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The status of infection by bonamia (*Bonamia exitiosa*) in Foveaux Strait oysters (*Ostrea chilensis*) in February 2013, estimates of summer disease mortality, and implications for the projections of future stock status made in the 2012 stock assessment for OYU 5

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Assessments of Foveaux Strait oyster stock (OYU 5) show that the future status of the fishery is primarily determined by the levels of bonamia (*Bonamia exitiosa*) mortality, and to a much lesser extent, recruitment. Recruit-sized oyster mortality from bonamia of about 10% has resulted in an increase in oyster abundance, while levels of bonamia mortality of about 20% are expected to reduce the oyster population size. Catching the current total allowable commercial catch (TACC) of 15 million oysters has no detectable effect on trends in the fishery. The fishery has shown an ability to rebuild rapidly in the absence of disease (assuming long-term average recruitment).

Oyster density in the Foveaux Strait oyster fishery (including eastern fishery areas) has been increasing in recent years, from a relatively low stock size in 2005. Areas with relatively high oyster densities are potentially at the greatest risk of higher mortality from bonamia. These areas are also of greatest importance to commercial fishers. In years between stock assessments of OYU 5, smaller, focused surveys of bonamia prevalence and intensity (bonamia surveys) are used to monitor the status of bonamia infection, and to estimate short-term (summer) mortality from bonamia in commercial fishery areas. The survey areas are chosen by oyster skippers on the basis that they are likely to be important in the next oyster season. The bonamia surveys do not attempt to sample the whole fishery area, but focus on areas of relatively high oyster density important to the commercial fishery.

The status of bonamia in the fishery is determined from the densities of shells of oysters that had died over the summer (new clocks), and moribund oysters (gapers), as well as the distribution of prevalence and intensity of bonamia infection. New clocks provide estimates of recent oyster mortality, gapers are indicative of oyster mortality at the time of sampling, and the prevalence and intensity of infection used to estimate the numbers of oysters with fatal infections. These surveys also estimate changes in recruit-sized oyster densities in the areas surveyed that allow the effects of disease mortality in commercial fishery areas to be quantified.

The February 2013 bonamia survey continues a series of surveys of bonamia infection in the oyster population. Sampling and operational procedures were the same as those in previous surveys. The survey sampled a randomly selected subset of 45 stations from those sampled in 2012 in strata designated as important for the 2013 oyster season by oyster skippers, and 12 target stations. Bonamia samples were taken from a random sample of 25 oysters at each station. Oysters were sampled using histology and quantitative polymerase chain reaction (qPCR); remnants of the oyster hearts sampled for heart imprints were taken for qPCR samples and replicate samples of gill tissue were also taken from the same oyster. qPCR samples were analysed using a method developed to detect bonamia in Foveaux Strait oysters in the MPI project OYS201101.

In 2013, oyster samples were initially tested for bonamia using the new molecular method. All heart imprint slides from those samples that tested positive for bonamia infection in either heart or gill samples were examined. At each station, at least three heart imprint samples that were qPCR negative were randomly selected and screened using histological methods.

New clock and gaper densities suggested relatively high levels of pre-survey mortality over the summer. The median percentage of recruit-sized new clocks was 3.4% in 2013 compared with 2.3%

in 2012. Pre-survey mortality was estimated to be higher in 2013, than in 2012. In 2013 there were 29.0 million new clocks (CV of 25%, 95% CI 13.4–49.4) and in comparable stations and strata in 2012, there were 17.6 million new clocks (CV of 22%, 95% CI 8.9–29.4).

The sensitivity of qPCR in the detection of bonamia was higher than heart imprints. The mean prevalence of infection was 12.1% (95% CI 0–32.8%) from heart imprints, 19.6% (95% CI 0–55.7%) from qPCR heart tissue samples, and 30.5% (95% CI 3.5–71.3%) from qPCR gill tissue samples. External contamination of gill tissues by water borne bonamia particles, especially in February samples when disease mortality is at its highest, could not be ruled out. Comparisons of estimates of the prevalence and intensity of infection between surveys used heart imprint data from randomly selected stations in strata common between surveys.

Most (88%) of the oysters examined for bonamia (from heart imprint samples alone) had no detectable infection, similar to surveys between 2010 and 2012. The total numbers of infected oysters in common strata estimated from the numbers of infected oysters in the catch increased from 65.3 million in 2012 (CV 0.32) to 81.9 million in 2013 (CV 0.15). The total numbers of infected oysters with fatal infections (category 3−5 intensity of infections from heart imprints) increased from 44.9 million in 2012 (CV 0.33) to 59.7 million in 2013 (CV 0.18). The percentage of the total recruit-sized oyster population infected with bonamia in common strata increased from 11.2% in 2012 to 12.7% in 2013; and fatal infections increased from 7.7% to 9.3% over the same period. The mean intensity of infection (for infected oysters only, determined from heart imprints) was similar to previous years at 3.1. Of all the recruit-sized infected oysters in the strata surveyed, 68.8% were fatally infected in 2012 and 72.9% were fatally infected in 2013.

The post-survey mortality of oysters estimated by the mean correction factor method was projected to reduce the recruit-sized oyster population from 644.9 million oysters (95% CI 394.8−997.5) at the time of the survey (February 2013) to 566.8 million oysters (95% CI 346.5−877.2), a loss of 78.1 million oysters (8.8%, 95% CI 48.2−120.3) by early in the new oyster season. This estimate applies a mean correction factor to the stratum, and the estimate of mortality is higher than that (59.7 million oysters) using the method that estimates the numbers of fatally infected oyster in the stratum from numbers of fatally infected oysters in each tow. Total summer mortality in the strata surveyed from three separate estimates (new clocks, gapers, and fatally infected oysters) was about 107 million recruit sized oysters.

There was a marked increase in the spread of bonamia infection in February 2013. Infection was more widespread than in 2012 and the prevalence of infection increased in the important central fishery areas, as did the intensity of infection. Bonamia infection was variable between sites; stations with no detectable infection were interspersed amongst stations with high prevalence of infection. The patterns of the distribution of the prevalence and the intensity of infection were similar or higher than that observed in February 2012.

Oyster densities estimated from randomly selected stations increased for recruit-sized oysters (from 1.76 oysters per m^2 in 2012 to 1.94 oysters per m^2 in 2013, but decreased for both pre-recruit (0.69 to 0.60 oysters per m^2) and small oysters (0.96 to 0.80 oysters per m^2). The recruit sized oyster population increased from 585.3 million oysters (95%CI 375.8–882.1) in 2012 to 644.9 million oysters (95%CI 394.8–997.5) in 2013. The commercial oyster population size (above a density of 400 oysters per survey tow) was similar at 412.7 million oysters (95%CI 236.9–660.4) in 2012 and 416.6 million oysters (95%CI 196.1–723.6) in 2013.

The distributions of recruited oyster densities has increased in recent years characterised by an increase in eastern fishery areas in 2012 and 2013 where it had been low since 2003. Generally, oyster densities are similar to 2012 or have increased slightly at stations in eastern and central fishery areas and are similar or have declined in southern and western fishery areas since then. The localised declines in western and southern fishery areas are probably a result of bonamia mortality, especially in western fishery areas where there has been very little fishing for many years.

This report provides a summary of information from the February 2013 bonamia survey undertaken under the MPI research programme OYS201201, Objective 1. 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 crews for the survey.

1. INTRODUCTION

The Foveaux Strait oyster fishery 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 important to the socioeconomics of Bluff and Invercargill. Mortality from the haplosporidian parasite *Bonamia exitiosa* (bonamia) is the principal driver of oyster population abundance during epizootics. Before the recent bonamia epizootics began (Doonan et al. 1994), the long-term, average landings from the fishery were about 80 million oysters. The first of two bonamia epizootics, between 1985 and 1992, 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 and 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. The recruit-sized oyster population continued to increase to 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 (Dunn et al. 2002, Dunn et al. 2003, Michael et al. 2004a, Michael et al. 2004b, Michael & Dunn 2005, Michael et al. 2005, Michael 2007, Michael 2008). Following the onset of the bonamia epizootic in 2000, a series of mostly annual surveys were implemented to determine the distribution, prevalence and intensity of infection by bonamia. These data have allowed patterns of disease to be tracked through the fishery by monitoring spatial and temporal changes in the prevalence and intensity of infection, the subsequent oyster mortality, and changes in oyster densities that determined catch rates and in the size of the areas affected that determined changes to the total population size.

These surveys showed an increase in the recruit-sized population from 408 million oysters in 2005 to 913 million oysters in 2012 (Michael et al. 2013). The catch limit in 1996 was set to 15 million oysters to allow the fishery to continue to rebuild, and the catch limit has remained unchanged since. The recreational and customary fishers take is about 1 million oysters annually in addition to the Total Allowable Commercial Catch (TACC). Between 2003 and 2008, the Bluff Oyster Management Company (BOMC) shelved half of the TACC, harvesting about 7.5 million oysters annually (Ministry of Fisheries 2008). As the fishery rebuilt, BOMC unshelved about 10% of the shelved quota each year from the 2009 oyster season landing 8.22 million oysters in 2009, 9.54 million oysters in 2010, 10.5 million oysters in 2011, and 12.06 million oysters in 2012 (Fu 2013).

Biennial and more recently triennial stock assessments estimate the status of the stock and make projections of future stock status based on expected levels of recruitment, harvest, catch rates, population size, and mortality (that includes mortality from bonamia). The stock assessment projections of future stock status and the size of the recruited oyster population are primarily driven by the levels of bonamia mortality, and to a lesser extent recruitment.

In years between stock assessments, smaller focused surveys of bonamia prevalence and intensity (bonamia surveys) are used to monitor the status of bonamia infection, and to estimate short term (summer) mortality from bonamia in designated commercial areas that are likely to be important to fishers in the following oyster season. These surveys are not intended to be stock assessment surveys, and do not attempt to sample all survey strata over the whole Foveaux Strait oyster survey area. Bonamia surveys focus on areas of relatively high oyster density important to the commercial fishery and to the stock, as the effects of heightened mortality are much higher there. In general, bonamia

mortality levels of about 10% of the recruit-sized population have resulted in an increase in population abundance, while levels of bonamia mortality about 20% are expected to reduce the population size. The fishery has shown an ability to rebuild rapidly in the absence of disease (assuming long-term average recruitment).

The status of bonamia in the fishery is determined from new clock and gaper densities, the distribution of prevalence and intensity of bonamia infection, and changes in oyster densities in three size groups. New clocks are the shells of oysters that have died since the beginning of summer, but before the survey, and these provide estimates of recent oyster mortality caused by bonamia. Gapers are moribund oysters indicative of oyster mortality at the time of sampling. The prevalence of infection provides estimates of the proportion of the oyster population with detectable infections, and the intensity of infection estimates the proportions of infected oysters with non-fatal and fatal infections. These surveys also estimate changes in recruit-sized oyster densities in the areas surveyed that allow the effects of disease mortality and current status of commercial fishery areas to be determined.

Surveys of the status of bonamia infection, mortality, and the oyster population between 2000 and 2005 found that bonamia had drastically reduced both the size and number of commercial fishery areas, reduced oyster density within them, and changed the distribution of oysters (Michael et al. 2009a). Bonamia surveys since 2006 found the levels of bonamia mortality had declined to about 10%, but infection was widespread, including eastern areas that had relatively high prevalence despite low oyster densities there. By February 2009, prevalence was generally low, but the infection was widespread and variable; stations with no detectable infections were interspersed with stations with low and high intensity infections. The distribution of infection was similar between 2010 and 2012, but prevalence was higher and infected stations generally had higher intensities of infection than in previous years, especially in central fishery areas (Michael et al. 2013).

Oyster density has been increasing across the Foveaux Strait oyster fishery in recent years, including eastern fishery areas. Areas with relatively high oyster densities are potentially at the greatest risk of mortality from bonamia. These areas are also of greatest importance to commercial fishers.

The annual monitoring of the status of bonamia in the oyster fishery provides a suite of information for management of the OYU 5 fishery and to the oyster industry. Initial bonamia surveys in March 2000 were focused on determining the spread of infection from what was thought to be a single area where the epizootic began. These surveys estimated densities of oysters and new clocks, and took oyster samples to determine the prevalence and intensity of infection along four transects extending down-tide through a number of fishery areas with high densities of oysters (Dunn et al. 2000). Sampling oysters to determine bonamia infection during stock assessment surveys (in the current epizootic) began in October 2001, and only a subset of the randomly selected stations that were sampled for oyster density were sampled for bonamia infection (Michael et al. 2004b). A subset of the randomly selected stations that were sampled for oyster density in the in October 2002 stock assessment survey were also sampled for bonamia infection (Michael et al. 2004a); and this became a feature of subsequent stock assessment surveys (Michael et al. 2005, Michael et al. 2008b, Michael et al. 2009b, Michael et al. 2013). Following the October 2001 stock assessment survey, the current series of bonamia surveys began, resampling a subset of stations from the stock assessment survey in small targeted surveys in January and March 2002. These surveys focused sampling effort in a subset of survey strata with relatively high oyster density (Michael et al. 2004b), and aimed to provide information on changes to the distribution of the prevalence and intensity of bonamia infection at resampled stations, and changes in oyster densities in these strata. Bonamia surveys since January 2002 have been undertaken with these primary objectives (Dunn et al. 2003, Michael et al. 2005, Michael et al. 2006, Michael et al. 2008a, Michael et al. 2009a, Michael et al. 2011, Michael et al. 2012a, Michael et al. 2012b). In 2006, estimates of short-term mortality derived from oysters with category 3 and greater intensity of infections were used to project changes in population size between the February bonamia survey and the start of the oyster season in March 2006, in the subset of strata surveyed Michael et al. 2008a. The stock assessment survey in February 2007 began to express the

estimated post-survey mortality as a percentage (Michael et al. 2008b) that could then be used to indicate the level of disease mortality in the oyster population mortality and the most likely option of the three projections of future stock status that were based on 0%, 10%, and 20% disease mortality (Dunn 2007). This estimate of post-survey mortality became a key result for both the stock assessment surveys and bonamia surveys that followed; and there was acceptance that bonamia surveys whilst not representative of the whole fishery area, gave a good indication of what would be likely to occur in the commercial fishery areas important to industry and stakeholders. Projections of future stock status determined selected by the levels of summer disease mortality were consistent with estimates of subsequent stock assessment surveys.

The objective of bonamia surveys has typically been to survey a subset of strata within the survey area considered important by oyster skippers, and to resample a randomly selected subset of stations from stock assessment surveys "to determine the distribution, prevalence and intensity of infection by *Bonamia exitiosa*, and to estimate the oyster density in the commercial areas sampled for bonamia". These surveys were never intended as stock assessments or to cover the entire fishery area. The information provided by oyster surveys has changed over time but, there has been no ranking of this information by the stakeholders who use it. In historical order of inclusion:

1. Since 2000, sample stations have been repeated in successive years to provide data for site by site comparisons of bonamia status and related mortality, and oyster density. Bonamia prevalence and intensity varies markedly over small spatial scales. Monitoring the spread of bonamia within strata was important from 2000–2006.
2. The random selection of stations sampled in previous stock assessment surveys and resampled in following bonamia surveys was introduced in 2002 to provide estimates of changes in oyster density by stratum when bonamia mortality often dramatically changed the distribution of oyster density. These estimates of stratum density were probably biased slightly upward, but could detect significant reductions in oyster density.
3. Changes in population size between the February surveys and the start of the oyster season calculated from short-term mortality derived from oysters with category 3 and greater intensity of infections were estimated from March 2006 onwards.
4. Estimates of total summer mortality based on pre-survey estimates of mortality from new clocks and gapers and the post-survey mortality were included in reports from February 2010 (Michael et al. 2011). It was acknowledged that these two estimates represented different estimates of mortality that are not directly comparable. However, the combined totals of these two estimates provide the only estimate available for total summer mortality that is important in determining which projection from the previous stock assessment is best able to inform future stock status.

This report provides a summary of information from the February 2013 bonamia survey undertaken under the MPI research programme OYS201201, Objective 1. This survey was undertaken in collaboration with the Bluff Oyster Management Company who provided information for the survey design, a vessel, dredge, and crews for the sampling.

2. OBJECTIVE

To carry out a bonamia survey in February 2013, to determine the distribution, prevalence and intensity of infection by *Bonamia exitiosa*, and to estimate the oyster density in the commercial areas sampled for bonamia.

3. METHODS

3.1 Sampling design

The February 2013 survey continues a time series of surveys using the 2007 Foveaux Strait oyster survey area (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 2013 survey retained the 2012 strata to continue a time series of oyster density data and data on the disease status of commercial fishery areas. In 2012, two of the 2009 strata were split to better define areas of similar oyster densities. Stratum B1 was split into B1b to the west, designated as a background area and B1 to the east, designated as commercial. Stratum B2a was split into B2a to the north, as a background stratum and B2 to the south, as an exploratory stratum (Figure 1).

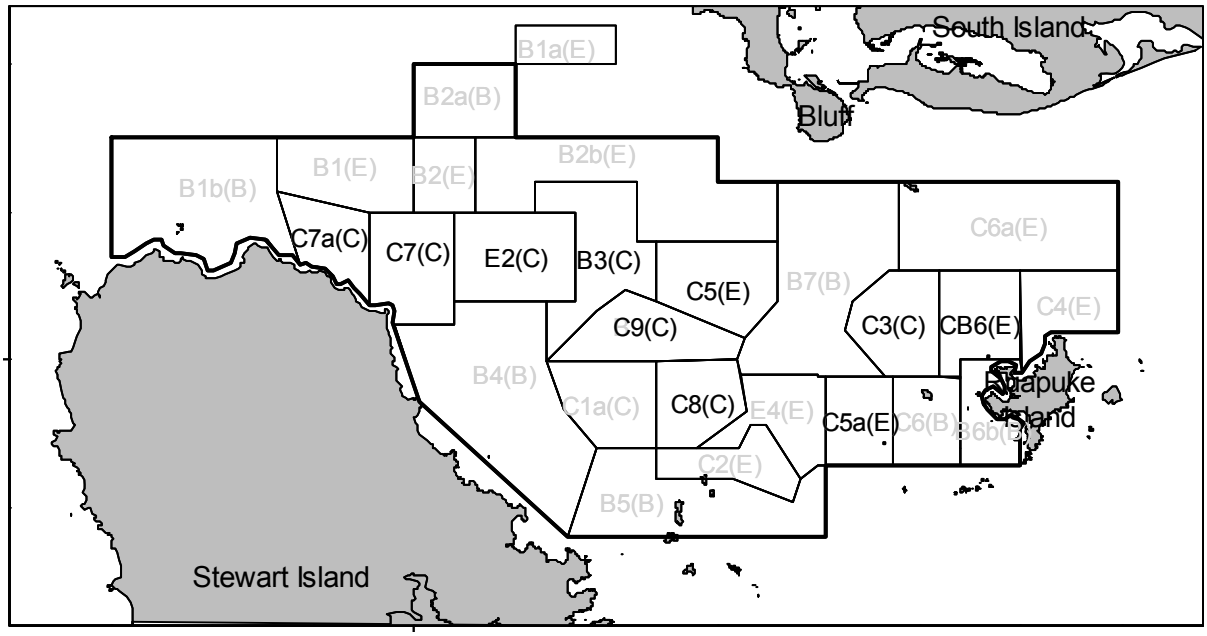

Figure 1: The 2013 survey area with the 2005 survey boundary shown as a heavy, black outer line, and the 2012 survey strata shown as black lines. Strata that were sampled in 2013 labelled with black text and those of the 2012 strata not sampled with grey text. Strata designated commercial in 2013 by 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.

In 2013, sampling was mainly focused in strata from the 2012 stock assessment survey that had the highest densities of oysters in 2012 (Figures 2–4) and were commercial fishery areas that were important to fishers (Figures 5 and 6). Some consideration was also given to bonamia status in recent surveys to determine the progression of disease, particularly in strata where there was increased risk of disease mortality from bonamia (Figures 7 and 8). Some sampling was carried out in the lower oyster density areas in the eastern fishery to investigate rebuilding and disease status. Ten of the 26 survey strata sampled in 2012 (B3, C3, C5, C5a, C7, C7a, C8, C9, CB6, and E2) were sampled again in 2013. These ten strata represented core commercial fishery areas accounting for 64.1% of the 2012 recruit-sized oyster population in 2012 and 46.2% of the survey area. All ten strata had mean recruit-sized oyster densities greater than 1 oyster/m^2. Although this approach will not provide estimates from the whole fishery area, , it allows the limited number of sample stations to be focused in the most important commercial fishery areas. Strata C2 and C6a were also sampled with previously defined target stations.

