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Foveaux Strait oyster and Bonamia surveys, February 2017

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The February 2017 Foveaux Strait oyster and Bonamia surveys informed the first stock assessment that has been done since 2013 when five-yearly assessments were introduced. The 2017 surveys and the stock assessment were funded by the Bluff Oyster Management Company (BOMC) and undertaken collaboratively between NIWA and BOMC.

The February 2017 surveys were completed despite a prolonged period of rough weather. Sampling was consistent with previous surveys. The surveys achieved low coefficients of variation (CVs) for population estimates, well below the 20% set by Fisheries New Zealand for stock assessment surveys.

All three size groups of oysters declined between 2012 and 2017. Recruit-sized oyster density declined by 42.6% (918.4 million oysters in 2012 to 527.4 million oysters in 2017) in the stock area, and 47.2% in the Bonamia survey area (688.1 million oysters in 2012 to 363.6 million oysters in 2017). Pre-recruit sized oyster density declined by 59.4% in the stock assessment survey area (414.3 million oysters in 2012 to 168.2 million oysters in 2017), and 58.6% in the Bonamia survey area (297.4 million oysters in 2012 to 123.1 million oysters in 2017). Small oysters declined by 40.9% in the stock area (612.2 million oysters in 2012 to 361.6 million oysters in 2017), and 42.0% in the Bonamia survey area (451.3 million oysters in 2012 to 261.9 million oysters in 2017). All three size groups of oysters have also declined between 2016 and 2017. Recruit-sized oysters declined by 6.0% and 5.6%, pre-recruit oysters by 12.0% and 2.2%, and small oysters by 0.7% and 2.3% in the stock and Bonamia survey areas respectively.

However, Bonamia mortality has declined to low levels that have not been recorded since 2005. Summer mortality over the stock area was about 5% in both 2016 and 2017. The densities of new clocks were low in 2016 and 2017 reflecting low pre-survey Bonamia mortalities and reduced oyster densities. Pre-survey mortality over the whole survey area was 1.5% of recruit-sized oyster population in 2017, slightly up from 0.5% in 2016. Prevalence of infection was low (5.4%) in 2017, and most of these were fatal infections. Moreover, non-fatal infections have declined to less than 1%, suggesting low Bonamia mortality in 2018. The low oyster densities and low non-fatal infection suggest reduced transmission of disease.

Overall, the slight increase in settler densities and low expected Bonamia mortality suggest that the fishery will be relatively stable in the short term and catch rates may not decline significantly.

1. INTRODUCTION

The Foveaux Strait oyster fishery (OYU 5, Figure 1) is over 150 years old. It is a high value, and nationally important fishery. Oysters (*Ostrea chilensis*) are an important customary (taonga), recreational, and commercial species, and the oyster fishery is important to the socioeconomics of Bluff and Invercargill. The OYU 5 stock is part of the Group 1 stocks in the Fisheries New Zealand (FNZ) draft National Fisheries Plan for Inshore Shellfish, which recognises the relatively high biological vulnerability of Group 1 stocks (including OYU 5) and prescribes a close monitoring approach. Accurate and frequent monitoring to support responsive management is essential to achieving maximum value from Group 1 stocks. Additionally, there is an approved collaborative fishery plan for the management of the fishery, the Foveaux Strait Oyster Fisheries Plan (Ministry of Fisheries 2009). A management committee collaboratively developed this plan with representatives from the Bluff Oyster Management Company (BOMC), customary and recreational fishers, and the then Ministry of Fisheries, now Fisheries New Zealand.

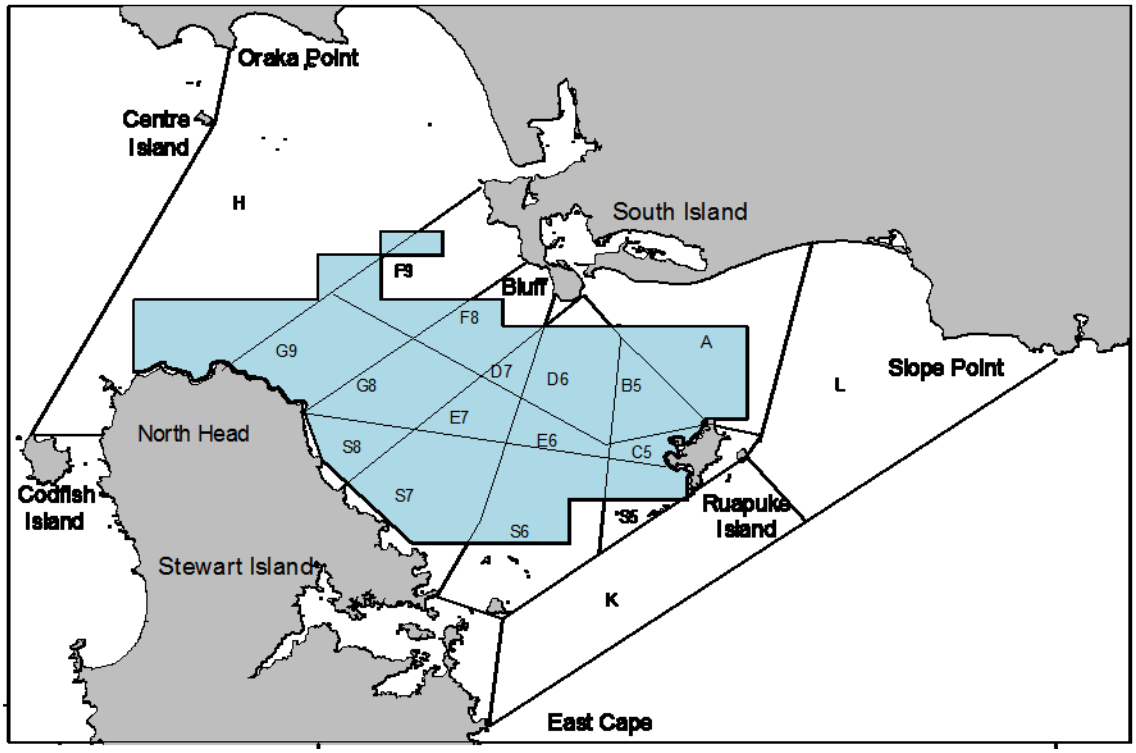

Figure 1: Foveaux Strait (OYU 5) stock boundary and oyster fishery statistical reporting areas, and the outer boundary of the 2007 stock assessment survey area (shaded blue) encompassing almost all the commercial fishery.

