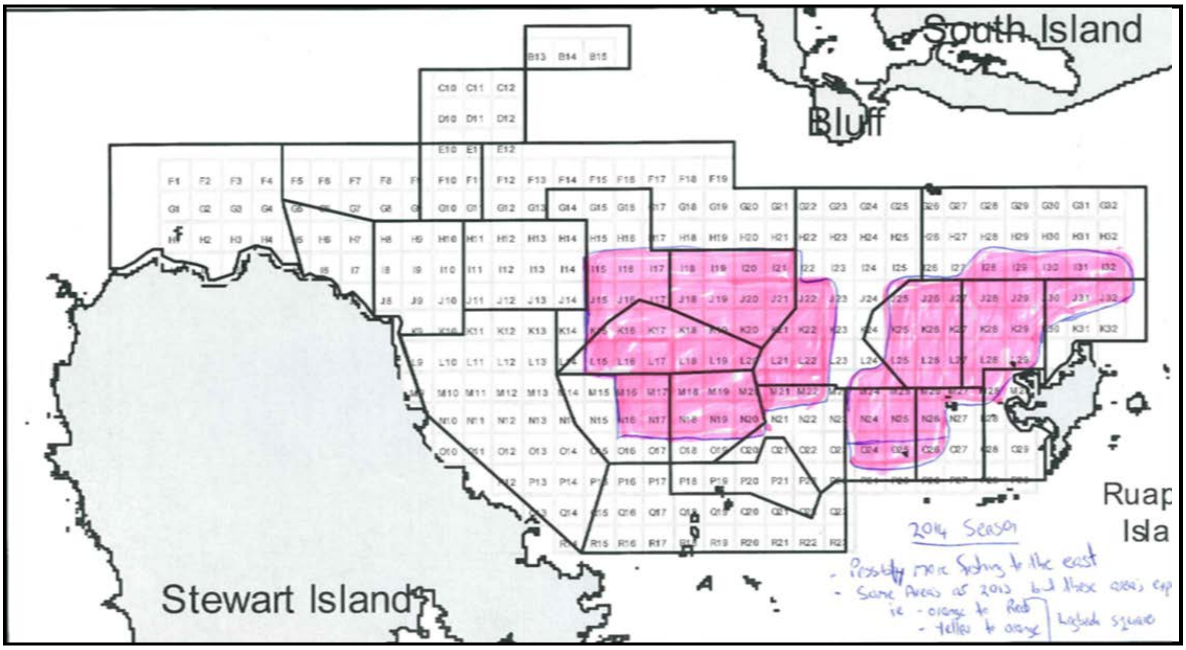

Figure A3.3: The commercial oyster fishery areas likely to be important in 2014 from fisher interviews to record skippers' knowledge.

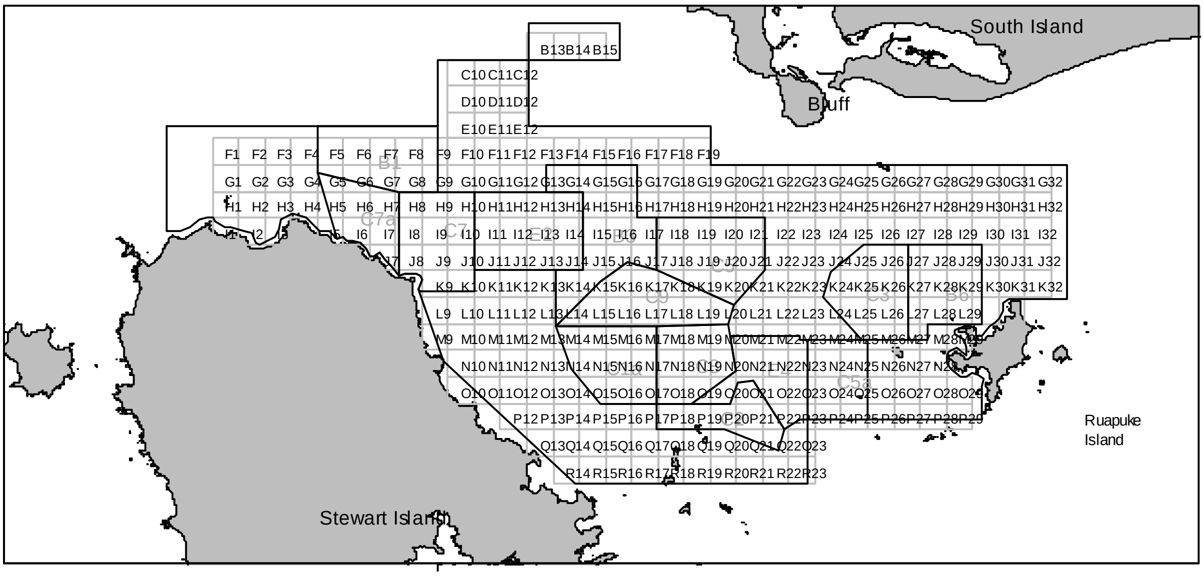

Figure A3.4: The 2012 survey area boundary and survey strata overlaid on the Foveaux Strait oyster skippers' logbook reporting grid (one nautical mile squares).