Bonamia infection and mortality varies over relatively small spatial scales, and historically the focus of bonamia surveys has been to determine changes in disease status and oyster density by station rather than by strata. Forty five stations within the ten strata were randomly selected from stations that had been

sampled in 2012. This approach is a compromise between stratum estimates and site specific information, and this approach may produce a bias in the scaled-up estimates of the infected population size in each stratum. The numbers of stations in each stratum were allocated based on the proportion of the total area accounted for by each stratum, their relative oyster density (Table 1), and on their infection status in 2012 (Appendix 1). At least three stations were randomly selected in each stratum (Table 1) and stations retained their 2012 survey numbering to avoid confusion in pairwise comparisons. In addition to these randomly selected stations, 12 target stations were also sampled. These target stations were chosen to investigate potential changes in bonamia infection at a set of consistent locations over time, some of which have been sampled since 2007. The location of stations is given in Figure 9.

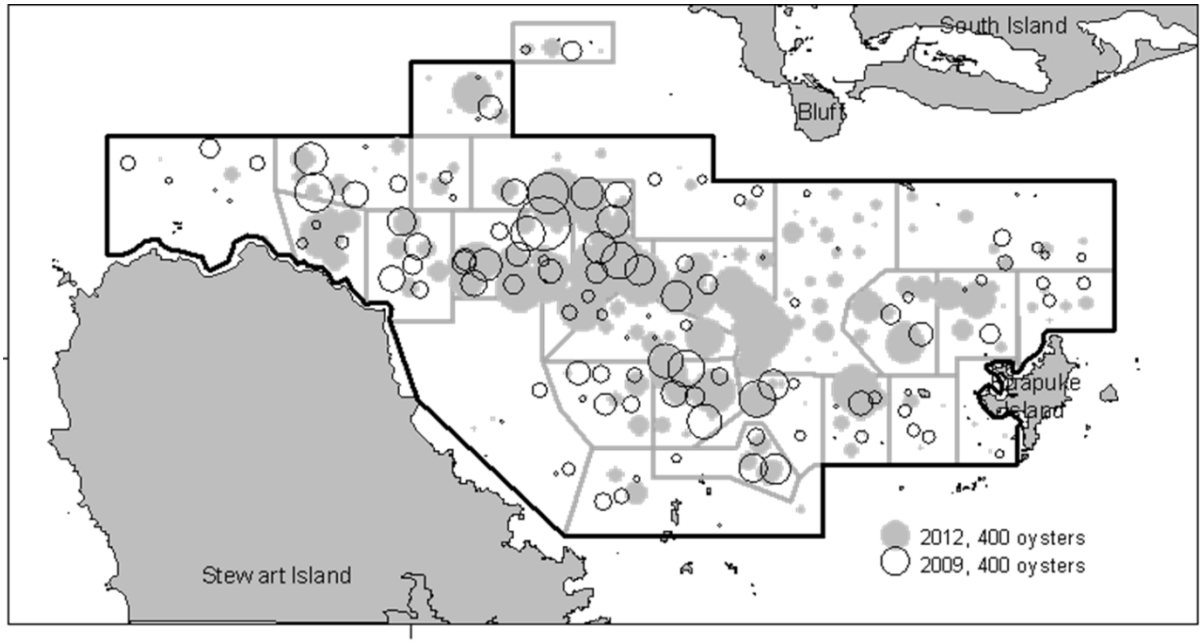

Figure 2: The densities (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled during February surveys in 2012 (filled grey circles) and in 2009 (open black circles).

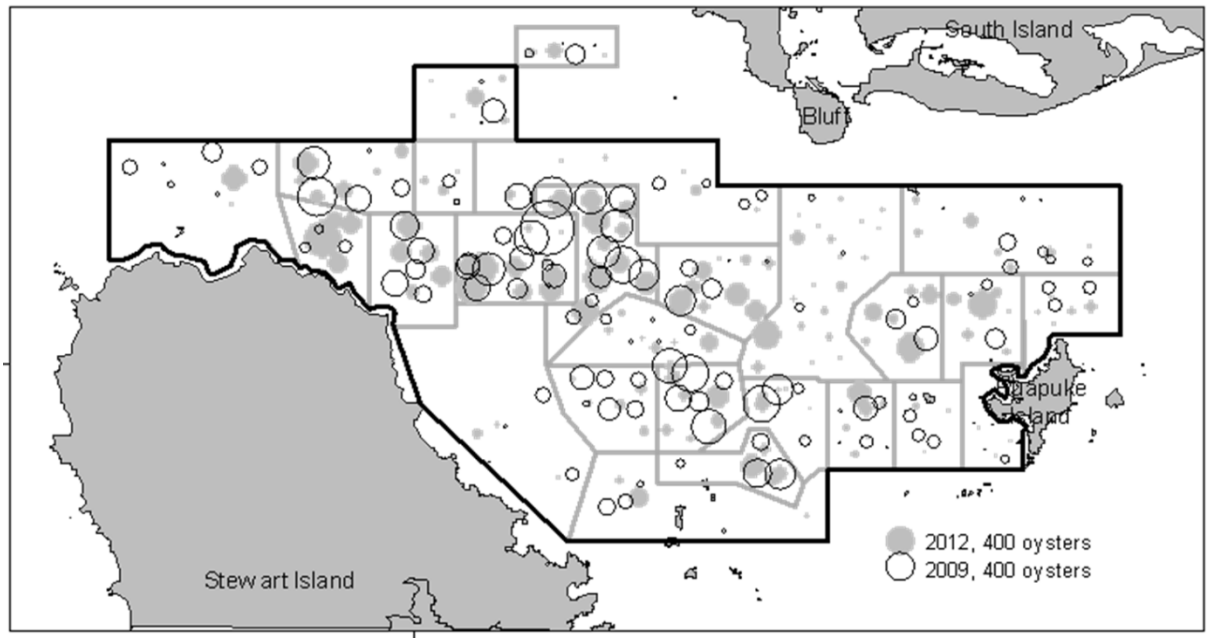

Figure 3: The densities (numbers of oysters per standard tow, 1221 m^2) of pre- recruit oysters sampled during February surveys in 2012 (filled grey circles) and in 2009 (open black circles).

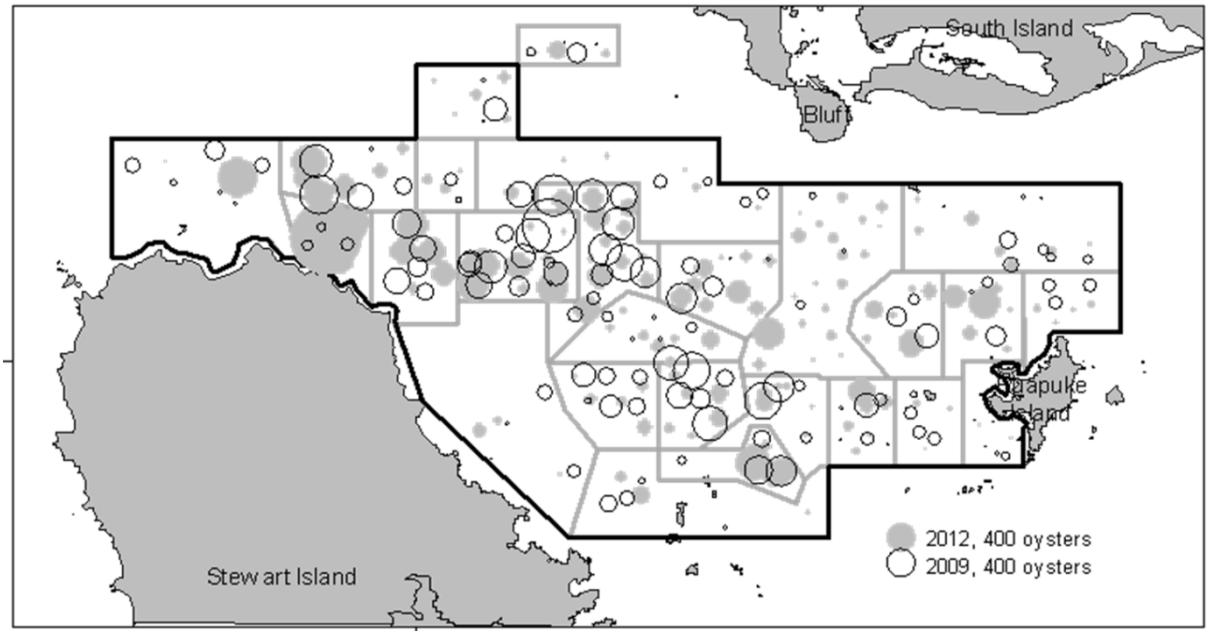

Figure 4: The densities (numbers of oysters per standard tow, 1221 m^2) of small oysters sampled during February surveys in 2012 (filled grey circles) and in 2009 (open black circles).

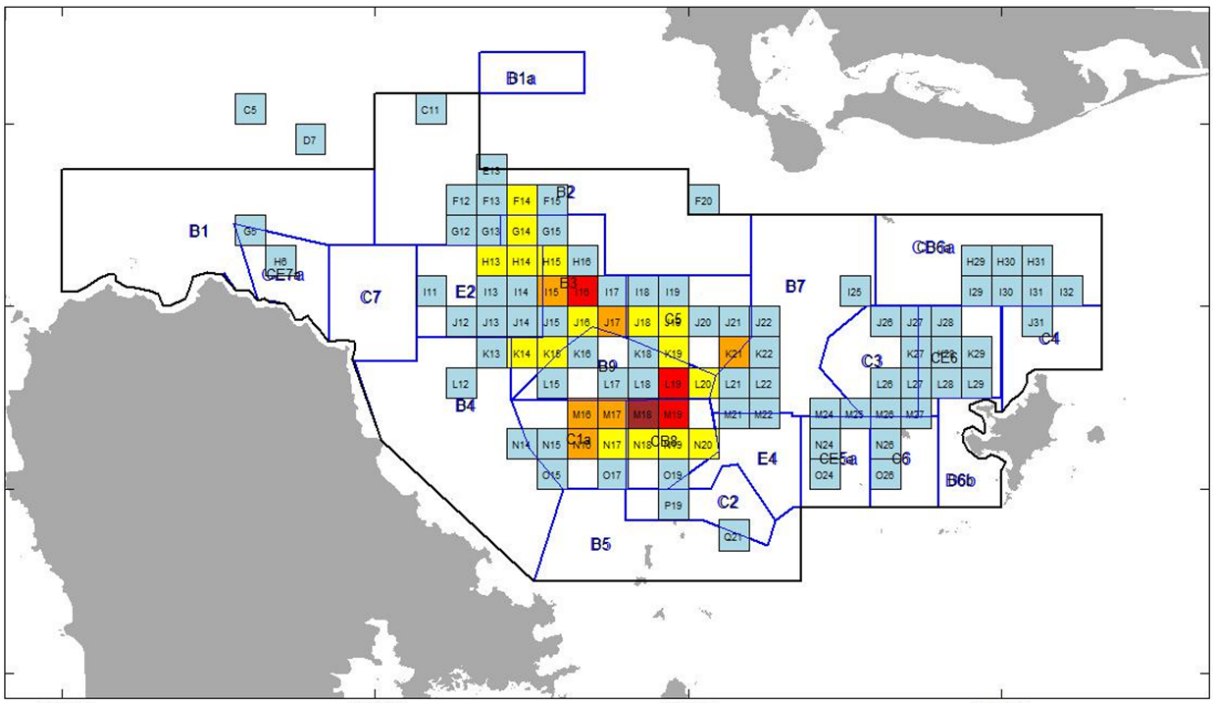

Figure 5: Distribution of catch as a percentage of the total annual catch from each grid in 2012; for commercial and prospecting tows: Greater than 10% shown in brown, 5–10% shown in red, 3–4.9% (orange), 1–2.9% (yellow), and <1% (light blue). Grid cells where no fishing took place are not shown and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

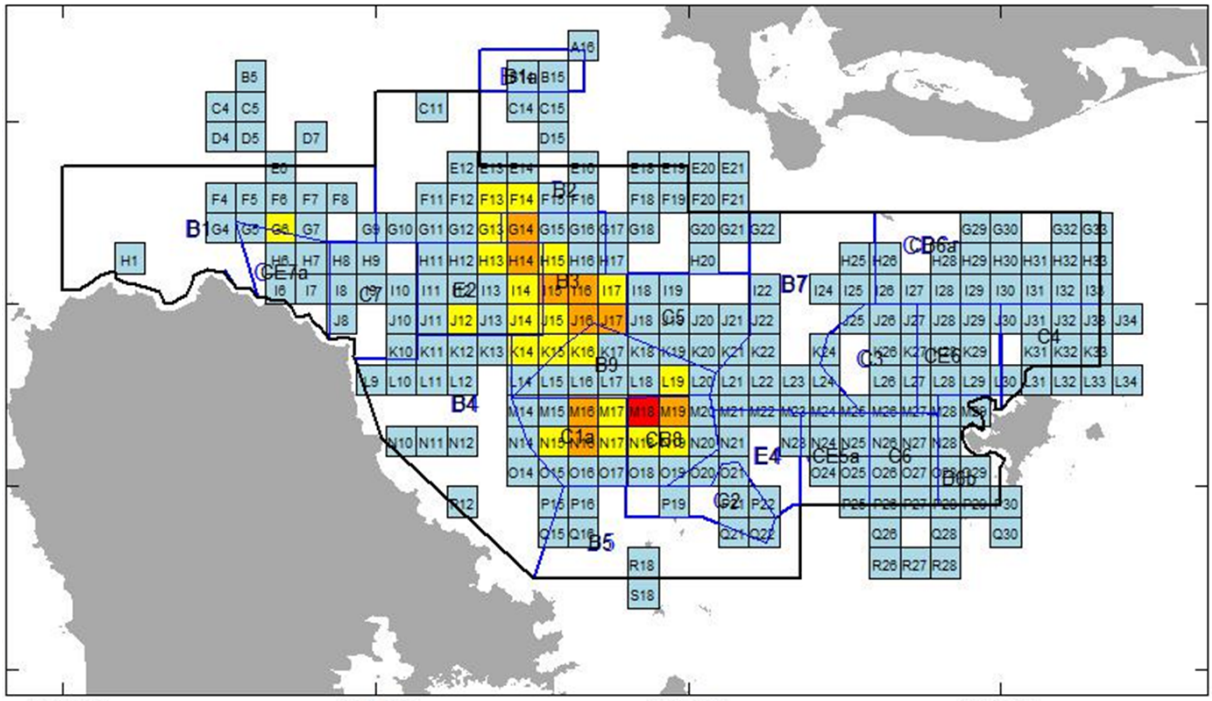

Figure 6: Distribution of catch as a percentage of the total annual catch from each grid in 2006–2012; for commercial and prospecting tows. 5–10% shown in red, 3–4.9% (orange), 1–2.9% (yellow), and <1% (light blue). Grid cells where no fishing took place are not shown and are represented by the white background.

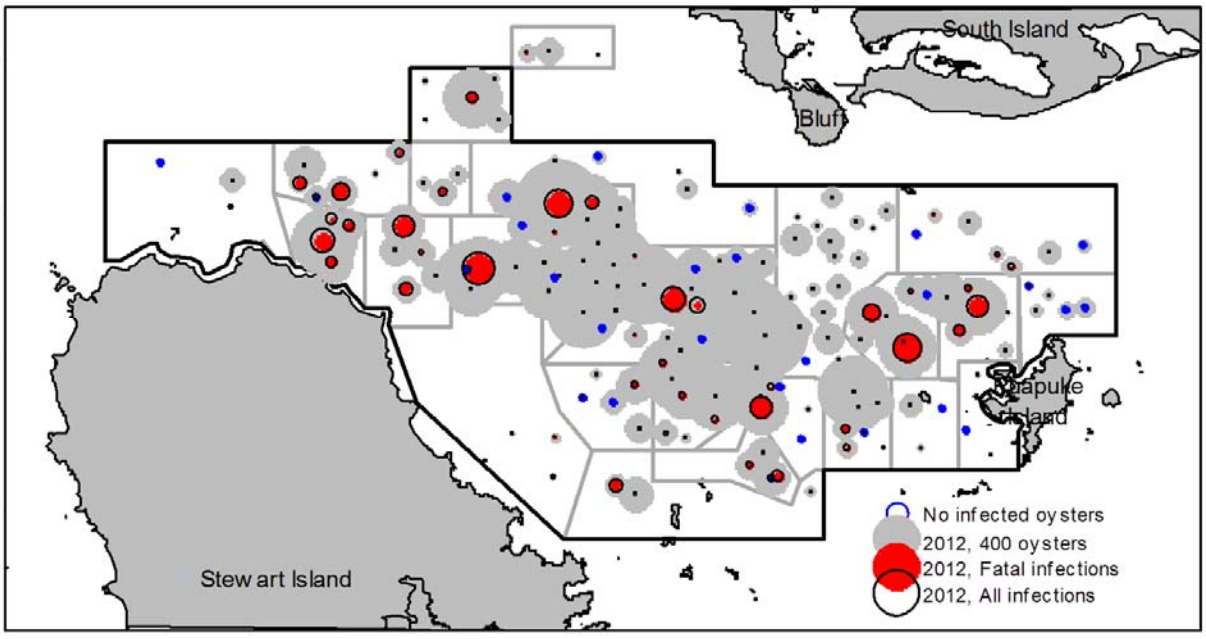

Figure 7: The distributions of oysters and bonamia infection in the February 2012 survey. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (open blue circles). The 2005 survey area (black outer line) and the February 2009 survey strata (grey lines) are shown.

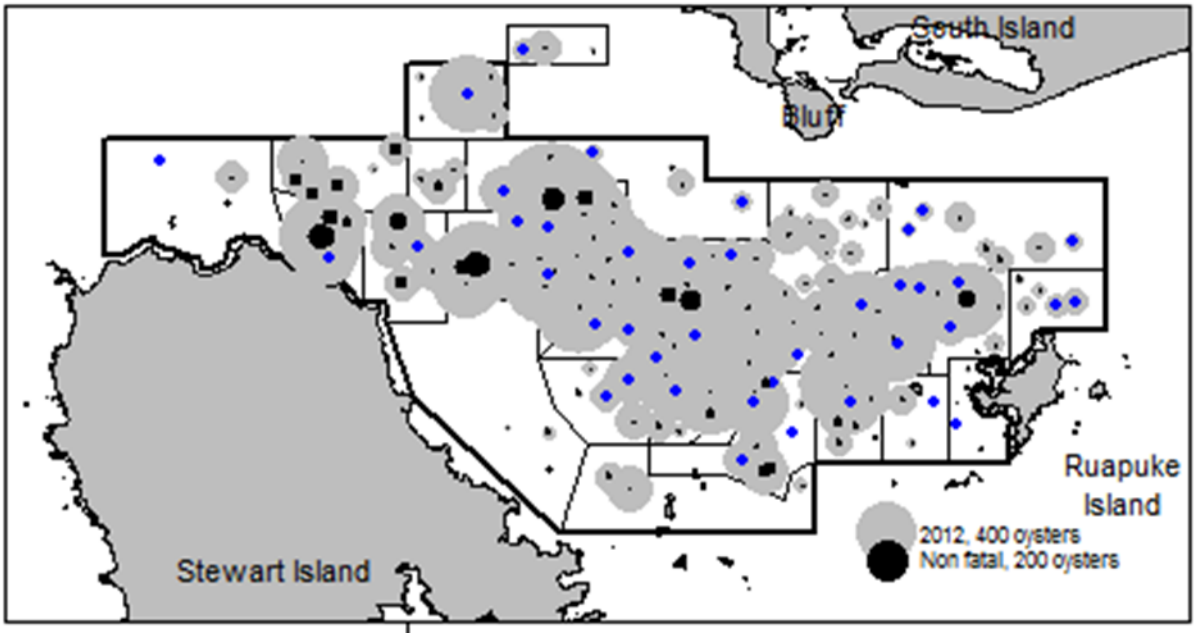

Figure 8: The distribution of recruit-sized oysters (filled grey circles showing numbers per standard tow) and oysters with non-fatal infections (filled blue circles, the numbers of oysters scaled to the size of the catch with intensity of infection category 1 and 2) in February 2012. Stations with no bonamia infection are shown by filled blue circles, and sample stations as black dots.