At relatively low levels of catch (less than 20 million oysters per year), the trend in the abundance of oysters in the Foveaux Strait fishery is driven by disease mortality from *Bonamia exitiosa* (Bonamia) and the levels of recruitment to the population (spat settlement). Bonamia is a haplosporidian parasite of flat oysters and is thought to be endemic to Foveaux Strait oysters. Three recent Bonamia epizootics in 1985–92, 1999–2005, and from 2013 to 2017, have shown that Bonamia mortality is a recurrent feature of the oyster population, and this mortality is the principal driver of oyster population abundance during epizootics. Anecdotal information from population surveys shows that pre-recruit sized oysters (expected to recruit in to the fishery within two years) are as vulnerable to Bonamia mortality as recruits. High Bonamia mortality can reduce short-term recruitment to the fishery as well as reduce recruit-sized oyster density (Keith Michael, NIWA, in prep.). A related and more destructive pathogen (*Bonamia*

ostreae), was first identified in 2015 as the cause of mortality in farmed *O. chilensis* in the Marlborough Sounds (Ministry for Primary Industries 2016). *B. ostreae* has reportedly caused 95–100% mortality of stock on some farms. The Ministry for Primary Industries (2016) implemented stock control measures but a surveillance survey in May 2017 detected a new *B. ostreae* infection in Big Glory Bay on Stewart Island. The Ministry for Primary Industries responded by removing all farmed oysters from Big Glory Bay (designated a single epidemiological unit) and from the Marlborough Sounds. Ongoing surveillance surveys will monitor the effectiveness of the response.

Management of the fishery recognises that when recruitment to the fishery is near long-term average recruit-sized stock abundance and future benefits from the fishery (harvest levels) are mainly determined by the levels of Bonamia mortality. At the current harvest levels (less than 15 million oysters per year), any effects of fishing on either oyster production or on exacerbating Bonamia mortality are not detectable.

Michael et al. (2016) summarise the status of Bonamia and its effects on the fishery. Recruitment to the oyster population (oyster spat settlement) has been low since the summer of 2009–10 (Figure 2) despite the population size of spawning sized-oyster densities increasing until 2012 (Figure 3). Consequently, the numbers of small and pre-recruit sized oysters have declined, reducing recruitment to the fishery.

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

Since 2004, a length based Bayesian stock assessment model has been used to assess the status of OYU 5 (Dunn 2005). Regular surveys of the oyster population that inform the stock assessments have sampled a consistent survey area (1054 km^2) since 1999. An additional stratum B1a (16 km^2) was introduced by oyster skippers in 2007. Since 2007, the size of the Foveaux Strait oyster survey area has remained at 1070 km^2. The original stratum boundaries have also remained similar since 1999. Between 1999 and 2017, some of the strata were subdivided to better define the areas with commercial densities of oysters. Stock assessment surveys have been undertaken in 1999, 2001, 2002, 2005, 2007, 2009 and 2012. The 2012 stock assessment survey sampled 26 strata (Figure 4). Five-yearly stock assessments were agreed to in 2013 and the February 2017 stock assessment survey is documented in this report.

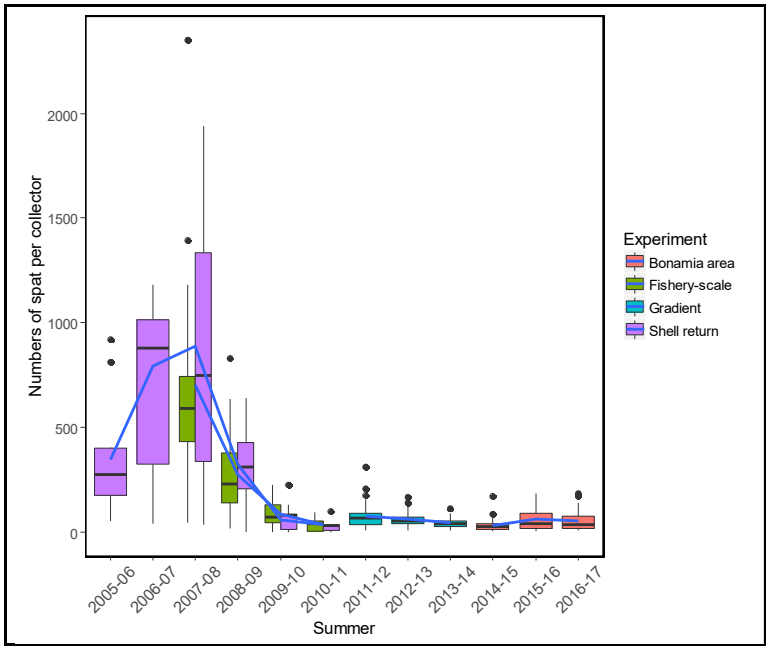

Figure 2: The total numbers of spat per collector sampled over the summers of 2005–06 to 2016–17. Spat settlement shows the success of spawning and indicates the levels of replenishment to the oyster population.

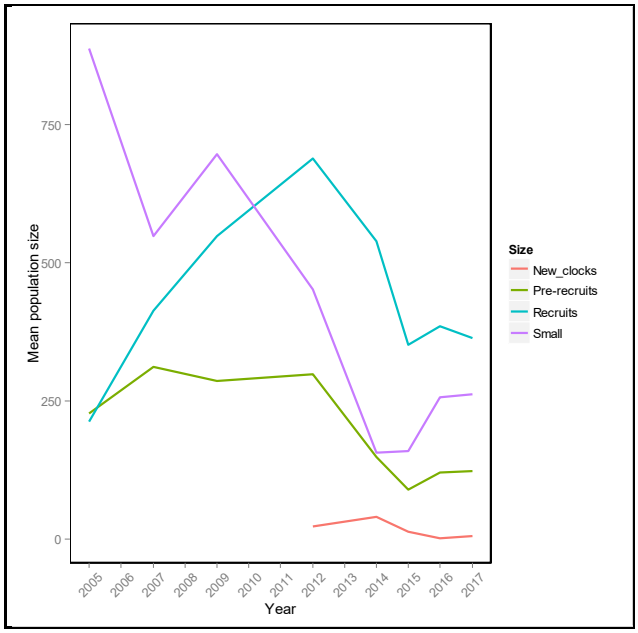

Figure 3: Trends in the population sizes of recruit-sized, pre-recruit, and small oysters, and recruit-sized new clocks in the Bonamia survey area between 2005 and 2017.