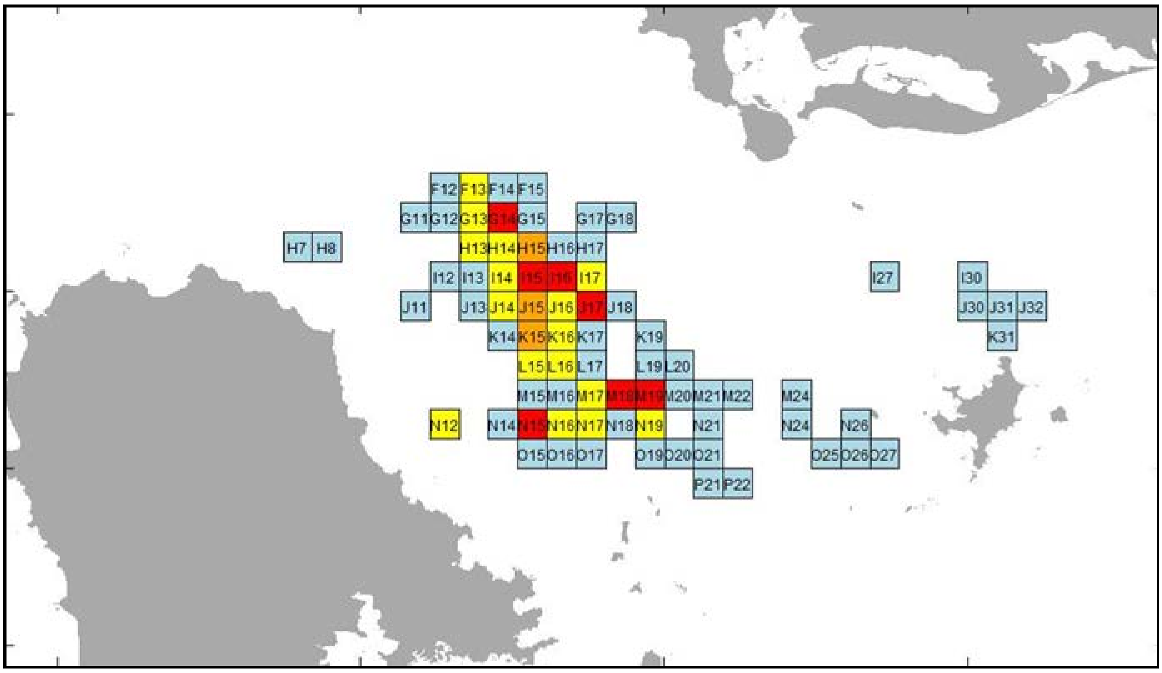

Figure A3.5: Distribution of catch as a percentage of the total annual catch from each grid in 2009; for commercial and prospecting tows: Greater than 10% shown in brown, 5–10% shown in red, 3–4.9% (orange), 1–2.9% (yellow), and <1% (light blue). Grid cells where no fishing took place are not shown and are represented by the white background.