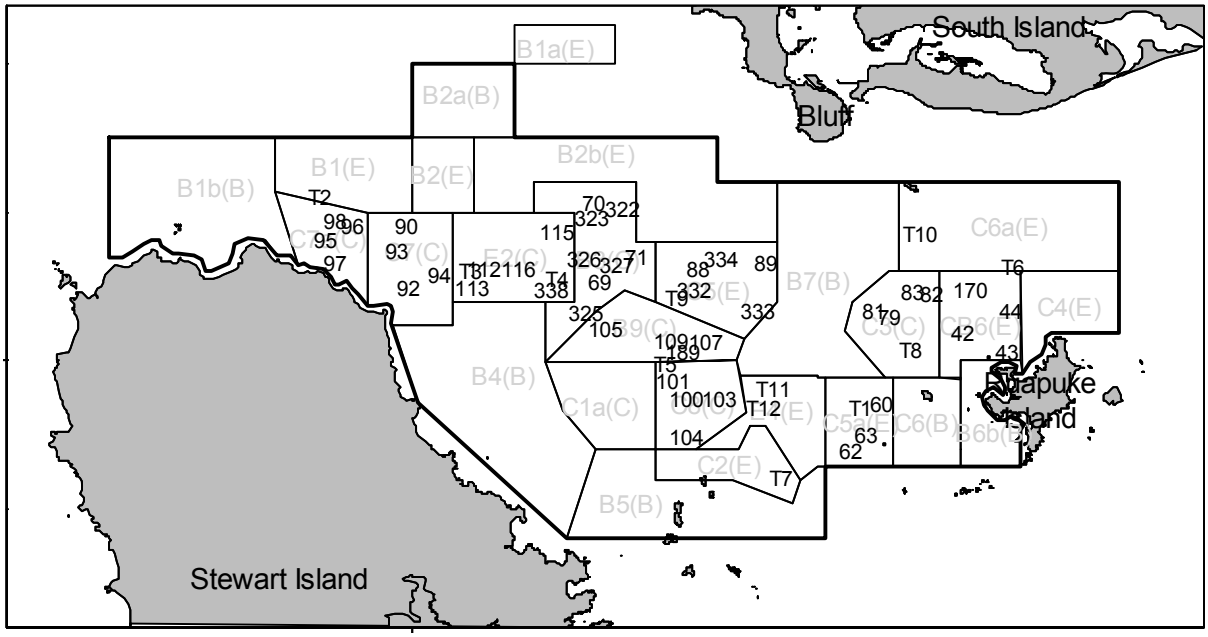

Figure 9: The 2013 survey area with the 2005 survey boundary shown as a heavy, black outer line, and the 2012 survey strata shown as black lines and labelled with grey text. Strata designated commercial in 2013 by oyster boat skippers have a "C" in brackets. Exploratory strata have an "E" in brackets, and background strata "B". B1A is an additional stratum added in 2007 survey. Station numbers shown in black text, target stations are shown with a "T" prefix.

Table 1: The allocation of the numbers of randomly selected stations to each stratum in 2013; rank by stratum area and density, the stratum area, percentage of the total area of the ten strata (% total area), mean density in 2012, coefficient of variation of the density estimate (CV), mean population size of recruit-sized oysters and the numbers of stations sampled in 2012.

Rank	Stratum	Area (km^2)	% total area	No. Stns 2013	Mean density	CV	Mean population	No. Stns 2012
1	B3	44.7	13.4	8	3.56	0.2	158.9	13
2	C5	37.7	11.3	5	1.98	0.27	74.5	8
3	E2	42.8	12.9	5	1.87	0.26	80.3	8
4	C7a	23.6	7.1	4	1.78	0.43	42	4
5	C8	26.8	8.1	4	1.65	0.39	44.3	6
6	C3	32.7	9.8	4	1.44	0.29	47.1	6
7	C5a	23.5	7.1	3	1.34	0.64	31.6	5
8	B6	30	9.0	4	1.15	0.44	34.4	6
9	C9	34.5	10.4	4	1.04	0.47	35.8	6
10	C7	36.1	10.9	4	1.01	0.18	36.4	5

3.2 Operational procedure

Sampling followed similar procedures to surveys in October 2002 (Michael et al. 2004a), February 2003 (Dunn et al. 2003), January 2004 (Michael et al. 2005), January 2005 (Michael et al. 2006), February 2006 (Michael et al. 2008a), February 2007 (Michael et al. 2008b), February 2008 (Michael et al. 2009a), February 2009 (Michael et al. 2009b), February 2010 (Michael et al. 2011), February 2011 (Michael et al. 2012a), and February 2012 (Michael et al. 2013). FV 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). NIWA staff ensured consistency of procedures.

3.3 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.

3.4 Survey tows

When it was possible, 2012 survey tows were repeated in 2013 over the same tow line and in the same direction, and started on station position where possible. Where the start of 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 (370 m), at each station. The start of tow was taken from when the winch brake was applied and tension came on to 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 mile, the end of tow position was taken, the winch brake released, and the dredge hauled aboard without washing.

Tows that could not be dredged because of foul ground were replaced with spare stations (randomly selected from the 2012 tows) in the same 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.

3.5 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 was 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.

New clocks are usually defined in the October surveys, as those shells that have clean inner valves and have retained their lustre without any sign of fouling (fouling organisms are thought to settle over the late spring and summer). 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 10). For this analysis, we assumed that new clocks were only those oysters that have 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 11 and 12). 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 (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.

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 wet in oyster sacks, transferred to poly bins, and flown to NIWA, Wellington, for processing.

Figure 10: New clock (with hinge intact), glossy inner valve with no fouling except a few white coralline specks.

Figure 11: Recent old clock (with hinge intact), glossy inner valve with light fouling.

Figure 12: Old clock with hinge intact. No gloss on inner valve and heavy fouling.

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 (Appendix 2). The presence/absence of bycatch species was also recorded directly from the dredge contents.

3.6 Processing of samples, heart imprints, and histology protocols

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.

Histology sampling methods

Station and sample data were recorded on bonamia sampling forms (Appendix 3), 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 to estimate the prevalence and intensity of bonamia in oysters. 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 13) 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. Oysters were recorded as either incubating white (early-stage) larvae, grey (late-stage) larvae; or with no larva present.

Heart imprints were made by removing the heart (dark organ adjacent to adductor muscle, see Figure 14) 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. A section was taken through the digestive gland (Figure 14) 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 histology 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 96well qPCR plates. Addition samples of gill tissues from the same oysters were placed into new uniquely labelled 96well 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 (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.

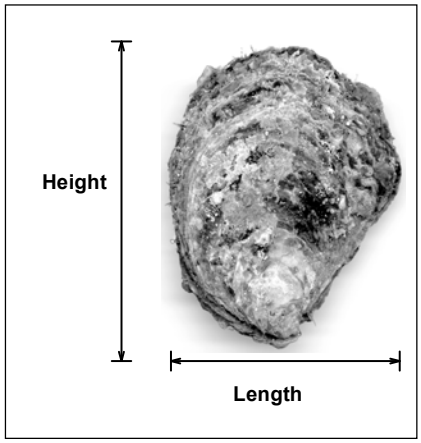

Figure 13: An oyster showing length (anterior-posterior axis) and height (dorsal-ventral axis) dimensions.

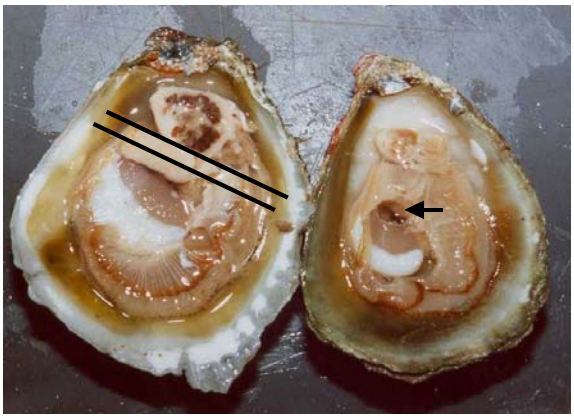

Figure 14: Lines on left oyster show location of 5 mm thick standard section taken for histology. The arrow on the oyster on the right shows the heart, a black organ adjacent to the adductor muscle.

Procedures were implemented to prevent contamination of the qPCR samples. Laboratory staff replaced gloves and rinse solutions 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 -20C in between use. 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 -20C then transferred to a -80C freezer for storage at the end of the day.

3.7 Analysis

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. 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 57 stations, and at least 3 randomly selected samples from each station that tested negative with qPCR. 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. Heart imprints were examined by two readers. A review of scoring protocols was undertaken before screening samples, initial samples were scored together, and thereafter three common stations were read by both readers.

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

We assume that category 0 oysters are not infected. Previous studies (Diggles et al. 2003) 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.

For each station, prevalence is defined as the proportion of oysters in a sample with at least one bonamia cell observed (i.e., the number of stage 1–5 oysters divided by the number of all oysters examined in the sample). Mean intensity is defined as the mean frequency of stages 1–5 oysters (i.e., the mean stage of all oysters examined that had at least one bonamia cell observed). The inclusion of the additional smaller oysters at sites 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 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:

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

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

Analysis of qPCR samples

A detailed account of qPCR methods and testing is given in (Maas et al. 2013). This novel qPCR method has been successfully developed to detect and quantify *Bonamia exitiosa* in *Ostrea chilensis* 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 qPCR data were analysed using BioRad CFX Manager™ software (Version 3.0). The quantification cycle (Cq), the fractional cycle number where fluorescence increases above the threshold was determined by the regression method as implemented in the option using the BioRad software. qPCR data from oyster heart and gill samples were assessed based on the information for each plate contained within the sample sheets, plots of relative fluorescence units (RFU) against Cq values, and Cq values for the positive (Bonamia ITS and β-actin gene) and the negative internal control reactions. Rules are proposed for repeating qPCR reactions for each sample or plate, and the rejection of data from analysis, and these are given in the results section. Decision rules were established to determine whether assays needed to be repeated (see Maas et al. 2013 for details), and there were decision rules to determine whether to omit data from analysis. Data were omitted when:

- Out of range Cq values for the bonamia positive and negative control wells (~ Cq of 28),
- the Bonamia ITS and internal control Cq values were both NAs (there were no values to show the reaction had worked),
- either the Bonamia ITS or internal control (β- actin) amplified very early in the cycles (Cq <10),
- or the internal control (β- actin) Cq values were late (Cq values ≥ 40) together with no Bonamia ITS amplification.

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.

Estimates of oyster and clock densities and population size

The February 2013 survey sampled only10 of the 26 strata from the 2012 stock assessment survey. Three or more randomly selected stations were sampled from those sampled in each stratum in 2012. Estimates of oyster and clock densities were made and compared with comparable strata from previous surveys. We note that these estimates of population abundance are based on a subset of strata and have relatively small numbers of samples, and that there is likely to be some bias as the 2013 stations were randomly selected from the 2012 stations (to provide better comparisons between stations).

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 important to catch rates in the oyster fishery.

Estimates of absolute abundance and variance were calculated using standard stratified random sampling theory (Jolly & Hampton 1990). We assumed a mean dredge efficiency, estimated from the 1990 data, of 0.17 (95% confidence interval 0.13–0.22), 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*. 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. Dredge efficiency for clocks is likely to be much lower and therefore underestimate clock densities.

The 95% confidence intervals of the population means are estimated by bootstrapping using the variance of the population size and the error of the estimated dredge efficiency (Cranfield et al. 1999), both were assumed to be normally distributed. Only the error in the relative population size is required when we compare population estimates between dredge surveys as the error in dredge efficiency cancels out.

The estimates of population size for recruit-sized, pre-recruits, and small oysters, and recruit-sized and pre-recruit new clocks are presented separately. The absolute population size of each size group of oysters was estimated using the combined population sizes in each stratum:

Patterns of recruitment

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 2009, February 2012, and February 2013 surveys.

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 numbers of small oysters per recruit-sized oyster were estimated to investigate trends in recruitment between 2009 and 2013.

Population estimates of bonamia infection

We used bonamia infection data from strata with three or more randomly selected stations only i.e., we did not include target stations. For each station, the total number of oysters in each bonamia infection category (1–5) was calculated based on the estimated proportion of oysters in each infection category in the sample, and scaled to the total catch. The population prevalence was calculated as the ratio of the estimated number of oysters with bonamia (category 1–5 combined) in the population to the total number of oysters (recruit-sized), and the overall intensity was calculated as the average bonamia level in the population. Variance for prevalence and intensity was estimated using standard methods as for population estimates.

Estimates of mortality

Total summer mortality is estimated as the sum of pre-survey mortality estimated from new clocks and gapers; and projections of post-survey mortality from the proportion of the population with category 3 and higher bonamia infections. Pre-survey mortality for the oyster population was estimated as the total, scaled numbers of recruit-sized new clocks and gapers. 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.

The catchability (dredge efficiency) and persistence of new clocks at the location of death varies spatially for new clocks, and their classification as new (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 as new clocks as their burial has preserved the articulation of the hinge and prevented the settlement of 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 separate new and old clocks, and reduce misidentification.

Bonamia studies (Diggles et al. 2003) suggest that category 3 bonamia infections 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, i.e. 1 - 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. Total projected mortality is the difference between the total population size at the time of survey and the population corrected for projected bonamia mortality (at the end of summer).

Pairwise comparisons were made in SIGMAPLOT 12 software by Systat Software Inc) using t tests or Mann-Whitney rank sum test where the data failed the Shapiro-Wilk test for normality.

4. RESULTS

4.1 Survey operational detail

The oyster vessel F V Golden Quest skippered by Stephen Hawke, her crew, and two NIWA staff, successfully sampled 45 stations from the 2012 survey, and 12 target stations. One station (62) couldn't be sampled and was replaced by station 61 in the same stratum. Sampling began on the 6th of February 2013 and was completed on the 10th of February, sampling on all five days over this period. Survey tows completed are shown in Figure 15, and the numbers of repeat and target stations sampled in each stratum are shown in Table 3.

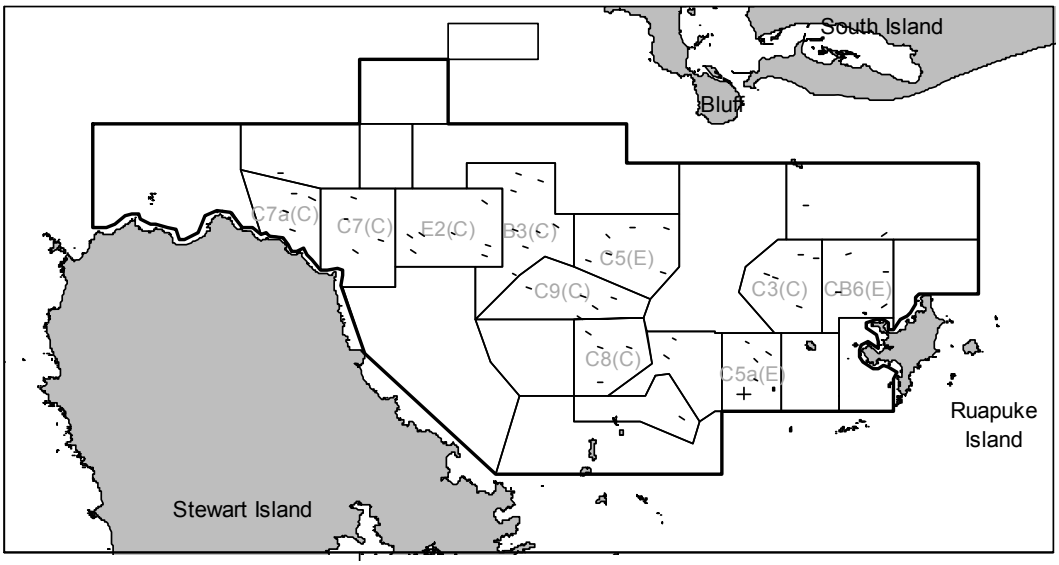

Figure 15: The survey tows (black lines) sampled in February 2013 to determine the status of bonamia infection and oyster density. The station that could not be dredged because of foul ground is shown as a cross. The 2005 survey area is bound by the outer black line and the February 2013 survey strata are bound by the grey lines; stratum B1a was added to the survey area in 2007. The 2013 survey stratum designations are shown in brackets. Commercial strata designated by oyster skippers in 2012 have a "C" suffix; exploratory strata have an "E" suffix, and background strata have a "B" suffix.

Table 3: The numbers of 2012 survey stations random selected and sampled in the February 2013, and the numbers of target stations by stratum.

Stratum	Random	Target	Total	Stratum	Random	Target	Total
B1	0	1	1	C7	4	0	4
B3	8	0	8	C7a	4	0	4
C2	0	1	1	C8	4	1	5
C3	4	1	5	C9	4	0	4
C5	5	1	6	CB6	4	0	4
C5a	3	1	4	E2	5	2	7
C6a	0	2	2	E4	0	2	2
				Total	45	12	57

Samples of up to 30 oysters were collected from all stations to determine the status of bonamia infection. Samples of oysters were also collected for Victoria University studies. Oyster samples were couriered to NIWA, Greta Point (Wellington) where they were processed for heart imprints and qPCR. Oyster tissues were also taken for histology and these were archived for future research.

Survey comparability

Dredge tow lengths were almost all about 0.2 nautical miles (371 m) in length (Figure 16). 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 and the remainder in moderate to rough sea conditions (Figure 17). The median wind force was 3 on the Beaufort scale (7–10 knots), with 5 and 95 percentiles of Beaufort scale 1 (1–2 knots) and 4 (11–15 knots) respectively, and 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.

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. In previous surveys, dredges have occasionally be landed more than 70% full and their dredge contents were unevenly, but symmetrically spread with contents lower in the middle of the dredge that at the edges. These observations and anecdotal evidence from video data recorded from dredge trials suggest that dredge saturation may occur below 80% dredge fullness. No adjustment of oyster density data is made for dredges considered to have become saturated during the tow.

No dredge was landed 80% full in 2013. Dredge fullness ranged from 1% to 70% with a median fullness of 30%, suggesting dredge saturation is not likely to have had a large effect on sampling effectiveness and the survey (Figure 18).

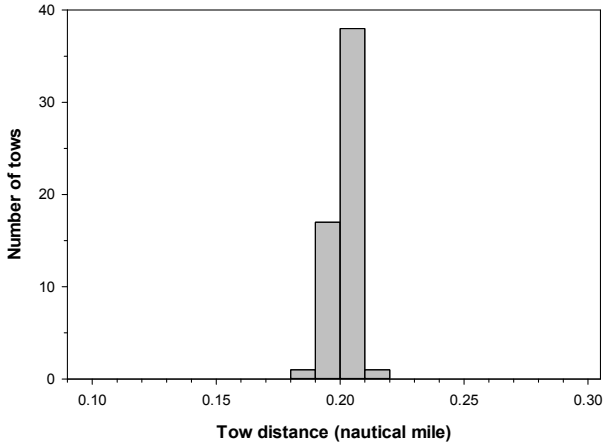

Figure 16: Distribution of tow lengths from the February 2013 survey. The standard tow length was 0.2 nautical mile (371 m).

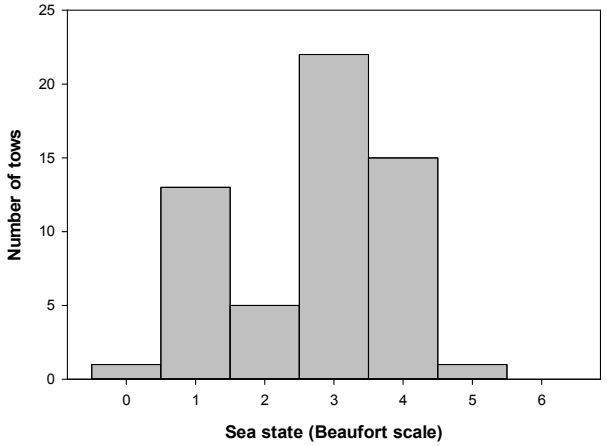

Figure 17: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2013. Beaufort scale: 0, < 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

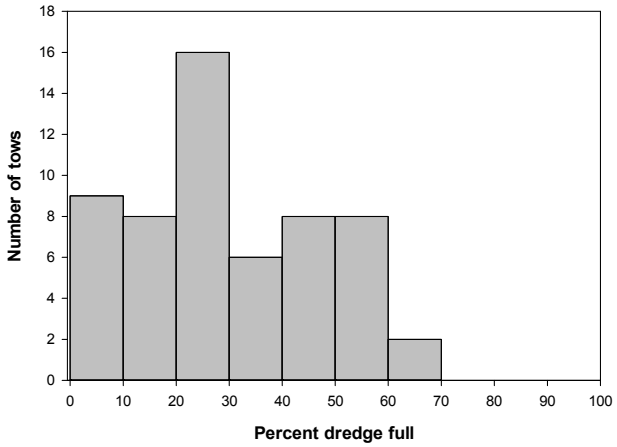

Figure 18: Distribution of dredge fullness recorded for survey tows in February 2013. No tows were landed with a dredge fullness of greater than 80%, suggesting that it may have saturated before the end of the tow leading to an underestimate of oyster density. Unpublished video data suggests that dredge saturation may occur below 80% full.