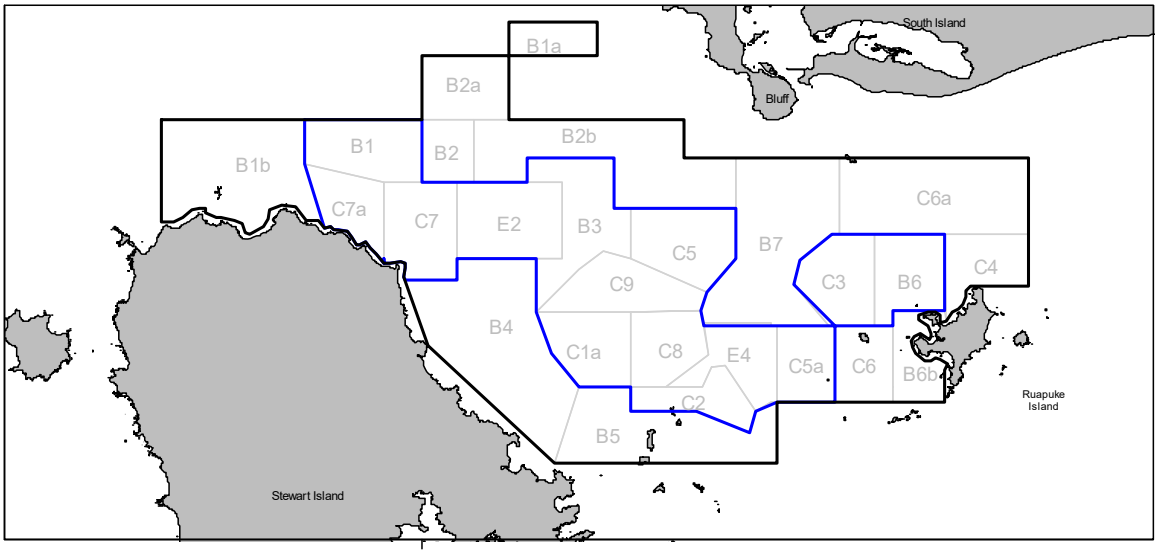

Figure 4: The 2007 stock assessment survey area (heavy, black outer line), and the Bonamia survey area (blue line). The 26 survey strata are bounded by grey lines and stratum labels are shown in grey text.

Five-yearly assessments have placed greater onus on the annual Bonamia surveys to monitor changes in the oyster population in commercial fishery areas (nominally the Bonamia survey area, Figure 4), as well as the status of and mortality from Bonamia (see Michael et al. 2016 for details). Bonamia surveys sample the prevalence and intensity of Bonamia infection and estimate summer mortality from the numbers of new clocks and oysters with fatal infections. These surveys also estimate the density of recruit-sized, pre-recruit, and small oysters in the Bonamia survey area, with limited additional sampling in the remaining stock assessment area. A new time series of surveys was established in February 2014 (Michael et al. 2015b). These surveys incorporated a fully randomised, two-phase sampling design aimed at better estimating oyster densities and population sizes of oysters and new clocks. To make these surveys comparable from year to year, a standard Bonamia survey area was established (Figure 4) primarily using survey data and fishers' logbook data. This area represents the core commercial fishery that has been consistent through the fluctuations in relative oyster abundance driven by Bonamia mortality. The Bonamia survey area comprises 14 core strata of the 26 stock assessment survey strata sampled in 2012. This area represented 75% of the recruit-sized oyster population and 46% of the stock assessment survey area in 2012. The 12 remaining strata have been combined into a single background stratum to allow these data to be incorporated into stock assessments i.e., population estimates for the whole survey area are treated as a single stratum (Michael et al. 2015a). The background stratum is sampled with five stations. Surveys since 2014 have achieved CVs of 8% to 11.2% for recruit-sized oysters in the Bonamia survey area, and 7% to 12% for the whole population (Michael et al. 2015b).

Until 2012, Bonamia killed 8–12% of the recruit-sized (legal-sized) oyster population, and fishing removed a further 1–2%. The recruit-sized oyster population was increasing, albeit slowly, despite this Bonamia mortality. The increased numbers of oysters killed by Bonamia since 2013 (about 200 million oysters in 2013), and the continued low replenishment of spat to the oyster population and recruit-sized oysters to the fishery, has resulted in a significant decline in the recruit-sized oyster population size. The 2016 Bonamia survey showed some promising signs for the fishery as Bonamia infection and summer mortality were down and were much lower than in previous years. Summer mortality was 16.2 million oysters representing 4.2% of the recruit-sized population. There was also an upward trend in the population sizes of all three size groups of oysters surveyed. There was also a slight increase in spat settlement over the summer of 2015/16. The declining trend in the fishery from 2012 to 2015 had

levelled off in 2016. Because of the relatively low numbers of pre-recruit and small sized oysters, any rebuilding of the recruit-sized population is likely to be slow.

The 2017 stock assessment programme was funded by the Bluff Oyster Management Company Ltd. The stock assessment and Bonamia surveys were completed in February 2017 in collaboration with the Bluff Oyster Management Company. These surveys provide estimates of population size for three size groups of oysters and clocks, information on Bonamia infection and mortality, and an estimate of the size structure of the population. This report details the methods and results from these surveys.

The overall objective was to undertake a five-yearly stock assessment of OYU 5, and to begin to incorporate into the stock assessment model an early recruitment index as well as the capability for spatially explicit assessments. The research programme has seven objectives. This report is on the first four objectives related to the stock assessment and Bonamia surveys only. Remaining objectives will be reported on separately.

1.1 Specific survey objectives are:

1. To develop designs for the stock assessment and Bonamia surveys
2. To complete the stock assessment and Bonamia surveys
3. To test oyster samples for Bonamia infection
4. To complete the analysis and draft a report of the survey results

2. METHODS

2.1 Survey design

Survey strata for the February 2017 survey were the same as those in the February 2012 survey (Figure 5), but some strata are labelled differently (Table 1). Simulations to determine the numbers of stations required to give a CV in the range of 8–12% for the recruit-sized population estimate in the stock assessment survey area used NIWA software ALLOCATE (Francis 2006). These simulations predicted that 55 stations would produce a CV of about 11%. Station allocation to Bonamia survey strata is shown in Table 1. Three stations were allocated to each of the remaining 12 strata classified as background strata for Bonamia surveys. Rand_Stn (Doonan & Rasmussen 2012) was used to generate the location of 90 random, first-phase stations (Figure 5) and sufficient stations in each stratum to sample 12 second-phase stations. The second-phase stations were allocated to better estimate recruit-sized oysters and pre-recruits with a maximum of 9 second-phase stations for each size class, 12 second-phase stations in total with common stations to both size groups allocated as a single station in each stratum (Figure 5). Stations were generated with an exclusion zone of 0.75 nautical miles to spread stations within strata to ensure good spatial coverage and to prevent the overlap of sample tows. The 12 fixed stations were also sampled in February 2017 (Table 1, Figure 5) to provide a time series of changes in oyster density and Bonamia status in localised areas. The Fisheries New Zealand Shellfish Working Group agreed that they add value to the information obtained from these surveys.

Table 1: Stratum labels from the 2017 and 2012 surveys, and the Bonamia survey of 2016 stratum classification (BK, background stratum comprises 12 stock assessment strata combined).The numbers of first-phase stations allocated to each stratum (First-phase), the numbers of second-phase (Second-phase) and targeted sample stations (Target) in each stratum, and the area of each stratum (Area). The numbers of first-phase stations not sampled because of foul ground (Not sampled) are also shown.