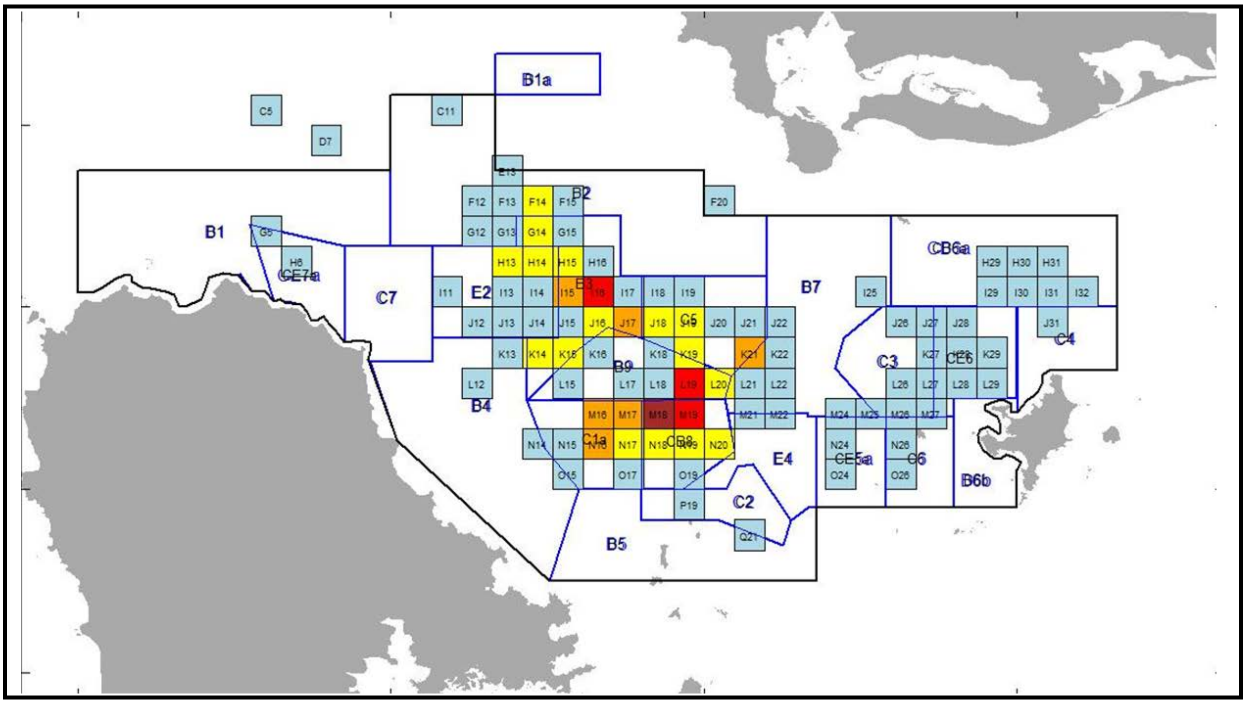

Figure A3.6: Distribution of catch as a percentage of the total annual catch from each grid in 2012; for commercial and prospecting tows: Greater than 10% shown in brown, 5–10% shown in red, 3–4.9% (orange), 1–2.9% (yellow), and <1% (light blue). Grid cells where no fishing took place are not shown and are represented by the white background.