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 2013 survey.

Recruitment to the commercial fishery areas surveyed appears to be variable in 2013 with about one quarter of the stations surveyed showing significant increases in dredge catches of recruit-sized oysters, about half showing no changes, and less than a quarter showing some decline. These increases and declines showed no clear spatial pattern over the fishery area surveyed and high variability over small-spatial scales. Most stations sampled in 2013 showed decreases in the numbers of pre-recruit sized and small oysters, suggesting continued poor settlement and survival of oyster spat, and this trend is consistent with both spat monitoring and the numbers of spat and wings on oysters sampled from the commercial catch.

4.2 Estimates of oyster mortality before and during the February 2013 survey

New clock numbers sampled at the 57 paired stations in 2012 and 2013 showed similar patterns of pre-survey mortality between years and across the fishery (Figure 19). The numbers of recruit-sized new clocks (Figure 19, panel A) were generally higher in February 2013 than in February 2012, but the percentage of new clocks or pre-survey mortality (the percentage of recruit-sized new clocks to recruit-sized new clocks and live oysters combined) was not significantly different (Mann-Whitney rank sum test, $P = 0.124$), although some individual stations showed marked changes in the numbers of clocks between 2012 and 2013 (Figure 19, panel B). The numbers of pre-recruit sized new clocks were low in both 2012 and 2013 (Figure 19, panel C), probably as a result of the low population sizes of pre-recruit oysters over the period. The pre-survey mortality of pre-recruit sized new clocks was significantly higher (Mann-Whitney rank sum test, $P = 0.009$) in 2013 than in 2012 (Figure 19, panel D).

Descriptive statistics for new clocks sampled at the paired stations in 2012 and 2013 are given in Table 4. The median percentage new clocks for both recruit-sized and pre-recruits was higher in 2013 than in 2012, and the numbers of stations with no new clocks was the same for recruit sized new clocks, but lower in 2013 than in 2012 for pre-recruit new clocks. These data suggest that bonamia mortality may be increasing and spreading.

Table 4: Descriptive statistics for the percentages of new clocks sampled at the 57 paired stations in 2012 and 2013. Recruit-sized new clocks (nc.r) and pre-recruits (nc.pr).

Percentage new clocks	2013		2012	
	nc.r	nc.pr	nc.r	nc.pr
Median	3.4	3.2	2.3	1.8
Min	0	0	0.0	0.0
Max	21.1	33.3	28.9	11.7
Lower 5th percentile	0	0	0.0	0.0
Upper 95th percentile	11.7	11.6	10.4	10.6
No. stations with no new clocks	5	8	5	13

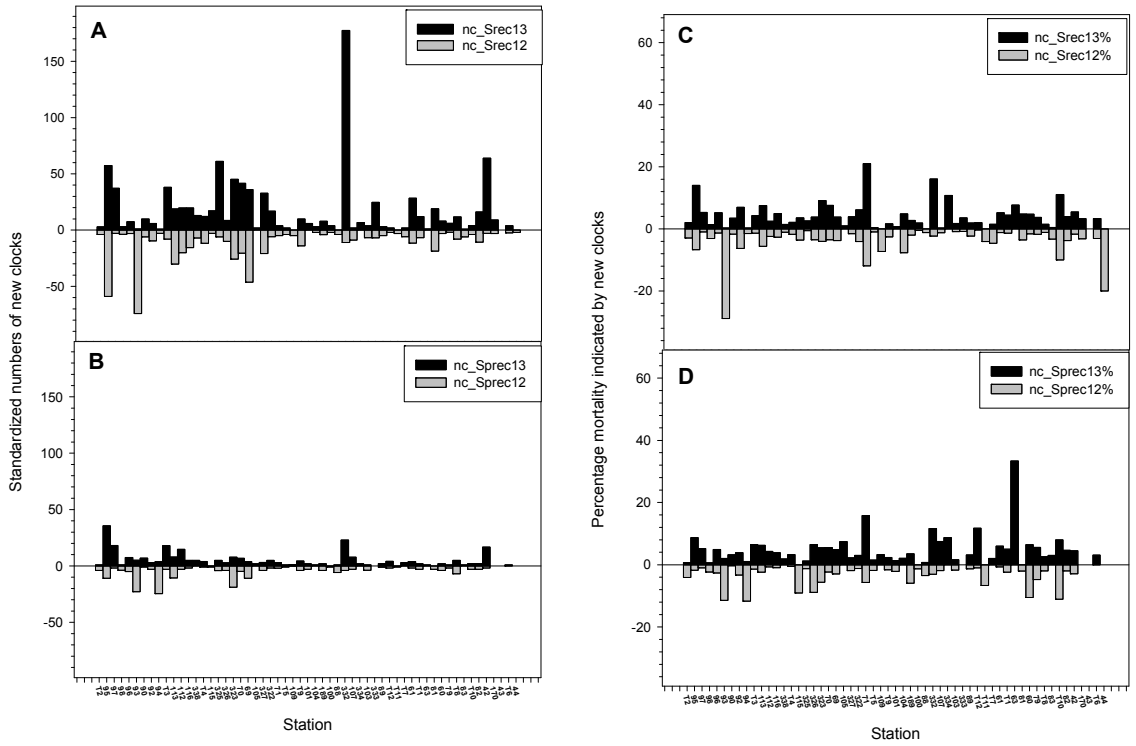

Figure 19: The numbers of new clocks standardized to a standard tow length of 0.2 nautical miles by station (west to east). The numbers of recruit-sized new clocks (nc_Srec12 and nc_Srec13) for 2012 and 2013 respectively are shown in panel A (top left), and pre-recruits (nc_Sprec12 and nc_Sprec13) in panel B (top right). The percentage new clocks (the percentage of recruit-sized new clocks to recruit-sized new clocks and live oysters combined) or pre-survey mortality are shown in panels C and D. The percentage recruit-sized new clocks (panel C lower left, nc_Srec12% and nc_Srec13%) and pre-recruits (panel D lower right, nc_Sprec12% and nc_Sprec13%) are also given for 2012 and 2013 respectively.

Similar numbers of gapers were observed between 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.

All stations sampled during 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 related to higher recruit-sized oyster densities found in strata mostly designated as commercial (E2, B3, C3, CB6, C7 , and C7a) (Figure 20). Figure 21 shows these data from the 2012 survey, and only for stations sampled in 2013. The 2013 survey shows a similar pattern of distribution (Figure 22), 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.

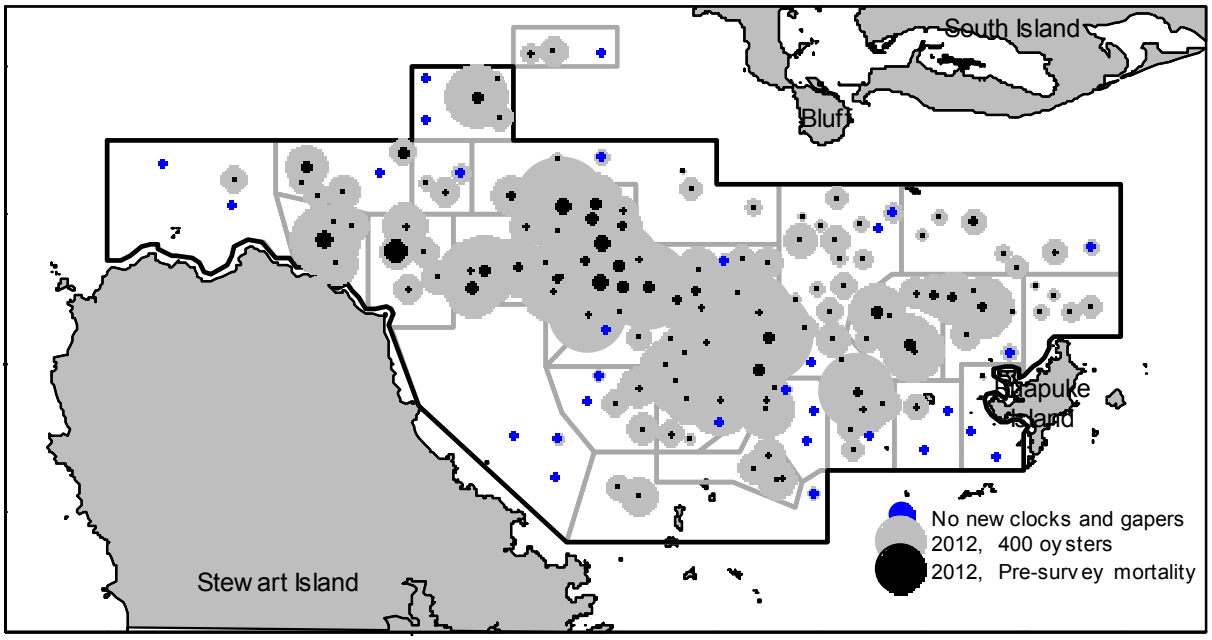

Figure 20: The distribution of recruit-sized oysters, new clocks, and gaper densities combined (filled grey circles) and the densities of recruit-sized new clocks and gapers combined (black circles) showing the pre-survey mortality in February 2012. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

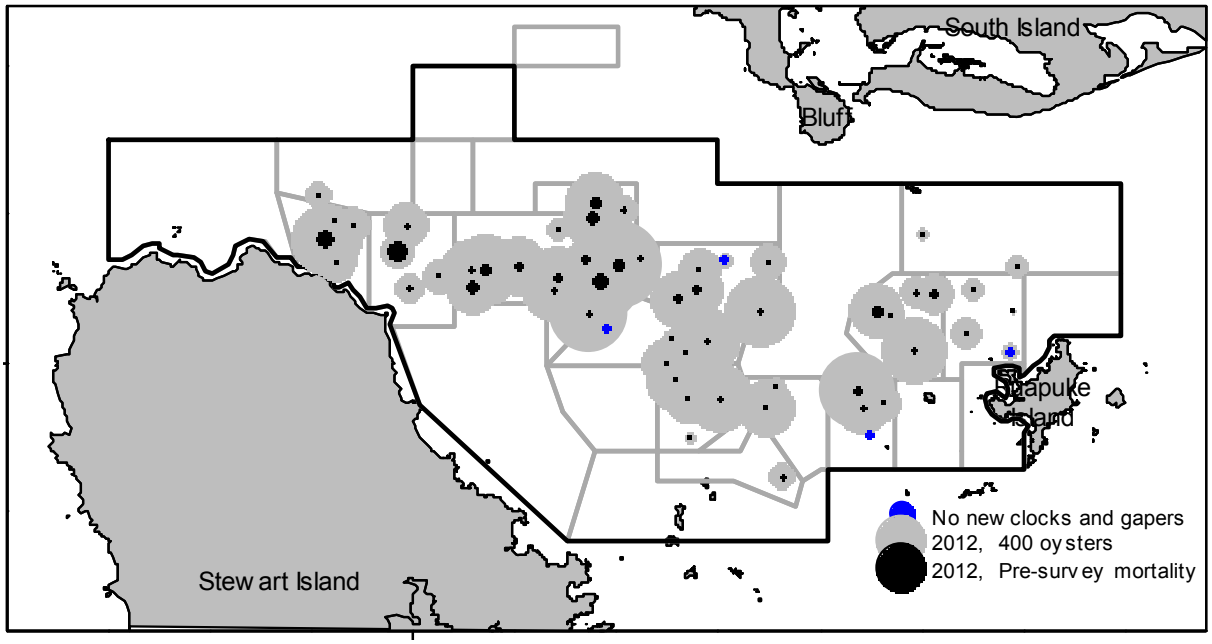

Figure 21: The distribution of recruit-sized oysters, new clocks, and gaper densities combined (filled grey circles) and the densities of recruit-sized new clocks and gapers combined (black circles) showing the pre-survey mortality at a subset of stations from the February 2012 survey, sampled in February 2013. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

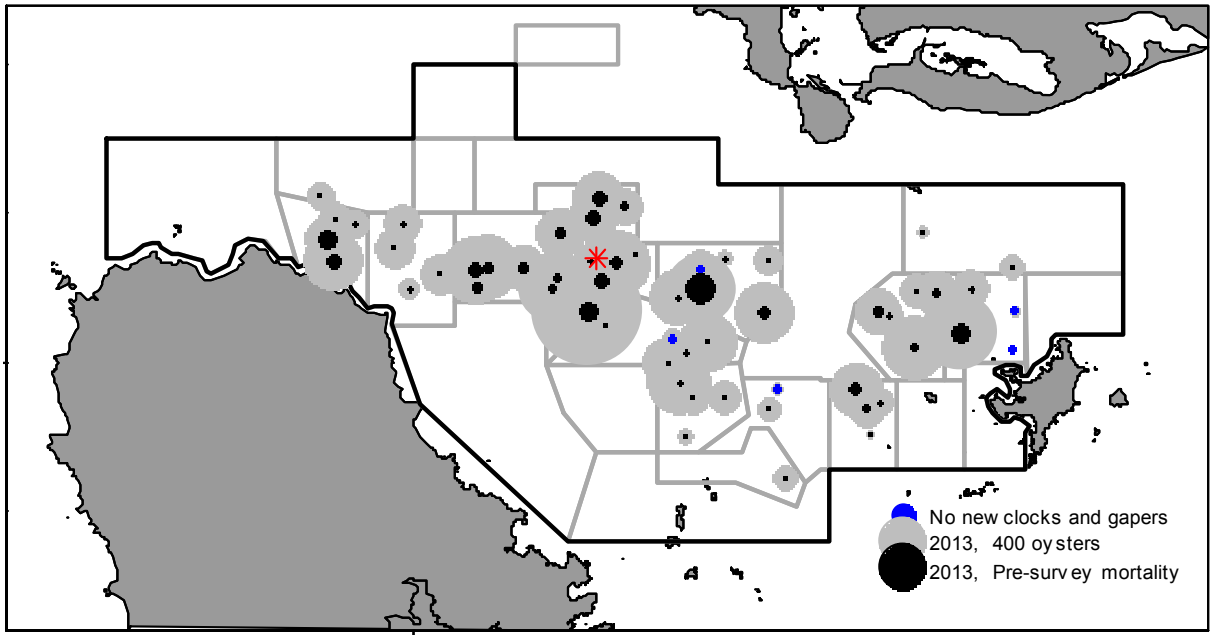

Figure 22: The distribution of recruit-sized oysters, new clocks, and gaper densities combined (filled grey circles) and the densities of recruit-sized new clocks and gapers combined (black circles) showing the pre-survey mortality in February 2013. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles. The red asterisk denotes the locations where the 1986 and 2000 bonamia epizootics are thought to have begun.

Estimates of recruit-sized new clocks sampled at randomly selected stations from common strata in 2012 and 2013 are shown in Table 5. Pre-survey mortality is estimated to be higher in 2013, 29.0 million new clocks (CV of 25%, 95% CI 134–49.4) than for comparable stations and strata in 2012, 17.4 million new clocks (CV of 22%, 95% CI 8.9–29.4), and higher for all stations sampled in 2012 in comparable strata 17.7 million new clocks (CV of 19%, 95% CI 9.5–28.2).

Recruit-sized new clock densities were more variable in 2013 than those in 2012. Strata B3 and C7 had the highest mean density of recruit-sized new clocks in 2012 and were also high in 2013. Stratum C3 had the highest densities of new clocks in 2013 (Table 5), suggesting relatively high mortality in eastern fishery areas.

Table 5: Recruit-sized new clocks estimated from randomly selected stations from common strata in 2012 and 2013. Population size (Popn.) is given in millions of new clocks, coefficient of variation (CV), upper and lower 95% confidence limits (Lower 95%C I and upper 95%CI) and the area of each stratum.

Recruit-sized new clocks			2012–all stns					2012–2013 stns					2013			
				Lower	Upper				Lower	Upper				Lower	Upper	Area
Stratum	Density	CV	Pop.n	95%CI	95%CI	Density	cv	Pop.n	95%CI	95%CI	Density	CV	Pop.n	95%CI	95%CI	(km^2)
B3	0.11	0.20	4.7	2.6	7.8	0.09	0.29	3.9	1.6	6.9	0.15	0.22	6.7	3.4	11.4	44.7
B6	0.03	0.45	0.9	0.1	1.9	0.01	0.35	0.3	0.1	0.6	0.05	0.40	1.7	0.4	3.4	32.7
C3	0.05	0.31	1.6	0.6	2.8	0.05	0.39	1.5	0.3	2.9	0.21	0.80	7.8	0.0	21.8	37.7
C5	0.02	0.27	0.8	0.4	1.5	0.03	0.34	1.0	0.3	1.9	0.06	0.66	1.4	0.0	3.7	23.5
C5a	0.02	0.51	0.5	0.0	1.0	0.02	0.72	0.6	0.0	1.5	0.02	0.48	0.8	0.1	1.7	36.1
C7	0.10	0.72	3.5	0.0	9.1	0.12	0.74	4.2	0.0	11.1	0.13	0.49	3.0	0.1	6.6	23.6
C7a	0.08	0.81	2.0	0.0	5.6	0.08	0.81	2.0	0.0	5.6	0.02	0.15	0.6	0.3	0.9	26.8
C8	0.02	0.45	0.4	0.1	0.9	0.01	0.54	0.4	0.0	0.8	0.01	0.57	0.5	0.0	1.2	34.5
C9	0.02	0.31	0.7	0.2	1.2	0.02	0.41	0.8	0.1	1.5	0.09	0.85	2.7	0.0	7.7	30.0
E2	0.06	0.31	2.4	0.9	4.4	0.07	0.32	3.2	1.1	5.9	0.09	0.07	3.7	2.5	5.6	42.8
All	0.05	0.19	17.4	9.5	28.2	0.05	0.22	17.7	8.9	29.4	0.09	0.25	29.0	13.4	49.4	332.4

4.3 Estimates of post–survey mortality from bonamia

Sampling effectiveness for the prevalence and intensity of infection by bonamia

In 2013, samples of 25 oysters were collected from all but one station (Stn 63) totalling 1422 samples of heart imprint slides and heart and gill tissue samples for qPCR. This sample comprised 1383 recruits, 23 pre-recruits, and 16 small oysters. Almost all of the samples (97%) were of recruit sized oysters, similar to previous surveys. Five stations had less than 25 recruit sized oysters: station 77 had mainly pre-recruit sized oysters; at stations 43 and 63, about one third of the samples were small oysters, and stations 44 and 79 had a few pre-recruit sized oysters in their samples.

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.

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

In 2013, oyster samples were initially tested for bonamia using qPCR (Maas et al. 2013). The 96 well plate format and the need to run controls only allowed 24 samples from each station to be run in the initial analysis. Some samples that showed anomalies in the qPCR data were rerun, and samples that failed a second assay were omitted from the data analysis (Table 6).

All matching heart imprint slides for those samples that tested positive for bonamia infection in either heart or gill samples were examined (Table 6). At least three heart imprint samples that were qPCR negative were randomly selected from each station, and all samples for the 25th heart imprint slide from each station (for which there are no qPCR data) were also examined (Table 6). Of these 56 samples, three tested positive for bonamia (stations 109 (intensity of infection 2), 325 (4), and 332 (3)). Details are given in Appendix 3. Heart imprint slides were scored by a single, experienced reader.