Stratum 2017	Stratum 2012	Bonamia survey 2016	First-phase	Second-phase	Target	Area (km^2)	Not sampled
B1	B1	Bon	4	0	1	78.2	0
B1a	B1a	BK	3	0	-	16.0	0
B1b	B1b	BK	3	6	-	36.2	2
B2	B2	BK	3	0	-	17.9	0
B2a	B2a	BK	3	0	-	29.8	0
B2b	B2b	BK	3	0	-	83.3	0
B3	B3	Bon	3	3	-	44.7	0
B4	B4	BK	3	0	-	98.7	0
B5	B5	BK	3	0	-	63.6	2
B6	CB6	Bon	3	0	-	30.0	1
B6b	B6b	BK	3	0	-	19.8	2
B7	B7	BK	3	2	-	86.1	0
C1a	C1a	Bon	3	1	-	31.3	0
C2	C2	Bon	4	0	1	21.9	1
C3	C3	Bon	3	0	1	32.7	0
C4	C4	BK	3	0	-	26.3	2
C5	C5	Bon	4	0	1	37.7	1
C5a	C5a	Bon	3	0	1	23.5	1
C6	C6	BK	3	0	-	23.5	1
C6a	B6a	BK	3	0	2	77.1	1
C7	C7	Bon	6	0	-	36.1	0
C7a	C7a	Bon	3	0	-	23.6	0
C8	C8	Bon	4	0	1	26.8	0
C9	B9	Bon	6	0	-	34.5	1
E2	E2	Bon	3	0	2	42.8	0
E4	E4	Bon	5	0	2	28.0	0
26	26	-	90	12	12	1070.3	15

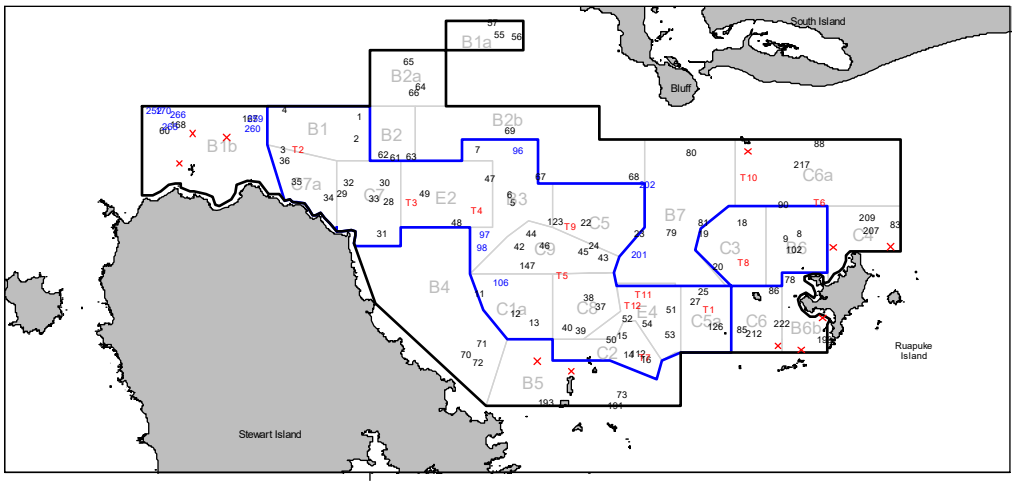

Figure 5: The 2017 stock assessment area with the 2007 survey boundary shown as a heavy, black outer line, the Bonamia survey area (core commercial fishery area) shown by heavy blue lines and the 2016 survey strata shown as grey lines.

Catch sampling

Dredge sampling followed standard procedures for stock assessment and Bonamia surveys between October 2002 and February 2016 (Michael et al. 2016). A commercial oyster vessel was used for these surveys (F.V. *Golden Quest*). Stephen Hawke (skipper) has run these surveys since 2011. Survey stations were sampled with the standard survey dredge (commercial dredge 3.35 m wide and weighing 430 kg) used since 1993 and rebuilt in 2014 to the same specifications. Since February 2016, dredges have been deployed using a hydraulic winch system that replaced the traditional friction winch used for surveys before 2014. Standard dredge sampling methods, standard methods for sorting the catch and recording data (data forms are shown in Appendix A.1 and A.2), and standard methods for sampling oysters to determine the status of Bonamia were used (see Appendix A.3).

In 2017, the catch was sorted into live oysters, gapers (live, but moribund oysters containing the whole oyster and valves remaining apart after the adductor muscle has lost its ability to contract), and clocks (the articulated shells of recently dead oysters with the ligament attaching the two valves intact) to estimate mortality. The catch was further sorted into two size groups: recruit-sized (unable to pass through a 58 mm internal diameter ring), and pre-recruits (able to pass through a 58 mm internal diameter ring, but unable to pass through a 50 mm ring). Live oysters were sorted into a third size group, small oysters (able to pass through a 50 mm internal diameter ring and down to 10 mm in length), see Appendix A.3 for details.

Estimates of oyster densities, population size and recruitment

Oyster densities and population sizes for the three size groups of live oysters were estimated for each of the 26 survey strata, for the 14 strata combined comprising the Bonamia survey area, for the 12 strata combined comprising the Bonamia survey background stratum, and for all 26 survey strata combined, which comprise the stock assessment survey area. Estimates are presented by strata where three or more randomly selected stations were sampled in February 2017 and these were compared with the estimates from strata sampled in the 2012 stock assessment and 2016 Bonamia surveys. Estimates for the three size groups of live oysters and recruit-sized 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. Estimates of the commercial population size (Michael et al. 2015a) are given for comparison.

Estimates of absolute abundance and variance were calculated using standard stratified random sampling theory (Francis 1984, Jolly & Hampton 1990). We used an estimate of dredge efficiency from Dunn (2005), 0.17 (95% confidence intervals 0.13–0.22) re-estimated from the 1990 data of Doonan et

al. (1992) as a single scalar, and hence calculated the absolute population size of recruit, pre-recruit, small oysters, and clocks using the combined population sizes in each stratum as,

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

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

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

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

where $s(\bar{x})$ is the standard deviation for the estimated population size and $s(\bar{x}_i)$ is the standard deviation for the mean density in stratum *i*.
The 95% confidence intervals of the population means for each stratum and the total population are estimated by resampling a normal distribution whose variance is based on a CV and the error of the estimated dredge efficiency. The total error of the estimates of the population mean has two sources: one is the sampling error from the survey, where the survey estimate of population size follows a normal distribution and this is based on standard survey sampling theory. The other source is error associated with dredge efficiency, which is assumed to be normally distributed (there are only three data points). If the two sources of error are independent, then the error can be estimated by simply adding the two variance components.

Recruitment to the fishery was summarized using plots of changes in the population estimates of pre-recruit and small oysters, and from changes in the patterns of distribution of small oyster densities, between the February 2012, February 2016 and February 2017 surveys.