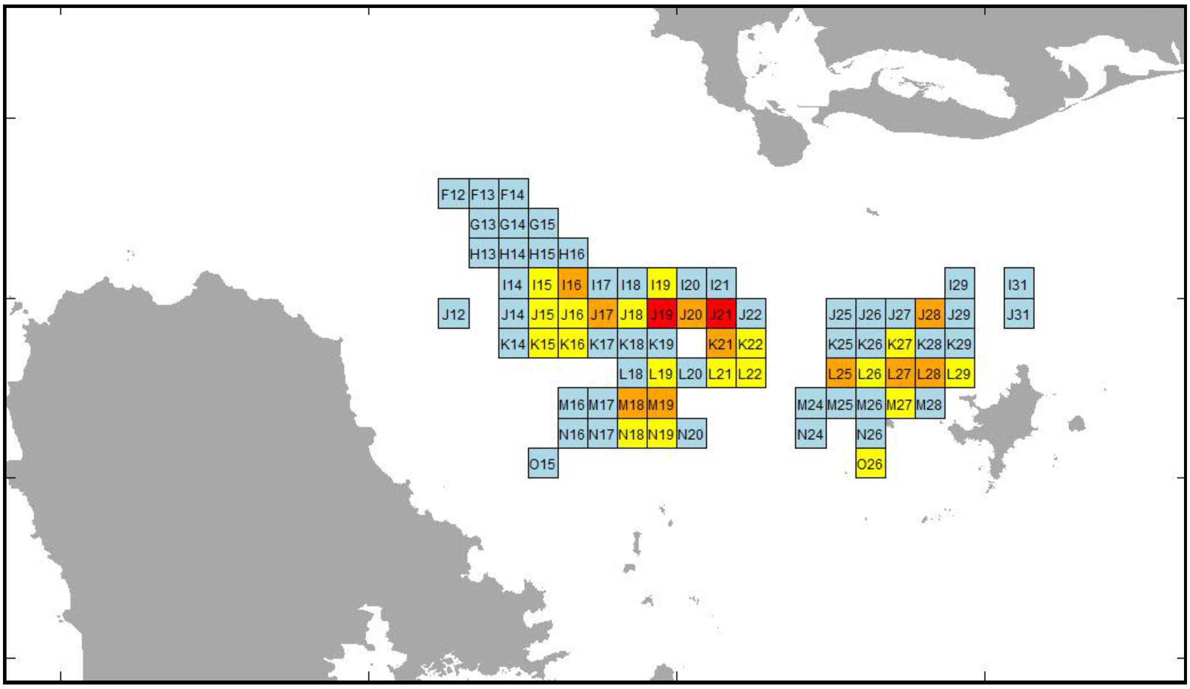

Figure A3.7: Distribution of catch as a percentage of the total annual catch from each grid in 2013; for commercial and prospecting tows. 5–10% shown in red, 3–4.9% (orange), 1–2.9% (yellow), and <1% (light blue). Grid cells where no fishing took place are not shown and are represented by the white background.

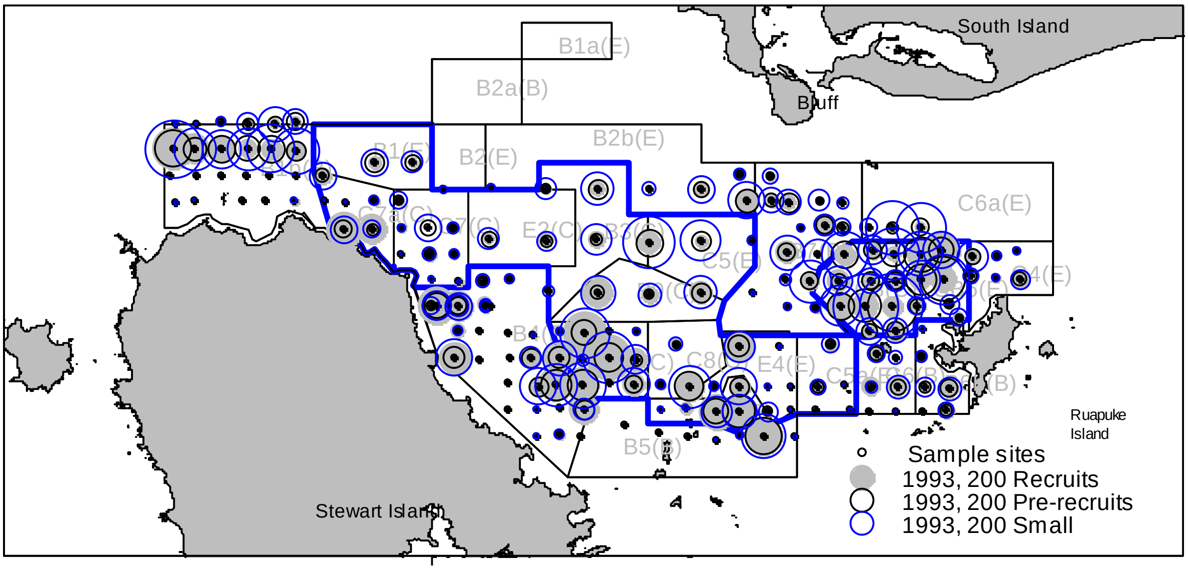

Figure A3.8: The 1993 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a “C” in brackets. Exploratory strata have an “E” in brackets, and background strata “B”.