Table 6: A summary of samples screened for bonamia. qPCR samples were run first on the first 24 of 25 oyster samples taken and the total numbers of tissue from matches, the numbers that were qPCR positive, numbers negative, and the numbers of qPCR samples where their data was omitted from analysis because they failed inclusion criteria after repeat sampling. The total number of histology slides sampled, the numbers score for bonamia infection based on their qPCR classification, and the numbers of those samples that were positive are also shown.

qPCR samples

Bonamia infection	**Sample (N)**	**Omitted**	**qPCR.N**	**Positive <35Cq)**	**Negative (>35 Cq)**
Heart	1365	55	1310	258	1052
Gill	1366	96	1270	388	882
Both H&G				215	

Histology samples

Number of slides	1422

qPCR infection	**Sample (N)**	**Histo+ve**
qPCR +ve	431	169
Heart qPCR +ve	258	169
Gill qPCR +ve	388	158
Selection qPCR -ve	165	0
all qPCR	631	169

Comparison of qPCR and heart imprint methods.

The qPCR method showed higher sensitivity in the detection of bonamia than heart imprints (Maas et al. 2013). More 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 23), which may either mean they are more sensitive (provide for better amplification) and or they are amplifying external contamination of gill tissue by water-borne bonamia particles. Heart tissues may provide better estimates of oyster infection and gill tissues better estimates of pathogen presence in the environment.

The quantification of bonamia cannot be directly compared between qPCR and histology as the qPCR Cq values estimate numbers of bonamia ITS region copies and histology scores categorise the average numbers of bonamia cells in oyster haemocytes. Of the heart imprint samples that were positive for bonamia infection, all but one of the Cq values from matched qPCR heart and gill samples were positive (below the 35 Cq cut-off). A boxplot 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 23).

There were a number of outliers in the boxplots of Cq values of heart and gill tissues by histological score (1−5). Outlier values greater than the medians may be the result of inhibition of the qPCR reaction, 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 23). Outlier values lower than the medians suggest that those individual tissues may have been more heavily infected than determined by heart imprints.

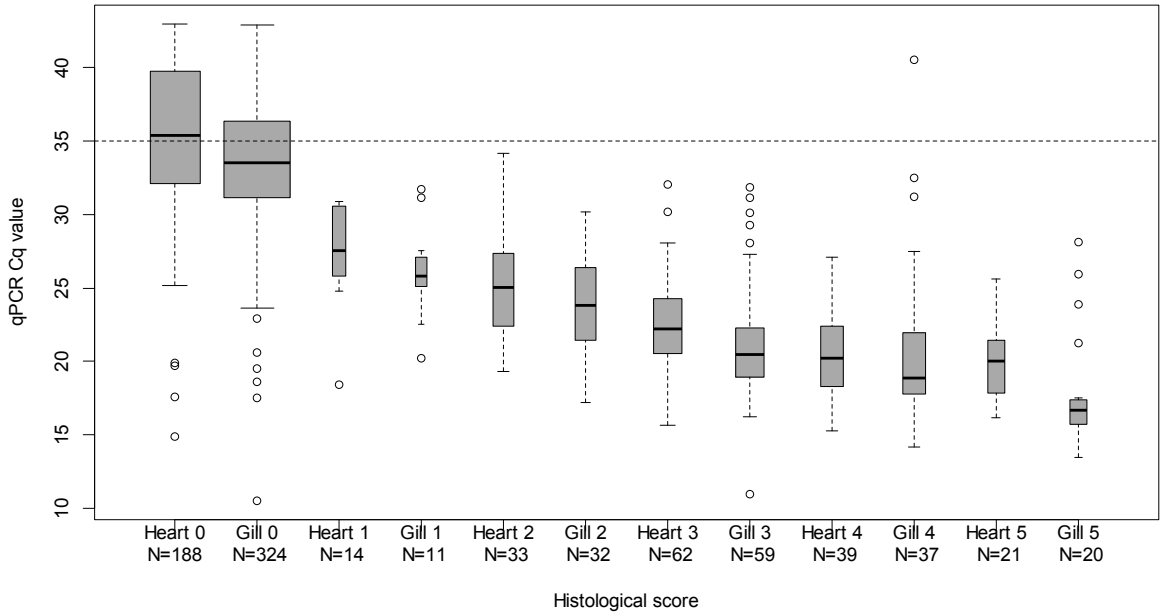

Figure 23: Boxplots of Cq values from qPCR analysis of the for bonamia ITS region for paired samples of heart and gill tissues by histological score. Cut-off levels set at 35 Cq (dashed line). Matching heart tissue sample for the outlier for gill 4 was strongly positive suggesting a reaction problem 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.

4.4 Prevalence and intensity of infection in oysters by bonamia

The prevalence and intensity of bonamia infection in February 2013

Median prevalence differed with the sampling method and tissue used to detect bonamia. Heart imprints are known to underestimate true prevalence, and the mean prevalence from heart imprints was 12.1% (N = 1422, 95% CI 0–32.8, median = 8.0%); similar to previous February surveys. 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 19.6% (N = 1310, 95% CI 0–55.7, median = 13.6%) and mean prevalence from qPCR analysis of gill tissues was 30.5% (N = 1270, 95% CI 3.5–71.3, median = 26.1%). We cannot rule out external contamination of gill tissues by water borne bonamia particle, especially at this time of year when disease mortality is highest.

Percentages of oysters infected indicated by heart imprints in 2013 were similar to 2010, 2011 and 2012; and of the 1422 oysters examined for bonamia in 2013, 88% had no detectable infection, similar to the 90%, 88%, and 89% in 2010, 2011, and 2012 respectively. Of the remaining 12% of oysters with detectable infections in 2013, 3% had light category 1 and 2 infections (3% in 2012 and 2011, and 4% in 2010), and 9% had category 3 and higher infections (8% in 2012, 7% in 2011, and 8% in 2010) that are normally fatal.

The prevalence of infection ranged from 4% to 44% in 2013, the same as in 2012. Peak prevalence was lower in 2012 and 2013 (44%) than in 2011 (52%). The median prevalence in 2013 was 8%, higher than in 2012 (5%) and the same as in 2011 (8%), but the mean prevalence in 2013 (12.1%) across all stations was similar, 10% in 2012 and 2011, and 12% in 2010. The percentage of stations with no detectable infection was lower than in previous years (11% in 2013), compared with 20% in 2012, 31% in 2011, 28% in 2010, and 29% in 2009. This suggests an increasing spread of infection, especially when only bonamia surveys that sampled similar areas are taken into account (2011 and 2010). In 2013, the percentage of stations with no detectable infection with qPCR analysis of heart tissues and gill tissues was 9% and 5% respectively.

The percentage of stations with prevalence less than 10% was lower in 2013, 44% compared with 58%, 28%, 30%, and 37% in 2012, 2011, 2010, and 2009 respectively. The percentage of stations with a 10–20% prevalence was 30% in 2013, similar to the 29% in 2012, and compared with 14%, 26%, and 27% in 2011, 2010, and 2009 respectively. The percentage of stations with more than 20% prevalence was 16% in 2013, lower than in 2012 (26%), and compared with 15%, 16%, and 8% in 2011, 2010, and 2009 respectively.

Intensity of infection has been determined from heart imprints only to maintain the time series of bonamia survey data. The median infection was category 3.0 (N = 1422, 95% CI 2.0–4.8). Infection levels were generally high with 50% or more of infected oysters expected to die within a few weeks.

The mean intensity of infection (3.1) was similar for the years 2009–2012. The proportion of stations with category 3 and higher infections increased from 74% in 2009 to 94% in 2010, decreased to 86% in 2011, decreased further to 67% in 2012, but increased to 81% in 2013. The intensity of infection was highly variable within stations, and patterns of variation were similar across the fishery area, in all years.

Details of recruit-sized oysters and densities by station, and bonamia infection status from histology and heart and gill tissue qPCR samples are shown in Table 7.

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. The median of prevalence is generally low (4−8%), but the upper 50% percentiles have a higher spread of infection, with a mean prevalence between about 7% and 13%. There were fewer stations with higher

prevalence between 2007 and 2009, and an increase in 2010 that has remained constant in 2011 and 2013 (Figure 24). The range of mean intensity of infection (stations with bonamia infection only) is also similar, showing no inter-annual trends. The mean and median intensity of infection is 3, showing that half the stations each year have some fatal infections. The numbers of stations with a mean intensity of infection of 3 and greater increased between 2007 and 2009, dropped in 2010, increased from 2011 and has remained similar since (Figure 24).
The trend in the percentage of stations with no detectable infection decreased between 2007 and 2010, and slightly increased in 2011, and remained at a similar level until 2012, but has dropped to a six year low in 2013 (Figure 25). The mean percentage prevalence of infection by station has been generally low between 2007 and 2013. The percentage of stations with a mean prevalence greater than 20% was high in 2007, declined in 2008 and 2009, increased in 2010, declined in 2011, and increased in 2012 and has remained similar in 2013 (Figure 25). The pattern of percentage mean prevalence of infection across all stations in February 2013 shows an increase in the numbers of stations with low prevalence. Even at higher percentage prevalence in recent years, the fishery has continued to rebuild.

Mean intensity of infection at stations with infection has been generally high since February 2007 (Figure 26), and in 2013 there were more stations with a high mean intensity of infection. The differences in mean intensity between February 2007 and 2013 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.

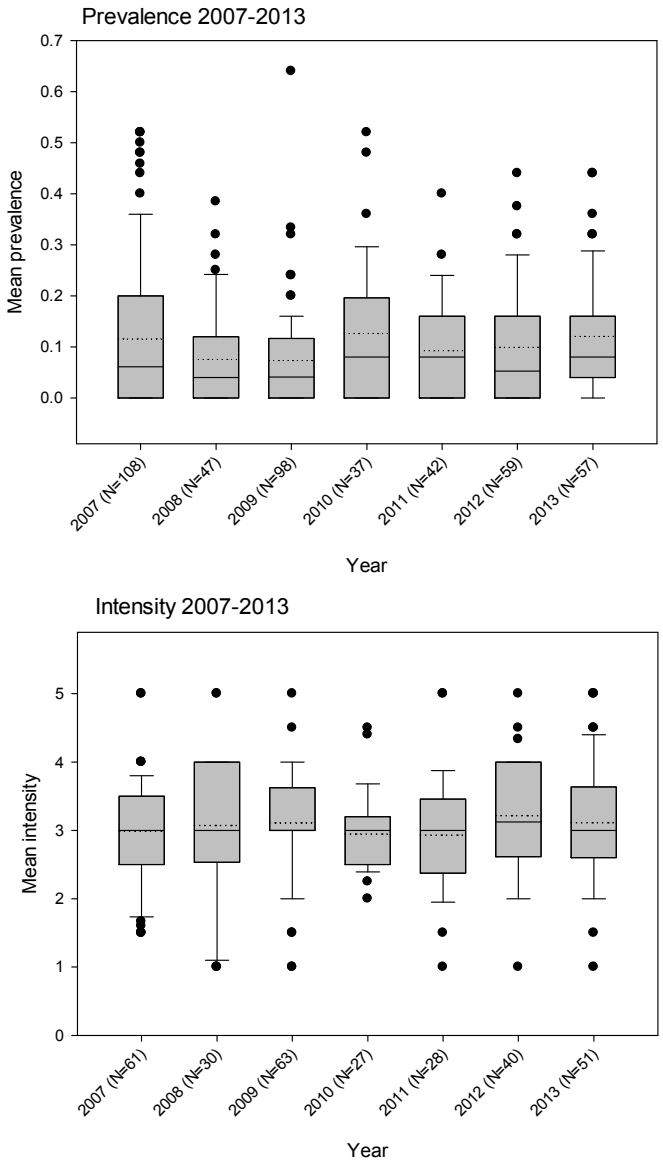

Figure 24: Boxplots of infection status by bonamia 2007–2013. The mean prevalence of infection at all stations (top), and the mean intensity of infection from those stations that had some infection (bottom). Medians shown as solid lines, means as dotted lines, boxes represent 50 percentiles and whiskers 75 percentiles, and outliers as filled circles.

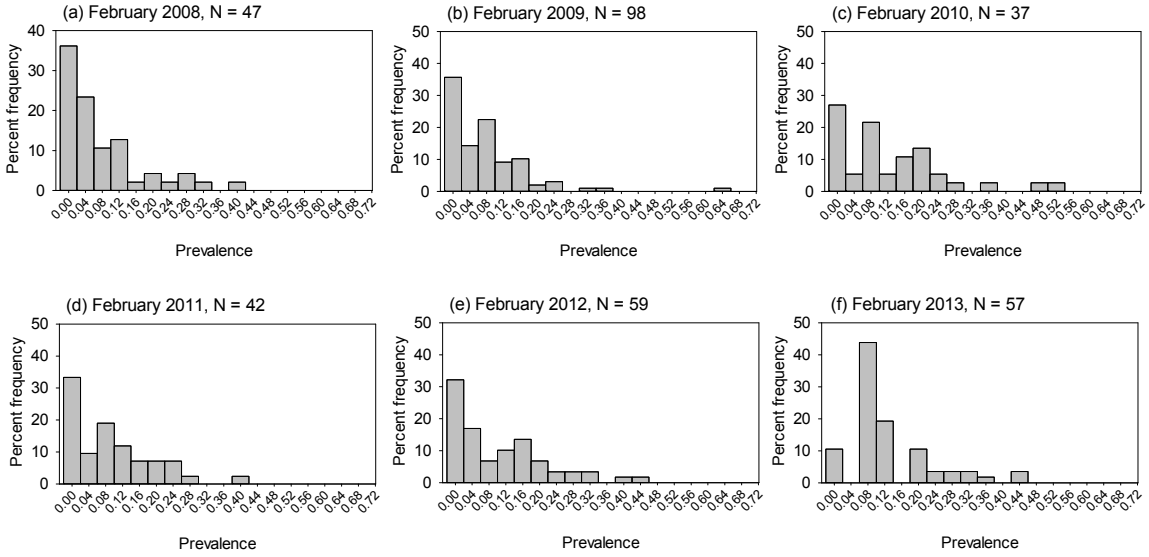

Figure 25: Percentage prevalence of bonamia infection at sites sampled in (a) February 2008, (b) February 2009, (c) February 2010, (d) February 2011, (e) February 2012 (f) February 2013.

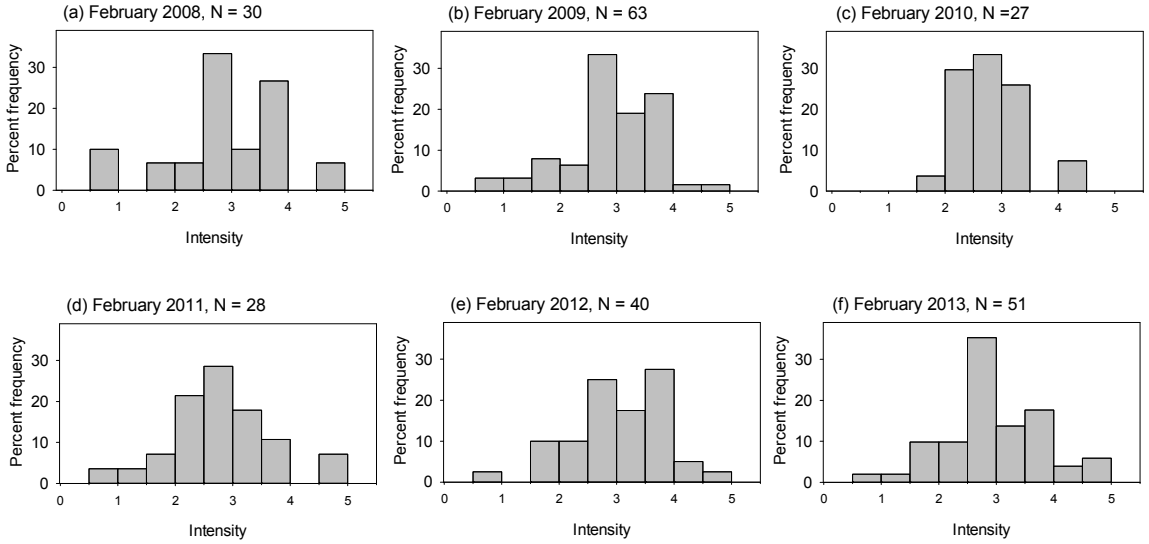

Figure 26: Percentage mean intensity of bonamia infection at sites sampled in (a) February 2008, (b) February 2009, (c) February 2010, (d) February 2011, (e) February 2012 (f) February 2013.

Table 7: Details of recruit-sized oysters and densities by station; the numbers of histology samples (heart imprint slides) and numbers of uninfected (Un.inf) samples, samples with non-fatal infections (NF.inf) and fatal infections (Fatal.inf) based on category 3 and higher infectives, and the prevalence and intensity of infection from heart imprints. The numbers of heart (Heart.No.) and gill (Gill.No) tissues where qPCR assays meet criteria for data inclusion and the prevalence of bonamia infection detected in heart (Prev.H (%)) and gill (Prev.G (%)) tissues.

			Histology						qPCR assays			
Station	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	Prev (%)	Intesity	Heart.No.	Prev.H (%)	Gill.No	Prev.G (%)
42	1131	3.00	25	23	0	2	8.0	3.5	24	37.5	23	95.7
43	16	0.04	25	25	0	0	0.0	-	21	4.8	19	5.3
44	20	0.05	25	25	0	0	0.0	-	23	0.0	22	31.8
60	160	0.43	25	24	0	1	4.0	4.0	24	8.3	23	8.7
61	525	1.39	25	21	1	3	16.0	3.5	23	21.7	23	60.9
63	12	0.03	22	21	0	1	4.5	5.0	22	4.5	20	15.0
69	928	2.44	25	22	0	3	12.0	3.7	24	16.7	23	26.1
70	526	1.37	25	22	1	2	12.0	2.7	22	22.7	22	22.7
71	15	0.04	25	22	0	3	12.0	3.7	24	25.0	23	39.1
79	156	0.42	25	23	2	0	8.0	2.0	24	12.5	23	13.0
81	377	1.01	25	20	1	4	20.0	3.0	24	25.0	23	52.2
82	389	1.06	25	23	1	1	8.0	3.0	24	8.3	23	39.1
83	217	0.58	25	25	0	0	0.0	-	21	0.0	20	40.0
88	293	0.78	25	22	1	2	12.0	3.3	23	13.0	24	16.7
89	156	0.42	25	24	0	1	4.0	3.0	22	9.1	22	18.2
90	280	0.75	25	19	0	6	24.0	4.0	24	33.3	23	47.8
92	80	0.21	25	16	3	6	36.0	3.1	24	62.5	23	69.6
93	321	0.88	25	23	1	1	8.0	2.5	18	33.3	23	21.7
94	330	0.86	25	17	1	7	32.0	3.1	24	33.3	23	43.5
95	356	0.95	25	18	1	6	28.0	3.6	24	29.2	23	47.8
96	219	0.37	25	23	1	1	8.0	2.5	22	13.6	23	26.1
97	657	1.78	25	23	1	1	8.0	2.5	24	8.3	23	17.4
98	215	0.58	25	20	2	3	20.0	2.6	22	31.8	19	57.9
100	207	0.55	25	24	0	1	4.0	3.0	17	11.8	17	5.9
101	795	2.10	25	23	1	1	8.0	2.0	24	8.3	23	17.4
103	225	0.60	25	22	0	3	12.0	4.0	24	29.2	23	34.8
104	59	0.16	25	21	1	3	16.0	3.0	24	20.8	23	34.8

			Histology						qPCR assays			
Station	**Recruits**	**Density**	**Total**	**Un.inf**	**NF.inf**	**Fatal.inf**	**Prev (%)**	**Intesity**	**Heart.No.**	**Prev.H (%)**	**Gill.No**	**Prev.G (%)**
105	202	0.54	25	23	0	2	8.0	4.5	24	12.5	19	21.1
107	614	1.63	25	24	1	0	4.0	2.0	23	8.7	23	4.3
109	96	0.25	25	22	1	2	12.0	2.7	24	8.3	23	13.0
112	790	2.10	25	19	2	4	24.0	3.0	23	47.8	21	57.1
113	234	0.63	25	14	3	8	44.0	2.8	23	69.6	22	81.8
115	489	1.25	25	21	2	2	16.0	3.0	21	23.8	23	30.4
116	382	1.02	25	18	4	3	28.0	2.4	24	37.5	22	36.4
170	264	0.72	25	23	0	2	8.0	3.0	24	16.7	22	31.8
189	283	0.76	25	22	2	1	12.0	2.0	24	12.5	23	17.4
322	259	0.70	25	23	1	1	8.0	3.0	24	16.7	23	26.1
323	457	1.21	25	14	2	9	44.0	3.6	24	54.2	23	78.3
325	2336	6.30	25	22	0	3	12.0	3.7	23	8.7	23	17.4
326	227	0.59	25	23	1	1	8.0	2.0	24	20.8	24	29.2
327	782	2.16	25	22	1	2	12.0	3.3	24	20.8	23	26.1
332	930	2.50	25	23	0	2	8.0	3.0	21	9.5	21	14.3
333	674	1.79	25	23	2	0	8.0	1.5	23	8.7	23	13.0
334	58	0.15	25	25	0	0	0.0	-	24	0.0	23	0.0
338	880	2.37	25	25	0	0	0.0	-	24	0.0	22	0.0
T1	257	0.69	25	21	0	4	16.0	3.8	24	20.8	22	27.3
T2	142	0.38	25	24	0	1	4.0	5.0	19	10.5	24	20.8
T3	842	2.27	25	22	1	2	12.0	3.3	22	22.7	22	31.8
T4	555	1.50	25	24	0	1	4.0	3.0	22	9.1	23	17.4
T5	500	1.35	25	25	0	0	0.0	-	23	0.0	23	0.0
T6	115	0.31	25	24	0	1	4.0	5.0	24	12.5	22	18.2
T7	131	0.35	25	17	3	5	32.0	2.9	21	61.9	21	52.4
T8	799	2.11	25	23	0	2	8.0	4.5	23	13.0	23	60.9
T9	531	1.57	25	22	1	2	12.0	3.0	24	25.0	22	31.8
T10	32	0.09	25	24	0	1	4.0	3.0	24	12.5	23	26.1
T11	35	0.10	25	24	1	0	4.0	1.0	24	8.3	22	9.1
T12	98	0.27	25	23	1	1	8.0	2.5	23	21.7	21	33.3

Changes in the distribution of prevalence and intensity of bonamia infection

The distribution of the prevalence of bonamia estimated by heart imprints, and qPCR analysis of heart and gill tissues (Figure 27) shows similar patterns of distribution with the qPCR heart tissues showing higher sensitivity than heart imprints, and the qPCR gill tissues showing the highest sensitivity. Gill tissues are detecting bonamia at sites where there was no bonamia detected by either heart imprints or qPCR heart tissues; and qPCR gill tissues show much higher prevalence in eastern fishery areas than western fishery areas, possibly as a result of external contamination of gill tissue by water-borne bonamia particles, suggesting that there could be higher levels of infection in eastern fishery areas (Figure 27).