2.2 Methods to estimate annual mortality from Bonamia

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

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

up numbers of fatally infected oysters in each station sample. Detailed methods are given in Appendix A.3.

Methods to estimate the prevalence and intensity of Bonamia infection

Definitions and details of the methods used to estimate the prevalence and intensity of Bonamia infection are given in Appendix A.3. The numbers of infected recruit-sized oysters were estimated from heart imprints and the quantitative polymerase chain reaction (qPCR) assays. Estimates of prevalence from heart imprints assumed that oysters that tested negative for Bonamia using qPCR for heart tissue analysis were also negative for heart imprints. The numbers of non-fatally and fatally infected oysters were estimated from Bonamia intensity scores derived from histology and scaled up to the size of the recruit-sized oyster population by strata and for the survey areas.

Histology and quantitative polymerase chain reaction sampling methods

Station and sample data were recorded on Bonamia sampling forms (Appendix A.2), and the total numbers of live and dead oysters in the samples noted. A subsample of up to 25 recruit-sized oysters from each station was taken for heart imprints and qPCR. Each oyster in the sample was assigned a unique number from 1 to 25, a size category using oyster size rings, and measured for length and height (see Michael et al. 2015c for details). In 2017, small oysters were denoted with an S (small oysters were denoted with an O in previous surveys). Gaping oysters were marked with an asterisk alongside the corresponding oyster number. Oysters were recorded as either incubating white larvae (early-stage), grey larvae (late-stage), yellow larvae (almost ready to settle); or with no larvae present.

Heart imprints were made using standard methods. Histological samples were taken from the first five oysters processed for heart imprints (see Appendix A.3 for details) as in previous surveys. Oysters sampled for heart imprints were also sampled using qPCR. Laboratory work sheets recorded sampling data including: date, name of sampler, plate number and station number and the date and time the sample was collected. The prevalence of infection was first determined by qPCR methods and then heart imprints. Samples of oysters to be scored for intensity of infection were determined from the results of the qPCR testing using standard methods (Michael et al. 2015c).

A detailed account of the qPCR method and testing is given in Maas et al. (2013) and summarised in Appendix A.3. This method includes a duplex qPCR assay (the co-amplification of the Bonamia target (ITS region of the ribosomal genes) and *Ostrea chilensis* β-actin gene as an internal control), a new master mix that is able to cope with inhibitors often found in crude extracts, and a system to delay the amplification of the internal control to prevent a low level Bonamia ITS amplification being outcompeted by the stronger internal control (β-actin) reaction. This method uses a 96 well-plate format, and analysis is undertaken with a BIORAD-CFX96 qPCR instrument.

Methods for qPCR quality control

Before the samples from the 2017 survey were analysed, quality control of reagents and methods was undertaken. A synthetic standard for Bonamia (dnature LTD) which incorporates the primer and probe sequences, was serially diluted in an oyster lysate (oyster DNA extracted using the same method used for testing Bonamia) down to 1 copy per µl. Two µl of this lowest dilution (i.e., containing two copies) was tested with the Bonamia duplex assay and the two copies of Bonamia were reliably detected.

The Bonamia qPCR primer/probe mix incorporated primers and probes for the Bonamia target and internal control as well as the BLOCK system to prevent the high level endogenous internal control outcompeting a low level Bonamia target. Resulting lots of this mix were tested on the synthetic template standard dilutions to ensure that the same sensitivity was maintained (i.e. detection of the 1 copy/ µl dilution). Aliquots of a 10^2 copies/µl dilution of synthetic standard were included as positive controls for each run of a 96-well plate as inter-plate calibrators to permit collation of data among multiple runs. The false-positive rate was estimated using a ddPCR test of oyster samples known to be negative for Bonamia. The risk of false positives was also monitored throughout the survey in negative template controls included on each plate and it did not exceed the detection limit determined by serial dilution.

The qPCR data were analysed using BioRad CFX Manager™ software (Version 3.0), and if needed, qPCR assays were repeated based on the criteria given in Maas et al. (2013). The cycle of quantification (Cq) cut-off to determine positives from false positives was derived from a standard curve analysis of serial dilutions of the standard to extinction and maintained at Cq 35.

In 2017 as in 2016, only 25 heart tissues were analysed for infection using qPCR. Previously, both heart and gill were analysed. All heart imprint slides for those samples that tested positive for Bonamia infection were examined. At least three samples that were qPCR negative were also randomly selected from the remaining samples from each station. Repeated samples that gave anomalous results such as flat-liners where no reaction was detected or those samples that showed early amplification (very low Cq values) were also scored from heart imprints.

Heart imprints were examined based on the methods of Michael et al. (2016), and imprints scored based on a categorical scale (Diggles et al. 2003). Heart imprints were examined by a single experienced reader, and a review of scoring protocols was undertaken before screening samples. Three good heart imprints containing oyster haemocytes were located and examined on each slide, and the number of Bonamia cells counted for each. If no Bonamia cells were found, further imprints were examined to confirm the absence of Bonamia.

Changes to the qPCR sampling method for the detection of Bonamia in oyster tissues

The oyster samples were tested for the presence of Bonamia infection using the qPCR method established in 2013 (Maas et al. 2013). Previous surveys tested heart and gill samples. In 2017, only heart tissues ($N \leq 25$ per station) were tested. Samples were tested on a 96 well plate format. All plates were run with serial dilutions of the synthetic standard as positive controls and a single well of deionised distilled water as a negative control. Samples that showed anomalies in the qPCR data were rerun. The repeat scores were used in the analysis for presence/absence.

After the initial qPCR screening for prevalence of Bonamia infection, heart imprint slides were selected to estimate prevalence and the intensity of infection, and false positives and false negatives based on four criteria:

1. All corresponding heart imprint slides for those qPCR samples that tested positive for Bonamia infection (heart samples only in 2017).

2. At least three heart imprint samples randomly selected from each station that were qPCR negative.

3. All corresponding heart imprint slides for heart qPCR samples that did not amplify both fluorophores (flatliners): FAM (6-carboxyfluorescein) used to detect Bonamia, and TR (Texas-red, sulforhodamine 101 acid chloride) was used as a cross check to ensure that the qPCR reaction occurred by detecting oyster tissue DNA in the sample.

4. All corresponding heart imprint slides for heart qPCR samples that were "early ampers", i.e., the samples where either or both fluorophores amplified very early in the cycling (Cq less than 10 cycles).

The standard methods of Michael et al. (2015b) were used to estimate prevalence and intensity of Bonamia infection and scaled to population estimates of Bonamia infection.