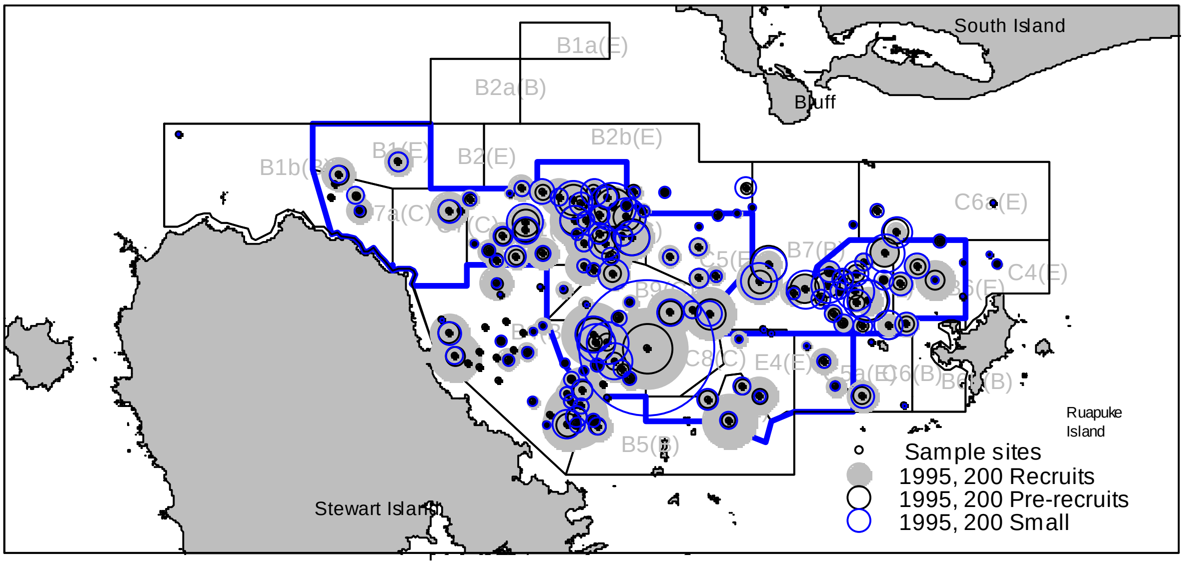

Figure A3.9: The 1995 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

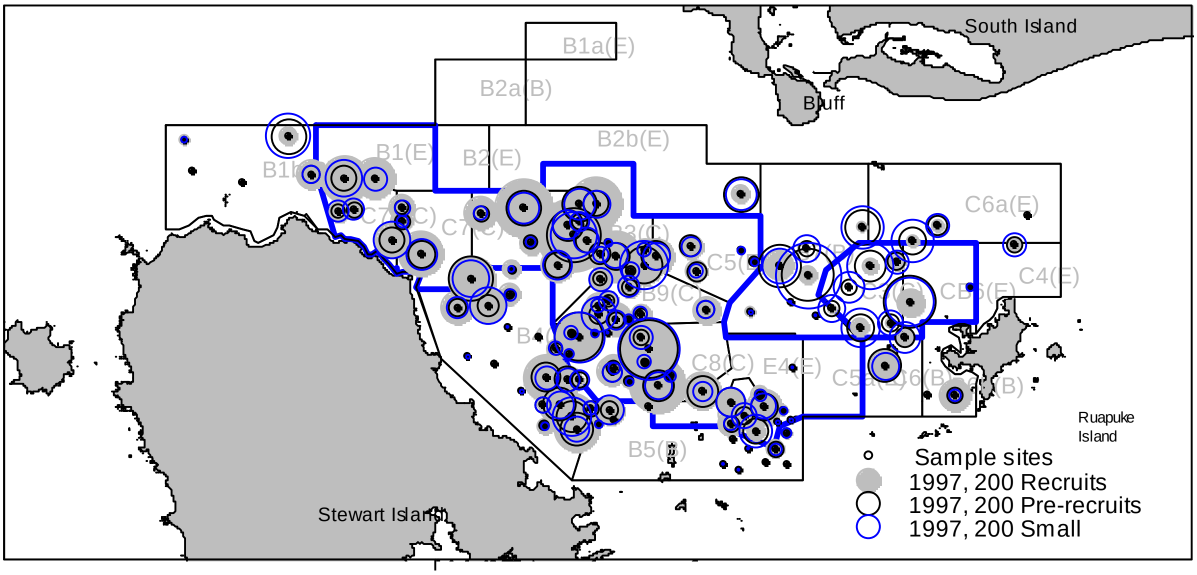

Figure A3.10: The 1997 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

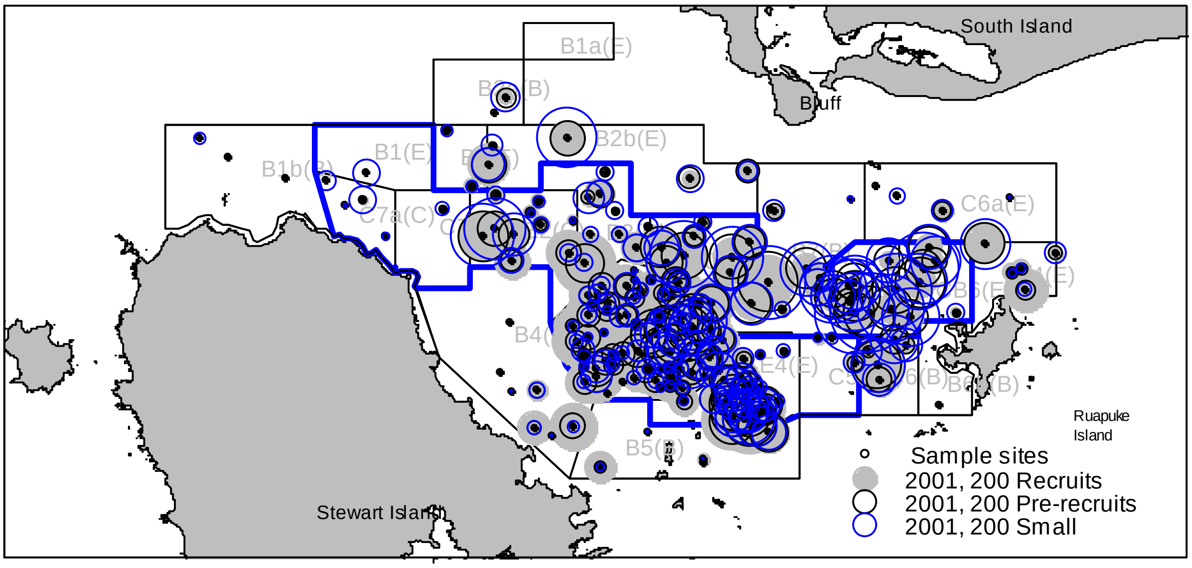

Figure A3.11: The 2001 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

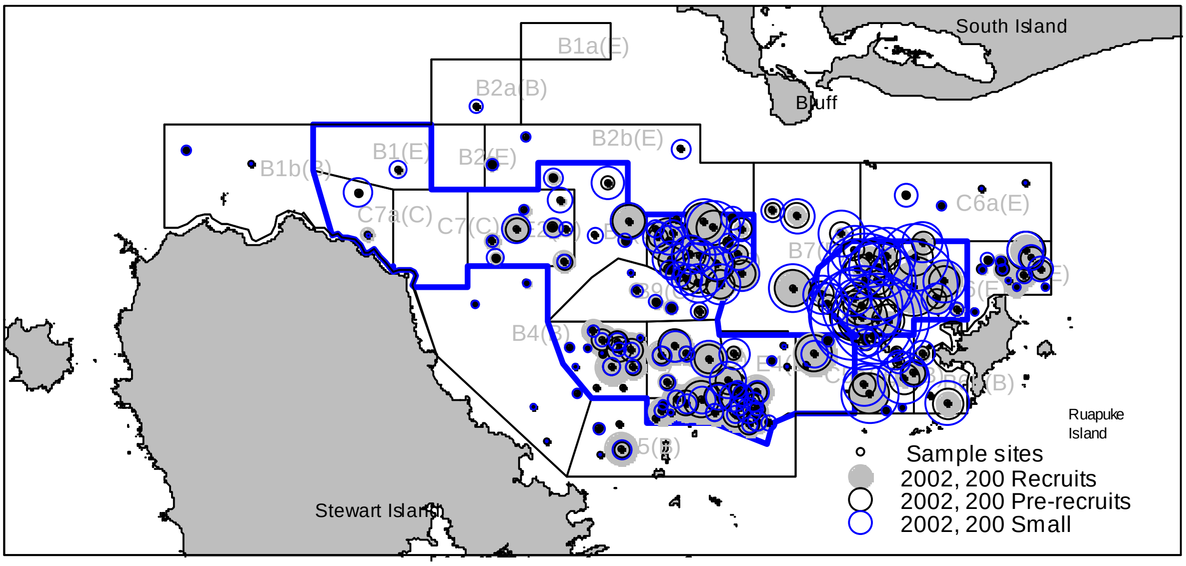

Figure A3.12: The 2002 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