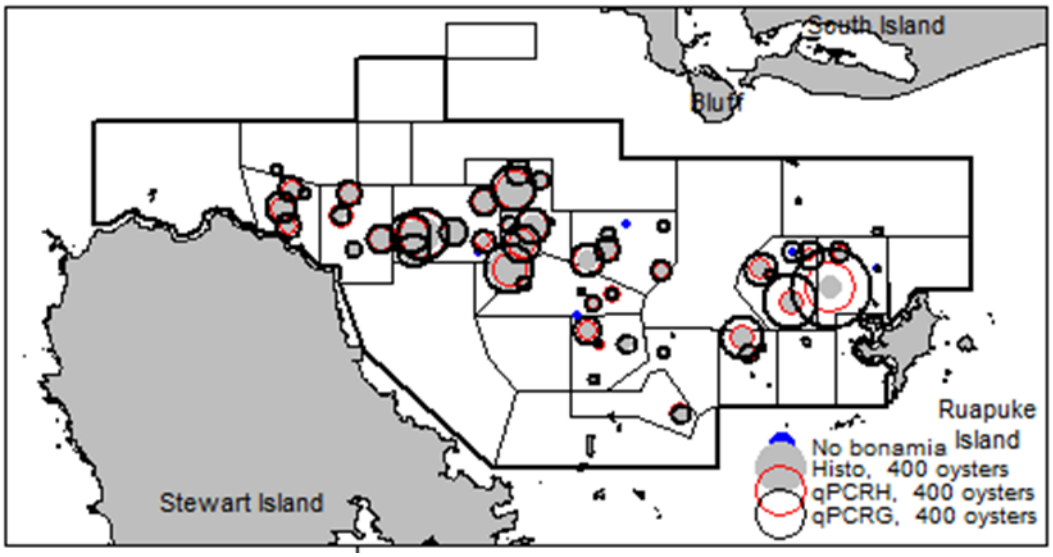

Figure 27: The distributions of bonamia infection in February 2013 estimated from heart imprints, and qPCR analysis of heart and gill tissues. Numbers of oysters with bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles), qPCR heart tissues (qPCRH, open red circles), and qPCR gill tissues (qPCRG, open black circles). Stations with no bonamia (filled blue circles). The 2005 survey area (black outer line) and the February 2012 survey strata (black lines).

During biennial stock assessment surveys (2007, 2009, and 2012) sampling is widespread throughout the fishery area, and in the years between stock assessments, sampling is limited to commercial fishery areas only (Michael et al. 2013). In February 2009, the prevalence of infection had declined in eastern fishery areas and had begun to increase in southern and western fishery areas where oyster density had also increased. Bonamia infection was widespread and patchy. There were localised sites with both high prevalence and high intensity infections in western and southern fishery areas. By February 2010, bonamia infection had become widespread throughout the fishery, but the prevalence and intensity of infection were highly variable at small spatial scales. Stations with high prevalence and high intensity of infection were interspersed amongst stations with no detectable infection. Generally, both prevalence and intensity of infection had increased especially in central fishery areas where recruited oyster density had probably reached pre-1985 levels at some stations. In February 2011, the distribution of infection was widespread and variable, similar to that in 2010, but the prevalence of infection had decreased (Michael et al. 2013).

In February 2012, the distribution of infection was more similar to previous years with increasing prevalence and likely mortality in western and eastern fishery areas, but decreased infection in the central and southern areas that support most of the commercial fishery (Figure 28). There was a marked increase in bonamia infection in February 2013, infection was more widespread than in 2012 and had increased in the important central fishery areas (Figure 29).

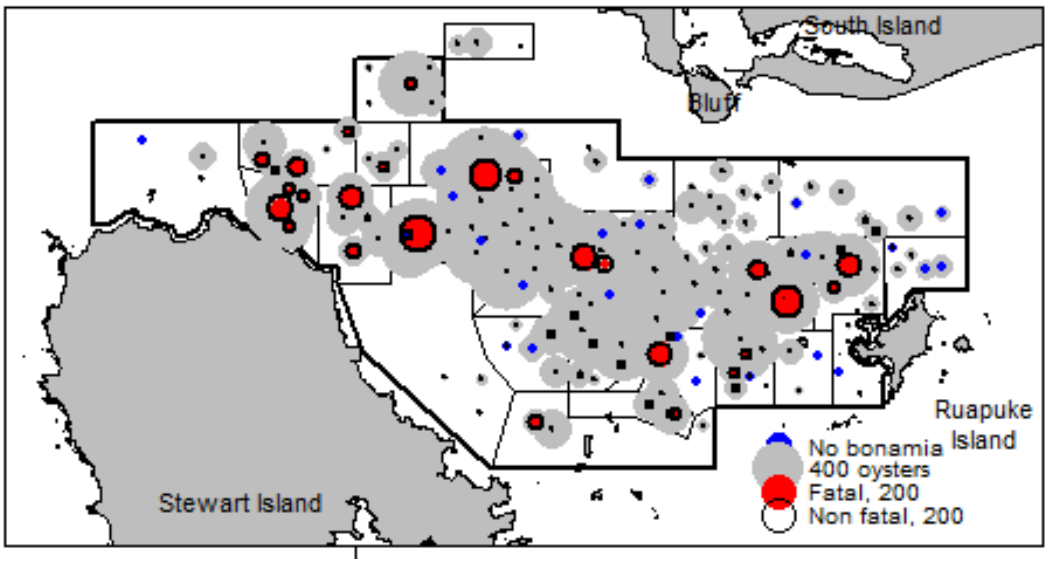

Figure 28: The distributions of oysters and bonamia infection in February 2012. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2005 survey area (black outer line) and the February 2012 survey strata (black lines).

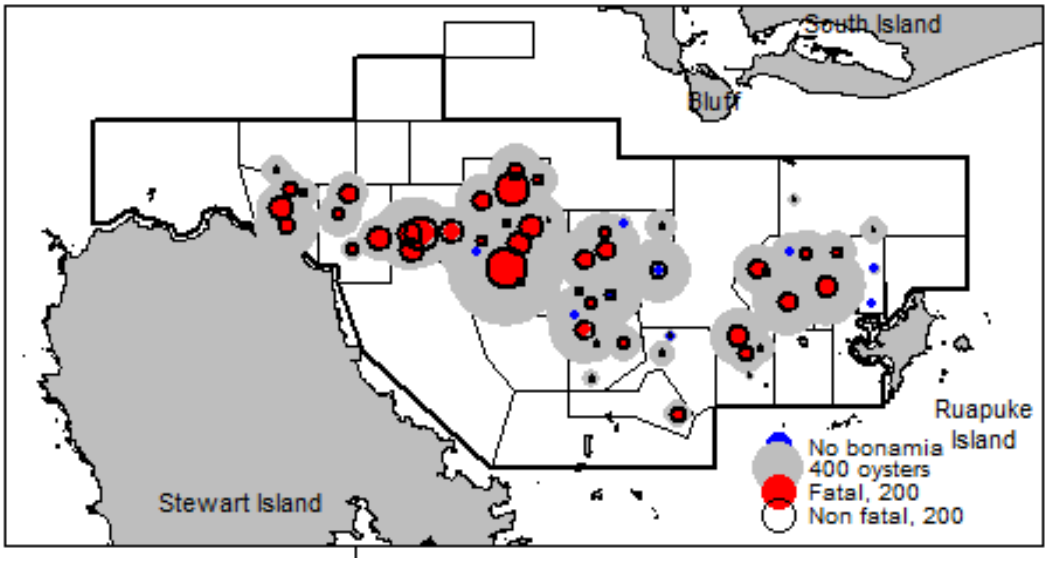

Figure 29: The distributions of oysters and bonamia infection in February 2013. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2005 survey area (black outer line) and the February 2013 survey strata (black lines).

The intensity of infection between January 2005 and February 2012 varied markedly within sites. Peaks in the mean intensity of infection also probably vary seasonally. The number of high intensity patches increased until January 2005. Since then infected oysters have had mainly high intensities of infection. These low prevalence, but high intensity infections have maintained a level of detectable disease mortality in the oyster population. Details of the distribution of prevalence and intensity of bonamia infection from January 2005 to February 2012 are given in Michael et al. 2013. The intensity of infection has increased markedly between February 2012 and February 2013, especially in the central commercial fishery areas (Figures 28 and 29).

Patterns in the distribution of prevalence and intensity of infection were not consistent with dredging patterns or oyster density; there were areas of high oyster density with relatively high prevalence and intensity of infection in areas that have not been fished from 2008 to 2012 because of the low meat quality there.

Changes in the numbers of oysters infected with bonamia

Estimates of the total numbers of recruit-sized oysters infected with bonamia in common strata were scaled up from the catches at randomly selected stations, and compared between the 2012 stock assessment survey (using common stations) and the 2013 bonamia survey. The total numbers of infected oysters increased from 65.3 million in 2012 (CV 0.32) to 81.9 million in 2013 (CV 0.15) (Table 8). The total numbers of infected oysters with fatal infections (category 3−5 intensity of infections) increased from 44.9 million in 2012 (CV 0.33) to 59.7 million in 2013 (CV 0.18), Table 8.

The percentage of the total recruit-sized oyster population infected with bonamia increased from 11.2% in 2012 to 12.7% in 2012 (Table 8). The percentage of fatal infections followed a similar trend increasing from 7.7% to 9.3% over the same period (Table 8). Mean densities and population estimates of infected recruit-sized oysters by stratum are given in Table 9, and fatally infected recruit-sized oysters by stratum in Table 10. Strata B3 and E2 have the highest densities of infected and fatally infected oysters, and are likely to account for half of the total expected mortality in the strata surveyed. Of all the recruit-sized infected oysters in the strata surveyed, 68.8% were fatally infected in 2012 and 72.9% in 2013.

Table 8: Mean and 95% confidence intervals (95%CI) for the total numbers of infected, recruit-sized oysters (millions) in the survey population (Prev.all) from common survey strata sampled each year, where three or more random stations were sampled in February 2012 and 2013. Survey population estimates (millions of oysters) and the percentages of populations infected, 95%CIs based on mean prevalence for upper and lower estimates of population size. Mean numbers of infected oysters with 3 and higher category infections (Prev3+), and the numbers (millions).

Comm. Str.	Prev. all			Population size			Percent of population infected		
	Mean	L95%CI	U95%CI	Mean	L95%CI	U95%CI	Mean	L95%CI	U95%CI
2012	65.3	21.9	121.1	585.3	375.8	882.1	11.2	5.8	13.7
2013	81.9	49.0	128.0	644.9	394.8	997.5	12.7	12.4	12.8

Comm. Str.	Prev.3+								
	Mean	L95%CI	U95%CI	Mean	L95%CI	U95%CI	Mean	L95%CI	U95%CI
2012	44.9	14.8	83.6	585.3	375.8	882.1	7.7	3.9	9.5
2013	59.7	33.4	95.8	644.9	394.8	997.5	9.3	8.4	9.6

Table 9: Absolute population estimates for recruit-sized oysters infected with bonamia (categories 1−5) from tows sampled for bonamia only in common strata: the number of randomly selected stations sampled (No. stations), the mean oyster density per m^2 (Mean density), standard deviation (s.d.) of the density estimate, coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Mean population), upper and lower 95% confidence intervals (CI), and the area of each stratum (Area), by stratum for the February 2012 and February 2013 surveys.

Stratum	No. Stations	Mean density	s.d.	Density CV	Mean population	Lower 95% CI	Upper 95% CI	Area (km^2)
B3	8	0.48	0.17	0.35	21.3	6.1	40.9	44.7
C3	4	0.15	0.08	0.55	4.8	0.0	10.8	32.7
C5	5	0.16	0.07	0.42	6.2	1.0	12.5	37.7
C5a	3	0.14	0.13	0.88	3.4	0.0	10.1	23.5
C7	4	0.27	0.09	0.32	9.8	3.5	18.2	36.1
C7a	4	0.25	0.09	0.35	5.9	1.7	11.2	23.6
C8	4	0.13	0.06	0.47	3.4	0.2	7.4	26.8
C9	4	0.10	0.02	0.23	3.6	1.8	6.0	34.5
CB6	4	0.13	0.10	0.76	4.0	0.0	10.9	30.0
E2	5	0.46	0.15	0.32	19.6	6.6	36.3	42.8
All	45	0.25	0.04	0.15	81.9	49.0	128.0	332.4

Table 10: Absolute population estimates for recruit-sized oysters with fatal bonamia infections (categories 3−5) from tows sampled for bonamia only in common strata: the number of randomly selected stations sampled (No. stations), the mean oyster density per m^2 (Mean density), standard deviation (s.d.) of the density estimate, coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Mean population), upper and lower 95% confidence intervals (CI), and the area of each stratum (Area), by stratum for the February 2012 and February 2013 surveys.

Stratum	No. Stations	Mean density	s.d.	Density CV	Mean population	Lower 95% CI	Upper 95% CI	Area (km^2)
B3	8	0.41	0.17	0.41	18.4	3.5	37.0	44.7
C3	4	0.09	0.07	0.75	3.0	0	8.0	32.7
C5	5	0.10	0.07	0.68	3.8	0	9.6	37.7
C5a	3	0.11	0.09	0.85	2.6	0	7.6	23.5
C7	4	0.23	0.09	0.39	8.2	1.9	16.4	36.1
C7a	4	0.17	0.08	0.48	4.1	0.3	8.8	23.6
C8	4	0.09	0.03	0.34	2.4	0.7	4.5	26.8
C9	4	0.04	0.02	0.39	1.5	0.3	2.9	34.5
CB6	4	0.13	0.10	0.76	4.0	0	10.9	30.0
E2	5	0.27	0.10	0.37	11.7	3.0	22.9	42.8
All	45	0.18	0.03	0.18	59.7	33.4	95.8	332.4

Projected short-term mortality from bonamia infections

Post-survey mortality of recruit-sized oysters was estimated for common 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 11) and this correction factor was

applied to the mean density estimated from all random tows. The post-survey mortality of oysters was projected to reduce the recruit-sized oyster population from 644.9 million oysters (95% CI 394.8−997.5) at the time of the survey (February 2013) to 566.8 million oysters (95% CI 346.5−877.2) by early in the new oyster season, a loss of 78.1 million oysters (8.8%), (Table 11).

Table 11: Absolute population estimates for recruit-sized oysters after projected mortality from bonamia based on category 3 and higher infections: the number of randomly selected stations sampled (No. stations), the correction factor applied to each stratum (Corr. factor), the mean oyster density per m^2 (Mean density), standard deviation (s.d.) of the density estimate, coefficient of variation (CV) of the oyster density, mean population size in millions of oysters (Mean population), upper and lower 95% confidence intervals (CI), and the area of each stratum (Area), by stratum for the February 2013 Foveaux Strait bonamia survey.

Stratum	No. Stations	Corr. factor	Mean density	s.d.	Density CV	Mean population	Lower 95% CI	Upper 95% CI	Area (km^2)
B3	8	0.80	2.69	1.01	0.38	120.1	29.5	235.0	44.7
C3	4	0.97	1.35	0.28	0.21	44.0	23.7	72.3	32.7
C5	5	0.97	1.99	0.78	0.39	75.2	15.9	148.3	37.7
C5a	3	0.93	1.05	0.69	0.65	24.7	0.0	62.3	23.5
C7	4	0.76	0.94	0.22	0.23	33.9	16.8	57.7	36.1
C7a	4	0.87	1.54	0.45	0.29	36.4	14.5	65.2	23.6
C8	4	0.94	1.45	0.73	0.50	38.9	0.0	86.5	26.8
C9	4	0.95	1.38	0.52	0.38	47.6	12.0	93.0	34.5
CB6	4	0.99	1.71	1.26	0.74	51.2	0.0	136.2	30.0
E2	5	0.82	2.21	0.49	0.22	94.8	47.5	160.2	42.8
All	45		1.71	0.23	0.14	566.8	346.5	877.2	332.4

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 density (Figure 30), it is assumed that these areas may eventually be subjected to heightened mortality. These infections are widespread throughout the fishery area sampled in 2013 (Figure 30).

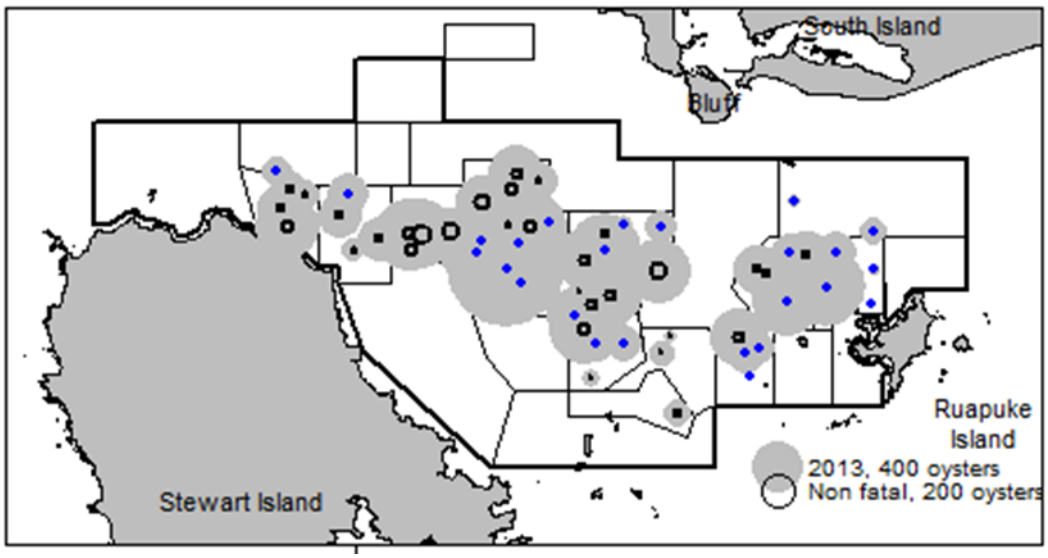

Figure 30: The distribution of recruit-sized oysters (filled grey circles showing numbers per standard tow) and oysters with category 1 and 2 infections (open black circles, the numbers of oysters scaled to the size of the catch with intensity of infection category 1 and 2) in February 2013. Stations with no bonamia infection are shown by open blue circles.