3. RESULTS

3.1 Summary of survey details

NIWA and the Bluff Oyster Management Company Ltd completed the 2017 stock assessment and Bonamia surveys between the 6th and 20th of February. Sampling was undertaken using the F.V. *Golden Quest* (an oyster vessel) and with oyster industry staff. A larger number of days were lost to rough sea conditions in 2017 than in most of the recent surveys. We completed sampling in eight survey days, in mostly good sea and tidal conditions for dredging. Sampling conditions and dredge efficiency were consistent with previous surveys (see Appendix B for details on survey comparability). Dredge tow lengths were almost all about 0.2 nautical miles. All oyster and clock densities were adjusted to the 0.2 nautical mile standard tow length for analysis. Most dredge sampling was undertaken in wind conditions less than 10 knots, in similar conditions to those of recent surveys. This wind range and the resulting sea conditions were below the level likely to affect dredge efficiency, but any gains in efficiency may have been moderated by the high tidal flows (spring tides) over some of the 2017 sampling period that reduce dredge efficiency (Stephen Hawke, oyster vessel skipper, pers. comm.).

Six stations (2, 3, 12, 28, 50, and 112) were landed over 70% full in 2017 with catches ranging from 31 to 261 recruit-sized oysters. Oyster densities may have been underestimated at these stations. Dredge efficiency is thought to be greatly reduced in areas densely populated with kaeos (*Pyura pachydermatina*) as the dredge skims above the seabed with little or no contact. Large numbers of kaeos were caught in strata E4 (stations 51 and 53), C6a (station T10), and, B6b (station 199). Very few oysters were caught there, and oyster density was most likely underestimated at these stations.

In all, we sampled 114 stations (see Table 1) and measured about 13 450 oysters for size structure. We aimed to collect 30 recruit-sized oysters from each of the 102 stations, to provide tissue samples for qPCR and heart imprints (N=25), and histology (N=5). Target sample size was achieved from 79 stations. At sites where fewer than 30 recruit-sized oysters were caught, samples included pre-recruit and small oysters: stations 1 (N=14), 70 (N=19), 71 (N=22), 35 (N=26), and 63 (N=27). At stations 80 (N=7), 69, 29 & 47 (N=12), 217 (N=15), 209 (N=17), and 20 & 126 (N=20) fewer than 30 oysters of all sizes were caught. We tested 83 samples of oysters for Bonamia. No oysters were sampled from second-phase stations, or at stations 57, 66, 72, 73, 78, 88, 199, 54, 55, 56, 65, 222, 51, 53, and 191. 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.

Observations from the survey suggest little pre-survey mortality, widespread distribution of oysters, but few high counts (500 – 1 000+ oysters per standard survey tow). Numbers of pre-recruit sized oysters are still low suggesting slow rebuilding of the population. There were some signs of heightened, localised oyster spat settlement. The numbers of spat were generally similar to 2016; however, a few stations had high counts.

3.2 Oyster abundance

Changes in oyster densities between 2012, 2016 and 2017

Figures 6 to 11 show plots of catches adjusted to the standard tow length (0.2 nautical miles) for the 2012 and 2017 stock assessment surveys, and for the 2016 and 2017 Bonamia survey areas. Each figure shows mean catches and 95% confidence intervals by stratum for each size group (recruit-sized, pre-recruit and small oysters). Strata are arranged west to east with northern strata at similar longitudes shown before those to the south.

Catches of recruit-sized and pre-recruit oysters in the stock assessment survey area have declined between 2012 and 2017 (Figures 6 and 8). The most notable change in recruit-sized oysters is the

absence of tows with 600–1500 oysters per tow in 2017 that were common in 2012. Catches in a few strata remained similarly low (e.g., B4 and B6b), while a few had consistently higher catches (e.g., C9 and B6). Catches of pre-recruit oysters were much lower than for recruit-sized oysters. Catches from many of the strata in 2017 were below 100 pre-recruit oysters per tow. Catches of small oyster were low in 2017 as they were in 2012 (Figure 10). Some western strata (C7 and E2) and central strata (C2, C5, C8) recorded higher catches of small oysters in 2017 than in 2012.

Catches of all three size groups of oysters in the Bonamia survey area were generally similar at stratum level between 2016 and 2017 (Figures 7, 9, and 11). Fewer catches above 500 recruit-sized oysters per tow were recorded in 2017 (Figure 7). Pre-recruit oysters were similar across all strata in 2016 and 2017 (Figure 9), as mostly were small oysters (Figure 11), however some western strata (C7, E2, and B3) had higher catches of small oysters in 2017 compared with 2016.

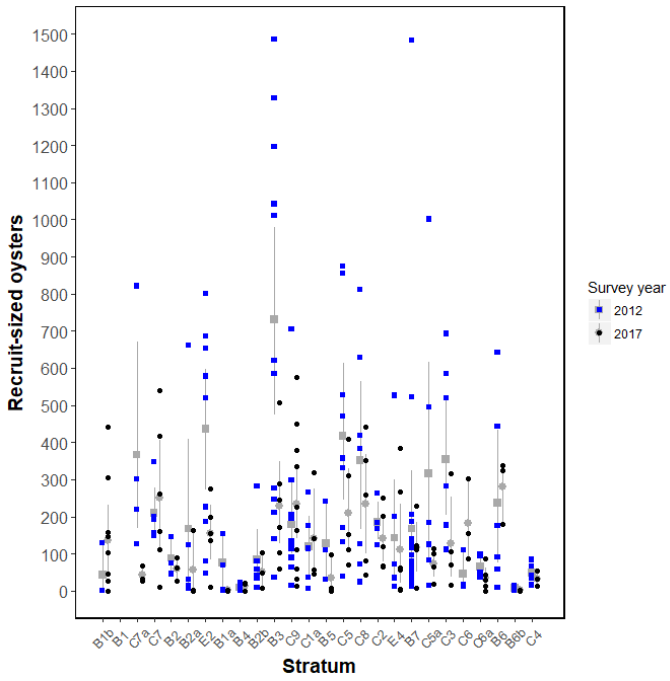

Figure 6: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized oysters, their means and 95% confidence intervals (grey line) by stock assessment survey stratum sampled during the 2012 (blue filled circles) and 2017 (black filled circles) surveys. Grey filled squares (2012) and grey filled circles (2017) represent annual strata means. Strata are arranged west to east with northern strata at similar longitudes shown first.