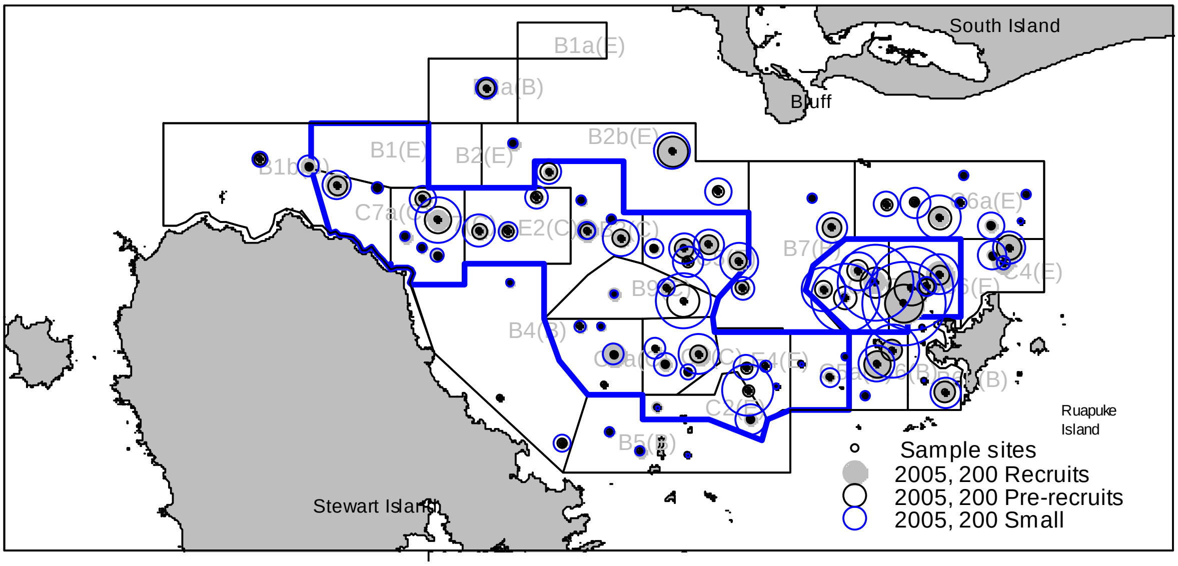

Figure A3.13: The 2005 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

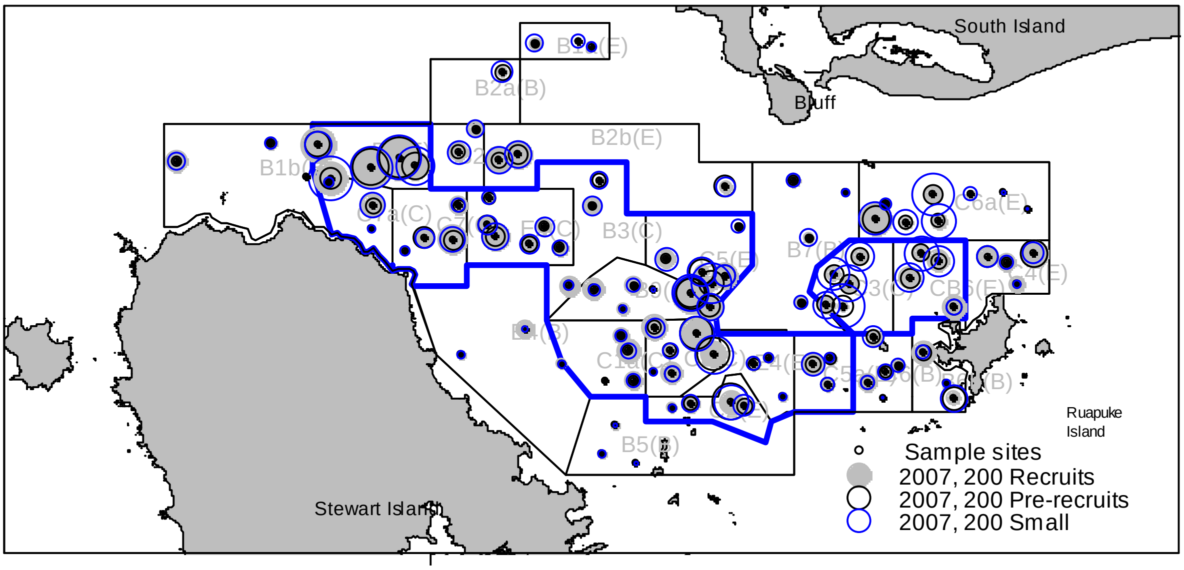

Figure A3.14: The 2007 survey data for recruit-sized, pre-recruit, and small oysters plotted over 2012 stock assessment survey area. The survey boundary, proposed boundary of the core strata (heavy blue line), and the survey strata are shown as black lines. Strata labelled with black text. Strata designated commercial in 2012 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B".

APPENDIX 4: The numbers of sample stations predicted to give CVs in the range of 10% to 20% for different combinations of merged strata and three survey data sets (2009, 2012 and 2014) estimated using allocate. The fourteen core strata (FS14) used for the 2014 Foveaux Strait oyster survey were merged into 1 (FS1), 2 (FS2), 4 (FS4), and 7 (FS7) strata respectively. Output for each survey shown in a separate table below.