4.5 Changes in oyster densities between 2012 and 2013

More random stations were sampled in common strata in 2012 (N=73) than in 2013 (N=45). Boxplots of oyster density from matched random stations sampled in 2013 and 2012, and for all stations sampled in common strata in 2012 (suffix all) show similar densities between years for recruit, pre-recruit, and small oysters (Figure 31).

Changes in mean oyster density (oysters per m^2) for recruit, pre-recruit, and small sized oysters from all common strata sampled in 2013 and 2012 show similar densities, and some regional trends (Figure 32). Oyster densities were not adjusted for dredge efficiency as this variable is probably different for recruit, pre-recruit, and small sized oysters. The three size groups showed some decline in density in eastern fishery areas. Recruit-sized oyster densities are similar in 2013 to those in 2012. Pre-recruit sized and small oysters show similar trends in densities between years and across the fishery. There are increasing densities in the far west (strata C7 and C7a), especially in small oysters and a decrease in southern and eastern areas (Figure 32). Stratum C7a shows some increase in oyster density in all size groups.

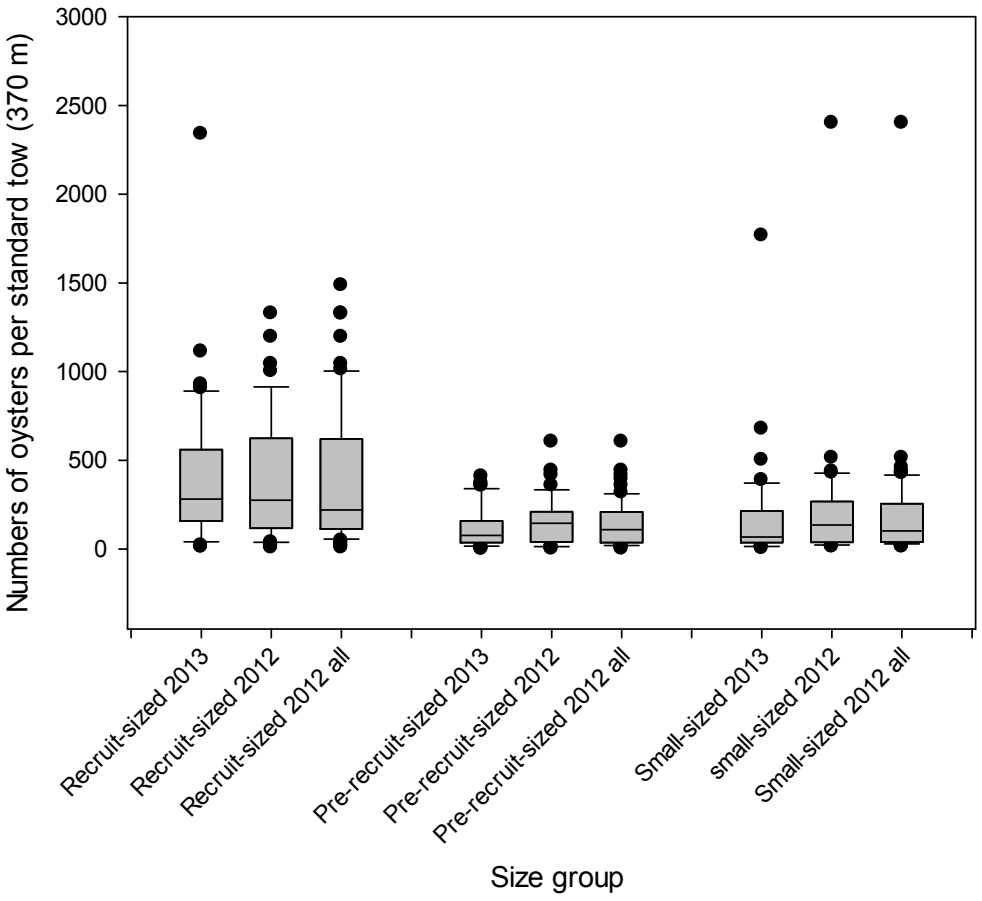

Figure 31: Boxplots of standardised numbers of oysters per tow for recruit, pre-recruit, and small sized oysters, from common strata sampled in 2013 survey (N=45) and 2012 survey (N=73). Medians shown as solid lines, boxes represent 50 percentiles and whiskers 75 percentiles, and outliers as filled circles.

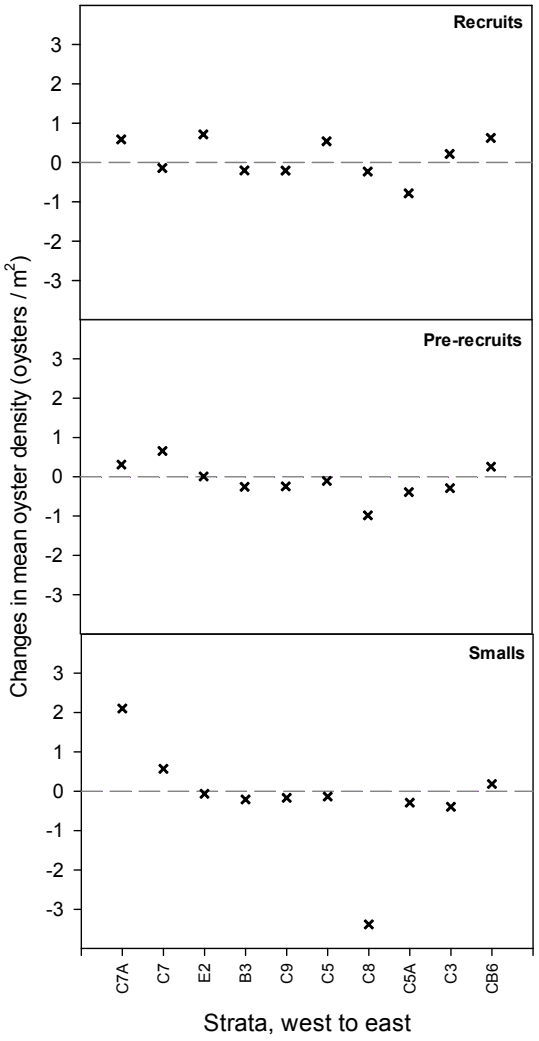

Figure 32: Changes in mean oyster density (oysters per m^2) for recruit, pre-recruit, and small sized oysters, from common strata sampled in 2012 and again in 2013. Mean densities have not been adjusted for dredge efficiency. Strata are shown from west to east on the x axis.

4.6 Survey estimates of population size

Survey estimates of population size for recruit-sized, pre-recruit, and small oysters from the February 2012 and February 2013 surveys by common strata (B3, C3, C5, C5a, C7, C7a, C8, C9, CB6, and E2) are shown in Tables 12–14. Table 15 compares survey estimates for recruit-sized, pre-recruit, and small oysters, and for recruit-sized new clocks.

In February 2012, the central fishery areas (strata B3, C5, and E2) that had sustained the most fishing over the last three years had the highest densities of recruit-sized oysters (2.0–3.6 oysters/m^2) followed closely by western (C7a, 1.8 oysters/m^2), southern (C8, 1.6 oysters/m^2), and eastern strata (C3, 1.4 oysters/m^2) (Table 12). The central area strata B3, E2, and C5 also had the highest population sizes 158.9, 80.3, and 74.5 million oysters respectively. Some eastern fishery areas are showing signs of rebuilding; stratum C3 had a population size of 47.1 million oysters. More bonamia mortality was expected in central areas in the summer of 2012/2013, and while some strata showed declines, others showed increases. Oyster densities of recruit-sized oysters and population sizes in strata B3, C5, and E2 were 3.4, 1.1, and 2.7 oysters/m^2 respectively and population sizes were 150.1, 26.6 (significant decline from 74.5), and 115.6 (increasing from 74.5) million oysters respectively. Stratum C3 in the eastern fishery showed an increase in density from 1.4 to 2.1 oysters/m^2) and a population size of 77.3 million oysters, up from 47.1 million in 2012. (Table 12).

Pre-recruit oyster densities (Table 13) are lower than for recruit-sized oysters. The highest pre-recruit densities were recorded in central and western areas, strata C7a, B3, C7, and E2 with (0.8−1.4 oysters/m^2), and they also had among the highest population sizes population sizes which ranged between 31.6 and 41.8 million oysters in February 2012. Strata in eastern fishery areas (C3 and CB6) that have historically had good recruitment and large numbers of small oysters had relatively low densities of about 0.5 oysters/m^2 and low population sizes (17.5 and 15.5 million oysters respectively) in 2012. Changes in mean densities and population size have varied within strata in similar regions in 2013, possibly due to the variable distribution of bonamia mortality that kills pre-recruit sized oysters at similar rates to recruit-sized oysters. C7a decreased in both density and population size, while C7, an adjacent stratum, increased in density in 2013 (Table 13). In the central area B3 declined while E2 remained about the same (Table 13).

The density and population sizes of small oysters had decreased in most strata between 2012 and 2013 (Table 14). The highest densities and population sizes of small oysters were in central and western areas in 2012 (C7a, C7, and E2, Table 14). Population sizes ranged between 9.8 million and 91.7 million oysters in these strata in 2013 compared with 47.5 million and 95.5 million oysters in 2012 (Table 14). Stratum C5a in the southeast showed an increase in density 0.5 to 1.2 oysters/m^2, and increase from 12.2 to 41.9 million oysters between 2012 and 2013.

Mean densities for all common strata combined (Table 15) show recruit-sized oyster densities and population size have increased by about 10% between 2012 and 2013, and pre-recruit and small oysters have declined by about 20% over the same period.

Table 12: Absolute population estimates for recruit-sized oysters for common strata sampled in 2012 and again in 2013. The 2012 section uses all random stations sampled in the strata, 2012a used only a subset of random stations that were sampled again in 2013, and 2013 the number of stations sampled, the mean oyster density per m^2 (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), and the area of each stratum (Area) in km^2, by stratum for the February 2012 and February 2013 Foveaux Strait oyster surveys.

	2012					2012a					2013					
Stratum	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area (km^2)
B3	3.56	0.20	158.9	86.3	261.4	3.18	0.27	142.3	61.8	251.0	3.36	0.38	150.1	36.9	293.7	44.7
CB6	1.15	0.44	34.4	5.4	70.7	0.40	0.42	12.1	2.1	24.6	1.39	0.21	45.6	24.5	74.8	32.7
C3	1.44	0.29	47.1	18.9	84.3	1.31	0.33	43.0	13.9	80.4	2.05	0.39	77.3	16.4	152.4	37.7
C5	1.98	0.27	74.5	32.1	131.6	1.85	0.36	69.5	19.3	135.3	1.13	0.65	26.6	0.0	67.1	23.5
C5a	1.34	0.64	31.6	0.0	78.1	1.91	0.76	44.8	0.0	120.3	1.24	0.23	44.6	22.1	75.8	36.1
C7	1.01	0.18	36.4	20.4	58.4	1.07	0.20	38.7	20.5	63.5	1.77	0.29	41.7	16.6	74.7	23.6
C7a	1.78	0.43	42.0	6.1	87.5	1.78	0.43	42.0	6.1	87.5	1.55	0.50	41.6	0.0	92.3	26.8
C8	1.65	0.39	44.3	10.2	87.6	2.24	0.37	60.0	16.1	116.6	1.45	0.38	50.0	12.6	97.8	34.5
C9	1.04	0.47	35.8	2.7	76.7	1.29	0.57	44.4	0.0	103.8	1.73	0.74	51.9	0	138.3	30.0
E2	1.87	0.26	80.3	35.4	140.8	2.56	0.23	109.5	53.5	186.6	2.70	0.22	115.6	58.0	195.5	42.8
All	1.76	0.10	585.3	375.8	882.1	1.82	0.12	606.3	378.7	928.0	1.94	0.14	644.9	394.8	997.5	332.4

Table 13: Absolute population estimates for pre-recruit sized oysters for common strata sampled in 2012 and again in 2013. The 2012 section uses all random stations sampled in the strata, 2012a used only a subset of random stations that were sampled again in 2013, and 2013 the number of stations sampled, the mean oyster density per m^2 (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), and the area of each stratum (Area) in km^2, by stratum for the February 2012 and February 2013 Foveaux Strait oyster surveys.

	2012					2012a					2013					
Stratum	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area (km^2)
B3	0.94	0.15	41.8	25.1	65.8	1.00	0.21	44.6	23.3	74.5	0.67	0.31	29.7	10.6	54.9	44.7
CB6	0.52	0.55	15.5	0.0	35.4	0.19	0.39	5.6	1.4	11.1	0.20	0.30	6.7	2.6	12.1	32.7
C3	0.53	0.29	17.5	6.9	31.4	0.48	0.27	15.7	6.8	27.5	0.43	0.33	16.3	5.2	30.4	37.7
C5	0.58	0.22	21.8	11.2	36.6	0.57	0.26	21.4	9.4	37.7	0.15	0.56	3.6	0	8.4	23.5
C5a	0.36	0.67	8.4	0.0	21.2	0.50	0.82	11.7	0.0	32.8	1.10	0.27	39.6	17.6	70.2	36.1
C7	0.87	0.17	31.6	17.9	50.5	0.90	0.22	32.3	16.5	53.8	1.34	0.18	31.6	17.8	50.6	23.6
C7a	1.39	0.37	32.9	8.1	65.0	1.39	0.37	32.9	8.1	65.0	0.33	0.38	8.9	2.1	17.8	26.8
C8	0.51	0.26	13.7	6.0	23.9	0.57	0.36	15.3	4.4	29.4	0.25	0.32	8.7	3.0	16.2	34.5
C9	0.26	0.40	9.0	2.0	18.1	0.32	0.47	11.1	0.8	23.7	0.55	0.75	16.4	0	44.2	30.0
E2	0.85	0.37	36.2	9.4	70.3	1.28	0.30	54.8	21.2	99.4	0.87	0.29	37.4	14.8	67.4	42.8
All	0.69	0.11	228.4	146.2	345.0	0.74	0.11	245.4	155.5	372.2	0.60	0.12	198.8	124.9	303.1	332.4

Table 14: Absolute population estimates for small sized oysters for common strata sampled in 2012 and again in 2013. The 2012 section uses all random stations sampled in the strata, 2012a used only a subset of random stations that were sampled again in 2013, and 2013 the number of stations sampled, the mean oyster density per m^2 (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), and the area of each stratum (Area) in km^2, by stratum for the February 2012 and February 2013 Foveaux Strait oyster surveys.

	2012					2012a					2013					
				Lower	Upper				Lower	Upper				Lower	Upper	Area
Stratum	Density	CV	Pop.n	95%CI	95%CI	Density	CV	Pop.n	95%CI	95%CI	Density	CV	Pop.n	95%CI	95%CI	(km^2)
B3	0.73	0.17	32.8	19.1	52.7	0.85	0.22	37.9	19.4	63.7	0.51	0.55	22.9	0.0	52.4	44.7
CB6	0.74	0.45	22.1	2.6	46.1	0.27	0.33	8.0	2.8	14.9	0.31	0.44	10.1	1.5	20.7	32.7
C3	0.49	0.32	16.1	5.6	29.6	0.43	0.33	13.9	4.4	26.1	0.35	0.34	13.3	4.1	25.1	37.7
C5	0.55	0.22	20.9	10.8	35.1	0.49	0.24	18.4	8.9	31.4	0.24	0.46	5.5	0.5	11.8	23.5
C5a	0.52	0.53	12.2	0.0	27.7	0.73	0.61	17.2	0.0	41.5	1.16	0.28	41.9	18.1	74.9	36.1
C7	1.36	0.21	49.2	25.6	81.2	1.34	0.27	48.2	20.5	85.0	3.89	0.42	91.7	15.2	187.3	23.6
C7a	4.04	0.63	95.5	0.0	236.2	4.04	0.63	95.5	0.0	236.2	0.37	0.34	9.8	3.0	18.7	26.8
C8	0.43	0.27	11.7	5.0	20.5	0.39	0.39	10.6	2.5	20.9	0.26	0.24	8.9	4.2	15.2	34.5
C9	0.30	0.30	10.3	3.9	18.8	0.35	0.39	11.9	2.8	23.8	0.51	0.73	15.2	0.0	40.2	30.0
E2	1.11	0.33	47.5	15.8	88.4	1.64	0.26	70.4	31.6	122.9	1.06	0.27	45.2	19.7	79.5	42.8
All	0.96	0.20	318.2	166.8	521.9	1.00	0.20	332.0	176.3	542.1	0.80	0.17	264.4	150.9	422.5	332.4

Table 15: Absolute population estimates for recruit-sized, pre-recruit, and small sized oysters, and recruit-sized new clocks for all common strata combined. The 2012 section uses all random stations sampled in the strata, 2012a used only a subset of random stations that were sampled again in 2013, and 2013 the number of stations sampled, the mean oyster density per m2 (Density), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n), and upper and lower 95% confidence intervals (95%CI) for the February 2012 and February 2013 Foveaux Strait oyster surveys.

	2012					2012a					2013				
Size	Density	CV	Pop.n	L95%CI	U95%CI	Density	CV	Pop.n	L95%CI	U95%CI	Density	cv	Pop.n	L95%CI	U95%CI
Recruits	1.76	0.10	585.3	375.8	882.1	1.82	0.12	606.3	378.7	928.0	1.93	0.14	644.9	394.8	997.5
Pre-recruits	0.69	0.11	228.4	146.2	345.0	0.74	0.11	245.4	155.5	372.2	0.59	0.12	198.8	124.9	303.1
Small	0.96	0.20	318.2	166.8	521.9	1.00	0.20	332.0	176.3	542.1	0.78	0.18	264.4	150.9	422.5

In 1995 and 1997, the commercial population used to estimate yield was estimated as the percentage of the population above a density of 400 oysters per tow (equivalent to about 6–8 sacks per hour during commercial dredging) over the entire survey areas. 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 catch at each station minus 400 oysters) from ten common strata (B3, C3, C5, C7, C7a, C8, C9, CB6, and E2) with three or more randomly selected tows sampled in 2012 and 2013 are given in Table 16. Nine of these strata supported commercial densities in 2012, and eight in 2013. The commercial population size estimated from paired stations was similar, 412.7 million oysters in 2012, compared to 416.6 million in 2013, and slightly lower than that estimated from all stations sampled in common strata in 2012, 442.6 million oysters. Oyster densities have increased in some strata (4) and decreased in others (6).

Table 16: Estimates of the size of the oyster population above a density of 400 oysters per survey tow (equivalent to about 6–8 sacks per hour in commercial dredging) from ten common strata (B3, C3, C5, C7, C7a, C8, C9, CB6, and E2) with three or more randomly selected tows sampled in 2012 and 2013. The mean oyster densities per m^2 (Density), coefficient of variation (CV) of the density estimate, mean population size (Pop.n), upper and lower 95% confidence intervals (CI), and the area of each stratum (Area), by stratum.

	2012					2012a					2013					
Stratum	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Density	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area (km^2)
B3	3.22	0.25	143.7	66.3	248.9	2.91	0.33	129.9	42.6	244.1	3.06	0.44	136.6	19.6	283.2	44.7
C3	0.88	0.65	26.4	0.0	65.2	0.00	0.00	0.0	0.0	0.0	0	0	0	0	0	32.7
C5	0.89	0.63	29.2	0.0	71.2	0.63	1.00	20.6	0.0	64.9	1.56	0.63	58.7	0	144.0	37.7
C5a	1.33	0.51	50.1	0.0	112.0	1.29	0.65	48.4	0.0	119.9	0.85	1.00	20.0	0	63.5	23.5
C7	0.95	1.00	22.3	0.0	70.8	1.58	1.00	37.2	0.0	119.6	0	0	0	0	0	36.1
C7a	0.00	0.00	0.0	0.0	0.0	0.00	0.00	0.0	0.0	0.0	0.81	1.00	19.1	0	61.4	23.6
C8	1.00	1.00	23.6	0.0	75.4	1.00	1.00	23.6	0.0	74.8	0.96	1.00	25.7	0	83.1	26.8
C9	1.16	0.64	31.2	0.0	77.5	1.74	0.59	46.7	0.0	110.2	0.75	1.00	25.8	0	82.7	34.5
CB6	0.58	1.00	19.9	0.0	63.3	0.87	1.00	29.8	0.0	95.4	1.37	1.00	41.0	0	129.8	30.0
E2	1.55	0.39	66.4	15.5	132.1	2.48	0.27	106.2	46.3	185.6	2.10	0.44	89.8	10.4	188.0	42.8
All	1.24	0.17	412.7	236.9	660.4	1.33	0.20	442.6	238.0	731.8	1.25	0.24	416.6	196.1	723.6	332.4

4.7 Changes in the distribution of live oysters

There was only limited sampling of random stations in commercial fishery areas in February 2013 and the sampling was insufficient to provide a consistent or complete coverage of the fishery area, and hence the survey is not likely to have estimated the distributions of oyster density for live recruit, pre-recruit, and small oysters well. The distribution of oyster densities for recruit-sized, pre-recruit, and small oysters from this limited sampling is shown in Figures 33–35. The distribution of oyster densities has become more widespread in recent years covering most of the fishery area. Densities of pre-recruit and small oysters are mostly lower than recruit sized oysters at most sites.