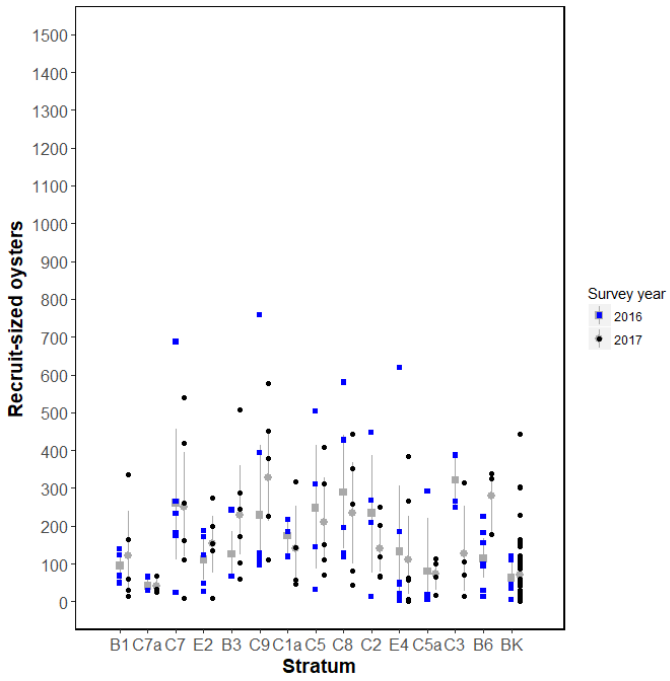

Figure 7: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized oysters, their means and 95% confidence intervals (grey line) by Bonamia survey stratum sampled during the 2016 (blue filled circles) and 2017 (black filled circles) surveys. Grey filled squares (2016) and grey filled circles (2017) represent annual strata means. Strata are arranged west to east with northern strata at similar longitudes shown first.

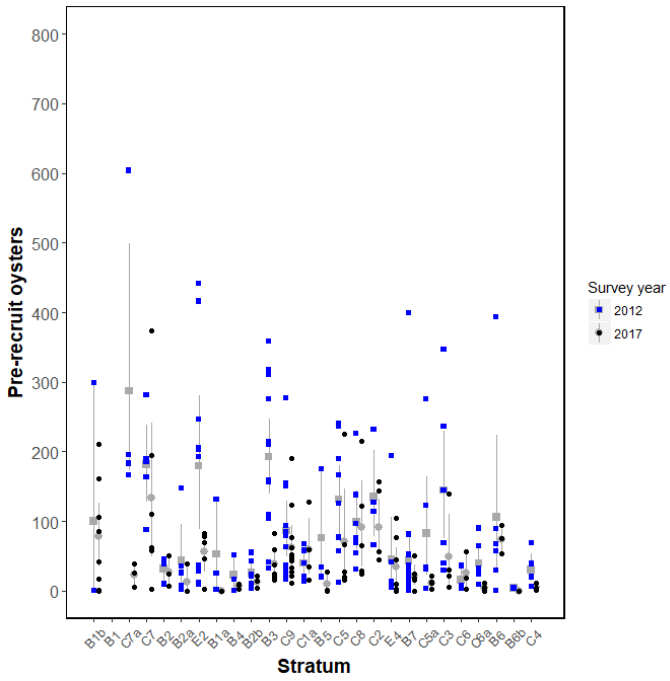

Figure 8: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for pre-recruit oysters, their means and 95% confidence intervals (grey line) by stock assessment survey stratum sampled during the 2012 (blue filled circles) and 2017 (black filled circles) surveys.Grey filled squares (2012) and grey filled circles (2017) represent annual strata means. Strata are arranged west to east with northern strata at similar longitudes shown first.

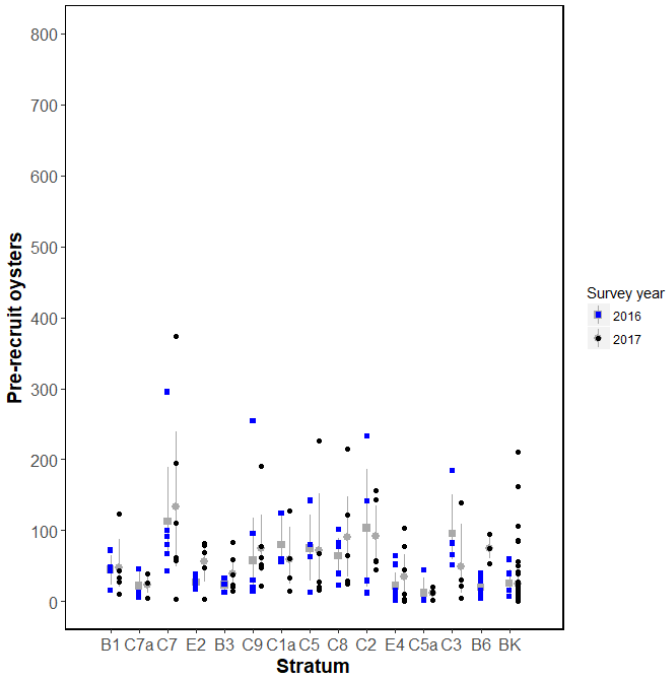

Figure 9: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for pre-recruit oysters, their means and 95% confidence intervals (grey line) by Bonamia survey stratum sampled during the 2016 (blue filled circles) and 2017 (black filled circles) surveys. Grey filled squares (2016) and grey filled circles (2017) represent annual strata means. Strata are arranged west to east with northern strata at similar longitudes shown first.

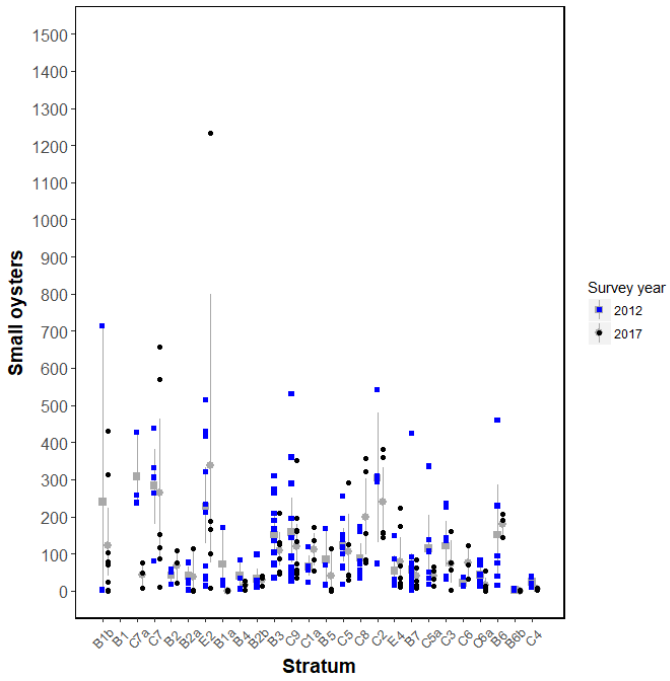

Figure 10: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for small oysters, their means and 95% confidence intervals (grey line) by stock assessment survey stratum sampled during the 2012 (blue filled circles) and 2017 (black filled circles) surveys. Grey filled squares (2012) and grey filled circles (2017) represent annual strata means. Strata are arranged west to east with northern strata at similar longitudes shown first.