Recruit-sized oysters 2014				Number of Stations	
CV	**FS1.R14**	**FS2.R14**	**FS4.R14**	**FS7.R14**	**FS14.R14**
10	83	74	64	51	50
11	69	61	53	42	45
12	58	52	45	36	43
13	50	44	38	31	42
14	43	38	33	27	42
15	37	33	29	25	42
16	33	29	25	23	42
17	29	26	23	22	42
18	26	23	20	21	42
19	23	21	18	21	42
20	21	19	17	21	42

Recruit-sized new clocks 2014				Number of Stations	
CV	**FS1.NC14**	**FS2.NC14**	**FS4.NC14**	**FS7.NC14**	**FS14.NC14**
10	91	85	80	72	65
11	75	71	66	60	56
12	63	59	56	50	50
13	54	51	48	43	46
14	47	44	41	37	43
15	41	38	36	33	42
16	36	34	32	29	42
17	32	30	28	26	42
18	28	27	25	24	42
19	26	24	23	23	42
20	23	22	20	22	42

Prevalence 2014				Number of Stations	
CV	**FS1.Prev14**	**FS2.Prev14**	**FS4.Prev14**	**FS7.Prev14**	**FS14.Prev14**
10	156	156	126	129	98
11	129	129	105	106	82
12	108	108	88	90	70
13	92	92	75	76	62
14	80	80	65	66	56
15	70	69	57	58	51
16	61	61	50	51	48
17	54	54	44	45	46
18	48	48	29	40	44
19	44	43	35	36	43
20	39	39	32	33	43

Recruit-sized oysters 2012

	Number of Stations				
CV	**FS1.R12**	**FS2.R12**	**FS4.R12**	**FS7.R12**	**FS14.R12**
10	106	100	85	83	69
11	88	82	70	69	59
12	74	69	59	58	52
13	63	59	51	49	46
14	54	51	44	43	44
15	47	45	38	37	42
16	42	39	34	33	42
17	37	35	30	29	42
18	33	31	27	26	42
19	30	28	24	24	42
20	27	25	22	23	42

Recruit-sized new clocks 2012

	Number of Stations				
CV	**FS1.NC12**	**FS2.NC12**	**FS4.NC12**	**FS7.NC12**	**FS14.NC12**
10	196	172	153	145	118
11	162	142	126	120	100
12	136	119	106	101	86
13	116	102	91	86	76
14	100	88	78	75	68
15	88	77	68	66	62
16	77	67	60	59	58
17	68	60	53	53	54
18	61	53	48	48	51
19	55	48	43	44	48
20	49	43	39	40	46

Prevalence 2012

	Number of Stations				
CV	**FS1.Prev12**	**FS2.Prev12**	**FS4.Prev12**	**FS7.Prev12**	**FS14.Prev12**
10	NA	214	156	133	129
11	190	177	129	111	110
12	160	149	108	94	95
13	136	127	92	81	84
14	117	109	80	71	75
15	102	95	70	63	67
16	90	84	61	57	61
17	80	74	55	51	57
18	71	66	49	47	53
19	64	60	44	43	50
20	58	54	40	39	48

Recruit-sized oysters 2009					Number of Stations
CV	**FS1.R09**	**FS2.R09**	**FS4.R09**	**FS7.R09**	**FS14.R09**
10	100	97	79	71	64
11	83	81	66	59	56
12	69	68	55	50	50
13	59	58	47	43	46
14	51	50	41	37	44
15	45	44	36	33	43
16	39	38	31	29	42
17	35	34	28	27	42
18	31	30	25	25	42
19	28	27	23	23	42
20	25	25	21	22	42

Recruit-sized new clocks 2009					Number of Stations
CV	**FS1.NC09**	**FS2.NC09**	**FS4.NC09**	**FS7.NC09**	**FS14.NC09**
10	NA	NA	417	324	206
11	NA	284	345	268	173
12	NA	236	290	225	147
13	NA	201	247	192	128
14	NA	173	213	166	112
15	NA	151	186	145	100
16	NA	133	163	127	90
17	NA	118	145	113	83
18	NA	105	129	101	76
19	NA	94	116	91	71
20	188	85	105	83	67

Prevalence 2009					Number of Stations
CV	**FS1.Prev09**	**FS2.Prev09**	**FS4.Prev09**	**FS7.Prev09**	**FS14.Prev09**
10	NA	304	240	158	124
11	NA	251	198	131	105
12	NA	211	167	110	91
13	NA	180	142	94	80
14	191	155	123	81	71
15	166	135	107	71	64
16	146	119	94	62	58
17	129	105	83	56	54
18	115	94	74	50	50
19	104	84	67	46	48
20	94	76	60	42	46