The distributions of recruited oyster densities has increased in recent years characterised by an increase in eastern fishery areas in 2012 and 2013 that had been low since 2003. Generally, oyster densities are similar or have increased slightly at stations in eastern and central fishery areas and are similar or have declined in southern and western fishery areas (Figure 33). The localised declines in the western and southern fishery areas are probably a result of bonamia mortality, especially in western fishery areas where there has been very little fishing over the last two oyster seasons (Figure 33).

The distribution of pre-recruit oyster densities in 2013, and 2012 (Figure 34) are similar to those for recruits. Generally pre-recruit densities across all stations are similar or have increased slightly. One station in Stratum C7a has increased significantly and this is the result of high densities of small oysters there in 2012 (Figure 35) growing through to the next size class. Another station in the southern fishery also showed a significant increase in density (Figure 34).

The distribution of small oyster densities show a similar pattern to larger size classes and the densities are similar or have declined (Figure 35). Small oyster densities are generally low except in stratum C7a.

Oysters may take one to two years to transition through the pre-recruit size class. Declines in pre-recruit numbers may also be attributed to oysters growing into the recruit size class, but not being replaced by small oysters growing through to pre-recruits.

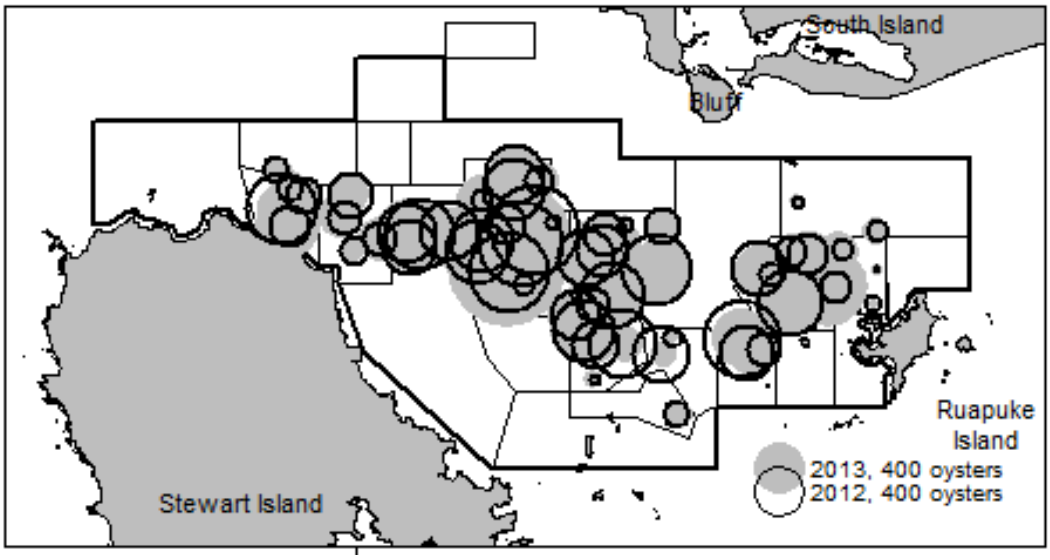

Figure 33: The densities (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled during February surveys in 2013 (filled grey circles) and in 2012 (open black circles).

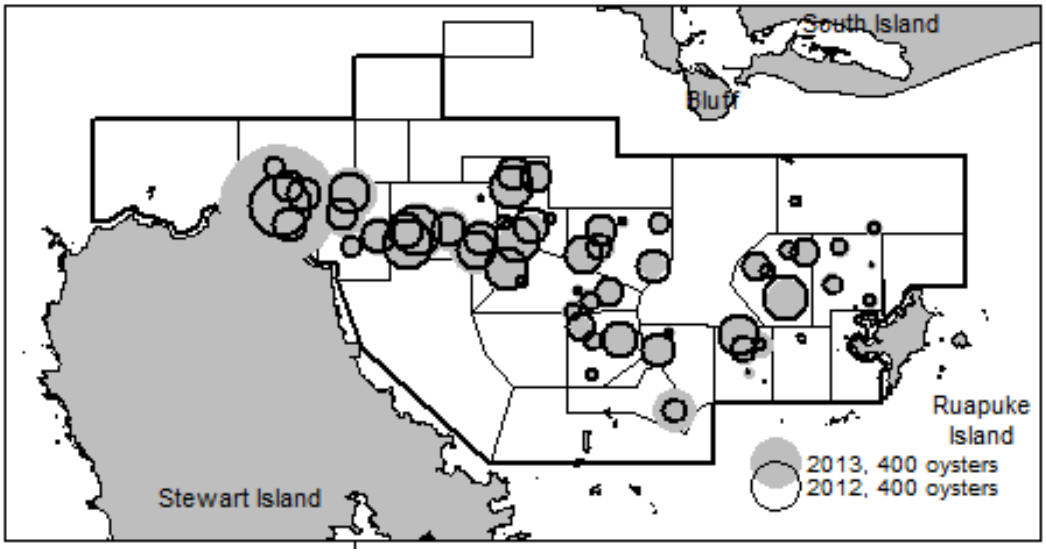

Figure 34: The densities (numbers of oysters per standard tow, 1221 m^2) of pre-recruit sized oysters sampled during February surveys in 2013 (filled grey circles) and in 2012 (open black circles).

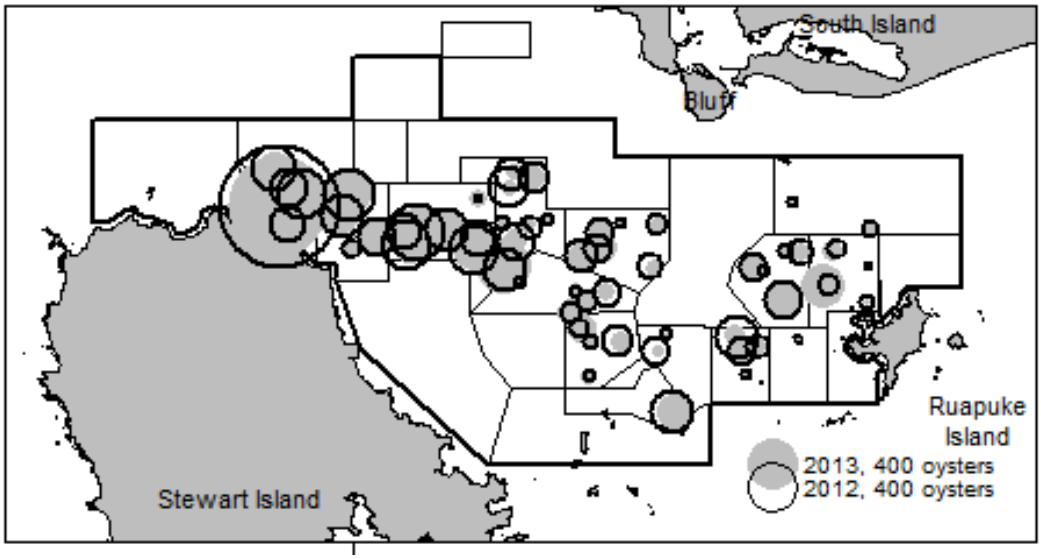

Figure 35: The densities (numbers of oysters per standard tow, 1221 m^2) of small oysters sampled during February surveys in 2013 (filled grey circles) and in 2012 (open black circles).

4.8 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 have declined slightly since 2009 (Figure 36), suggesting that recruitment to the commercial population may be low. This is consistent with the trend of declining numbers of small oysters sampled from the commercial catch between 2009 and 2013 (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 ENREF 28).

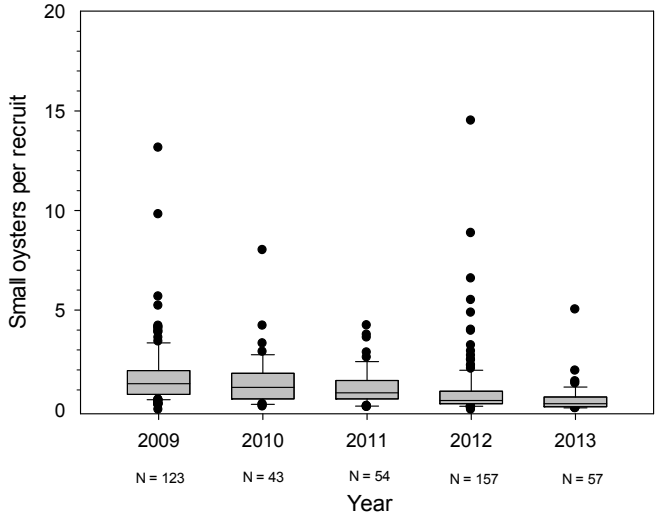

Figure 36: The numbers of small oysters per recruited oyster sampled at common stations between 2009 and 2013. The numbers of stations sampled each year varies.

5. DISCUSSION

The 2013 survey design provides a broad range of information to the Bluff Oyster Management Company skippers and to the Ministry for Primary Industries. The bonamia surveys are not intended to be stock assessments or to cover the entire fishery area. The subset of survey strata chosen represent fishery areas important to oyster skippers and provide data to inform decisions about fishing strategies for the next oyster season. The survey strata generally have the highest oyster densities and are therefore more vulnerable to heightened disease mortality from bonamia. The overall objective of these surveys is to determine the distribution, prevalence and intensity of infection by *Bonamia exitiosa*, and to estimate the oyster density in the commercial areas sampled for bonamia. Information from these surveys includes site by site comparisons of bonamia status and recent oyster mortality, and changes in oyster density and population size in strata. These surveys also provide projections of short-term mortality estimated from oysters with category 3 and greater intensity of infection; and indicative estimates of total summer mortality for all survey strata combined.

Demand for oysters is high at the beginning of the season, and oyster skippers use these survey data to determine the status of the commercial fishery areas just before the start of the oyster season and as a "weather forecast" for how disease mortality may change that status. The estimates of total summer mortality also provide some indication of which of the stock assessment projections (based on levels of bonamia mortality) is most likely for the coming year.

The survey design could be greatly improved if there were clear and specific ranked objectives for future bonamia surveys. If future surveys have a greater emphasis on stratum by stratum comparisons and less emphasis on site by site data, the survey design could fully randomise sample sites within strata. Given the constant, relatively low level of disease mortality, and the potential for another epizootic as oyster densities increase, bonamia surveys will provide up-to-date information on factors that increase or decrease population size. The current Fishery Plan for the OYU 5 fishery and strategic research plan currently include the provision of annual bonamia surveys.

The availability of a sensitive, specific and cost effective molecular method (qPCR) for the detection of bonamia (Michael et al. 2012b; Mass et al. 2013) has increased the ability to detect low level infections in oysters. The 2013 February survey samples were analysed using a combination of qPCR

and histological methods. The initial screening of samples using qPCR identified samples that tested positive. Of the heart tissue samples that tested positive, 65.5% were positive from heart imprint scores and 40.7% of gill tissue qPCR positives were positive from heart imprint. None of the of randomly selected qPCR negative samples (for both heart and gill tissues) were positive using heart imprints. In all, about 20% of the oyster heart imprint slides were scored. This combination of 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. 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.

The limited sampling in February 2013 shows a spread and intensification of bonamia infection and an increase in the level of summer mortality. New clocks increased in common strata from 17.6 million in 2012 to 29.0 million in 2013; and estimated mortality from scaled up population estimates of infected oysters with fatal infections increased from 44.9 million in 2012 to 59.7 million in 2013, and using another method (mean correction factor) mortality increased from 48.5 million in 2012 to 78.1 million in 2013. Total mortality increased between 44% and 62% between 2012 and 2013. The percentage mortality of about 12% in 2013 is within the range of bonamia mortality estimated in the oyster population since 2007 (Michael et al. 2013), and there appears to be a pattern of alternating years of relatively high and low mortality, with 2013 representing a relatively high year.

Despite the relatively high mortality in 2013, mean recruit-sized oyster density has increased across all strata sampled. The recruit-sized oyster population also increased from 585.3 million oysters in 2012 to 644.9 million oysters in 2013. The commercial population size remained similar between 2012 and 2013 at 412.7 million oysters and 416.6 million oysters respectively. Levels of recruitment to the recruit-sized population have been relatively low in recent years and the mean density and population size of both pre-recruit and small oysters declined in common strata in 2013. The fishery continues to rebuild, albeit more slowly than it has done in the absence of disease mortality and near long-term average recruitment.

6. ACKNOWLEDGMENTS

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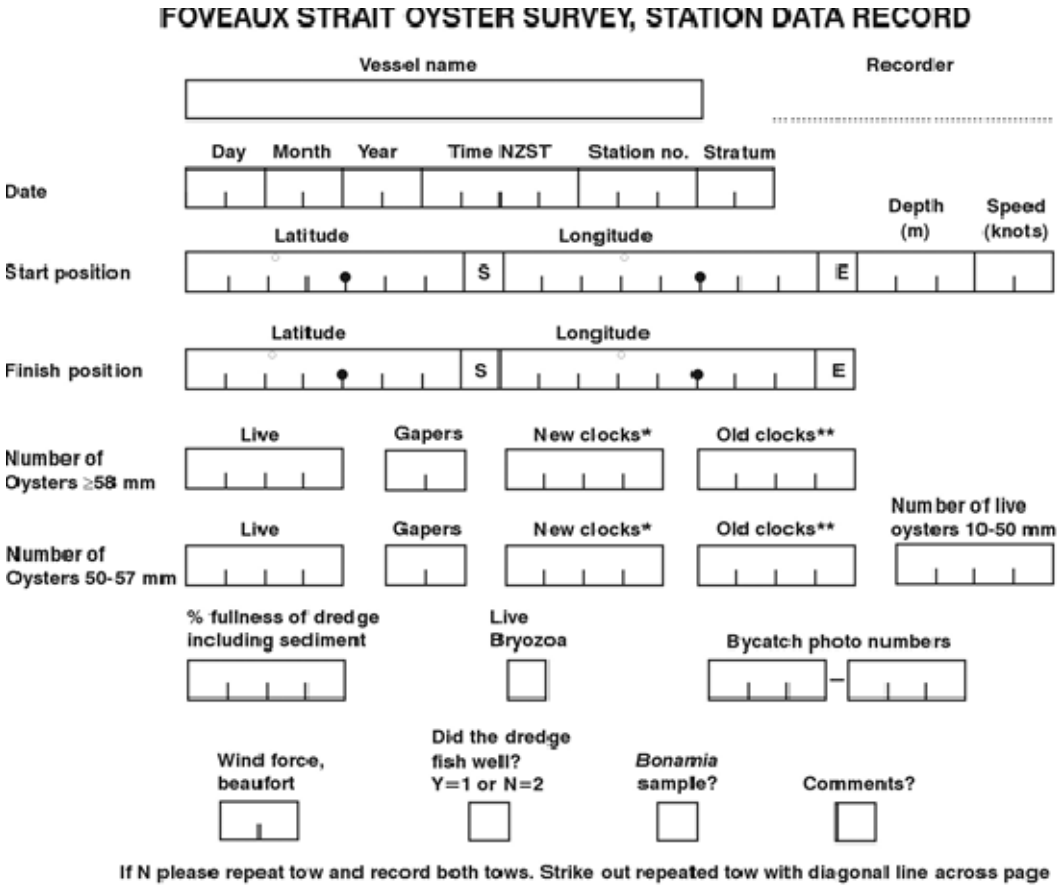
APPENDIX 1: Infection status of stations in 2012 by stratum (Str) and station (Stn). Number of recruit-sized oysters (Rec) and density in 2012 (Den.12) corrected for dredge efficiency (0.17), number of oyster in the bonamia sample (N), prevalence of infection (Prev), numbers with no detectable infections (Un inf), total numbers infected (No. inf), numbers of oysters with Light and Fatal infections.

Str	Stn	Rec	Den.12	N	Prev	Un inf	No. inf	Light	Fatal
B3	69	NA	5.8	NA	NA	NA	NA	NA	NA
B3	70	571	2.8	25	0.08	525	46	23	23
B3	71	37	0.2	24	0.08	34	3	0	3
B3	322	NA	0.7	NA	NA	NA	NA	NA	NA
B3	323	NA	3.0	NA	NA	NA	NA	NA	NA
B3	325	NA	5.0	NA	NA	NA	NA	NA	NA
B3	326	NA	1.3	NA	NA	NA	NA	NA	NA
B3	327	NA	6.4	NA	NA	NA	NA	NA	NA
C3	79	NA	0.5	NA	NA	NA	NA	NA	NA
C3	81	509	2.5	25	0.12	448	61	0	61
C3	82	274	1.4	21	0.00	274	0	0	0
C3	83	175	0.9	25	0.04	168	7	0	7
C5	88	324	1.6	25	0.00	324	0	0	0
C5	89	NA	0.8	NA	NA	NA	NA	NA	NA
C5	332	NA	2.3	NA	NA	NA	NA	NA	NA
C5	333	NA	4.1	NA	NA	NA	NA	NA	NA
C5	334	NA	0.2	NA	NA	NA	NA	NA	NA
C5a	60	NA	0.9	NA	NA	NA	NA	NA	NA
C5a	62	81	0.4	25	0.16	68	13	3	10
C5a	63	14	0.1	10	0.10	12	1	1	0
C7	90	350	1.7	25	0.28	252	98	28	70
C7	92	144	0.7	25	0.32	98	46	12	35
C7	93	NA	0.9	NA	NA	NA	NA	NA	NA
C7	94	NA	1.0	NA	NA	NA	NA	NA	NA
C7a	95	821	3.9	25	0.16	689	131	66	66
C7a	96	219	1.1	25	0.12	193	26	9	18
C7a	97	293	1.4	25	0.12	258	35	0	35
C7a	98	121	0.6	25	0.24	92	29	19	10
C8	100	376	1.8	25	0.04	361	15	0	15
C8	101	NA	3.0	NA	NA	NA	NA	NA	NA
C8	103	NA	3.9	NA	NA	NA	NA	NA	NA
C8	104	NA	0.1	NA	NA	NA	NA	NA	NA
C9	105	89	0.4	25	0.00	89	0	0	0
C9	107	697	3.4	25	0.00	697	0	0	0
C9	109	NA	0.3	NA	NA	NA	NA	NA	NA
C9	189	NA	0.9	NA	NA	NA	NA	NA	NA

Str	Stn	Rec	Den.12	N	Prev	Un inf	No. inf	Light	Fatal
CB6	42	173	0.8	25	0.16	145	28	0	28
CB6	43	NA	0.3	NA	NA	NA	NA	NA	NA
CB6	44	NA	0.0	NA	NA	NA	NA	NA	NA
CB6	170	NA	0.4	NA	NA	NA	NA	NA	NA
E2	112	802	3.8	25	0.28	577	224	64	160
E2	113	NA	2.5	NA	NA	NA	NA	NA	NA
E2	115	78	0.4	25	0.04	75	3	0	3
E2	116	NA	2.8	NA	NA	NA	NA	NA	NA
E2	338	NA	3.1	NA	NA	NA	NA	NA	NA

APPENDIX 2: SURVEY STATION FORM



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 3: SURVEY BONAMIA FORM

FOVEAUX STRAIT OYSTER BONAMIA DATA RECORD

Page ___ of ___

Date

Station no.	Day	Month	Year	Time NZST

Recorder

.....

Comments

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

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