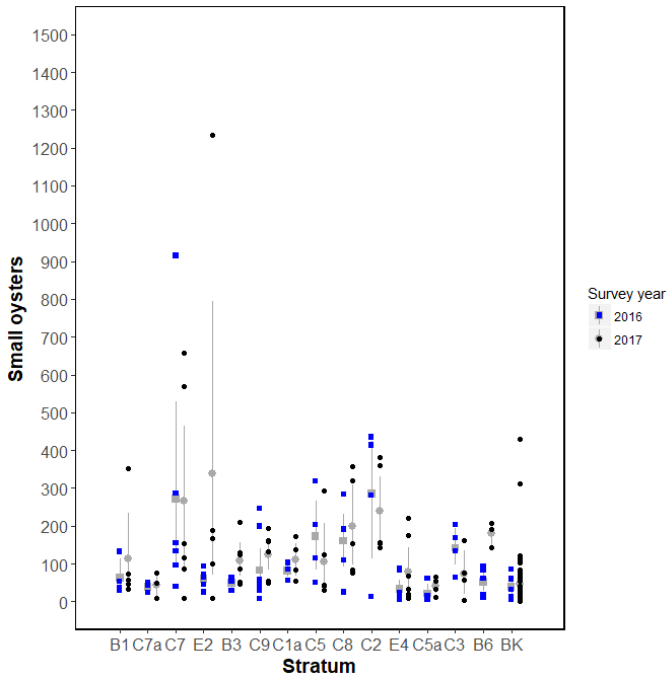

Figure 11: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for small oysters, their means and 95% confidence intervals (grey line) by Bonamia survey stratum sampled during the 2016 (blue filled circles) and 2017 (black filled circles) surveys. Grey filled squares (2016) and grey filled circles (2017) represent annual strata means. Strata are arranged west to east with northern strata at similar longitudes shown first.

Estimates of population size

Estimates of absolute population size for recruit-sized, pre-recruit, and small oysters from the February 2012, 2016, and 2017 surveys are shown in Tables 2 to 5. These tables show population estimates for the core strata (N = 14: B1, B3, B6, C1a, C2, C3, C5, C5a, C7, C7a, C8, C9, E2, and E4), all core strata combined, the background stratum (all background strata combined, N = 12: B1a, B1b, B2, B2a, B2b, B4, B5, B6b, B7, C4, C6, and C6a), and the whole 2007 stock assessment survey area. Ninety five percent confidence intervals are bootstrapped estimates from resampling a normal distribution whose variance is based on a CV and the error of the estimated dredge efficiency. Bootstrapped estimates are likely to better represent the true range of the population estimates. These tables also show changes in the population sizes between 2012, 2016 and 2017 by stratum (where available); and the percentages of population size between 2012 and 2017, and 2016 and 2017 for the Bonamia survey area (core strata), background strata, and the stock assessment survey area.

Changes between the 2012 and 2017 stock assessment surveys

All three size groups of oysters have declined between 2012 and 2017 (Tables 2 to 5). However, on a stratum by stratum basis, oyster numbers increase in some strata. Strata that recorded increases for recruit sized oysters generally had increases of pre-recruit and small oysters.

The 2012 and 2017 surveys of the population size of recruit-sized oysters in the stock assessment survey area attained low CVs (8–9%), well below the Fisheries New Zealand target CV of 20% (Table 5). Recruit-sized oyster density declined by 42.6%, as did population size from 918.4 million oysters in 2012 to 527.4 million oysters in 2017 (Table 2). Recruit-sized oyster density also declined by 47.2% in the core commercial strata (Bonamia survey area), as did the population size from 688.1 million oysters in 2012 (CV 9%) to 363.6 million oysters in 2017 (CV 11%). The recruit-sized oyster population increased in five (B6, C1a, C7, C9, and E4) of the 14 Bonamia survey strata and three (B1b, B4, and C6) of the background strata (Table 2).

Pre-recruit sized oyster density declined by 59.4% in the stock assessment survey area and population size declined from 414.3 million oysters in 2012 (CV 10%) to 168.2 million oysters in 2017 (CV 10%, Tables 3 and 5). Pre-recruit sized oysters in the core commercial strata declined 58.6%, from 297.4 million oysters in 2012 (CV 10%) to 123.1 million oysters in 2017 (CV 12%). Population size increased in four strata (C1a, C6, C9, and E4, Table 3).

Small sized oyster density declined by 40.9% in the stock assessment survey area and population size declined from 612.2 million oysters in 2012 (CV 14%) to 361.6 million oysters in 2017 (CV 9%, Tables 5 and 6). Small sized oysters in the core commercial strata declined 42.0%, from 451.3 million oysters in 2012 (CV 16%) to 261.9 million oysters in 2017 (CV 10%). The population of small oysters increased in five (B6, C1a, C8, C9, and E4) of the 14 Bonamia survey strata and in two (B2 and C6) of the background strata (Table 4–3).

Changes between the 2016 and 2017 in the Bonamia survey area (core strata)

Changes in densities and population sizes for the three size groups of oysters in the Bonamia survey area and all background strata combined are given in Tables 2 to 5. Oyster population sizes in the background stratum are not likely to be well estimated because of the small numbers of stations sampled (N=5) over a large area (578.4 km^2).

The recruit-sized oyster population size declined by 6.0% over the stock assessment survey area from 561.1 million oysters (CV 7%) in 2016 to 527.4 million oysters (CV 9%) in 2017 (Table 2). Recruit-sized oysters also declined by 5.6% in the Bonamia survey area from 385.2 million oysters in 2012 (CV 9%) to 363.6 million oysters in 2017 (CV 11%). The recruit-sized oyster population increased in five strata (B3, B6, C5a, C7a, and C9) of the Bonamia survey strata (Table 2).

Pre-recruit sized oyster density declined by 12.0% in the stock assessment survey area and population size declined from 191.2 million oysters in 2016 (CV 3%) to 168.2 million oysters in 2017 (CV 10%, Tables 3 and 5). Pre-recruit sized oysters in the Bonamia survey area increased 2.2%, from 120.5 million oysters in 2016 (CV 3%) to 123.1 million oysters in 2017 (CV 12%). Population size increased in eight Bonamia survey strata (B3, B6, C5a, C7, C7a, C8, C9, and E2, Table 3).

Small sized oyster density declined by 0.7% in the stock assessment survey area and the population size declined from 364.3 million oysters in 2016 (CV 5%) to 361.6 million oysters in 2017 (CV 9%, Tables 4 and 5). Small sized oysters in the Bonamia survey area increased 2.3%, from 256.1 million oysters in 2016 (CV 7%) to 261.9 million oysters in 2017 (CV 10%). Population size increased in nine Bonamia survey strata (B3, B6, C1a, C5a, C7a, C8, C9, E2, and E4, Table 4).

Table 2: Changes in the population size of recruit-sized oysters (millions of oysters) between 2012, 2016 and 2017 by stratum (where available); 95% confidence intervals for the 2017 estimates, and the 2017 population size expressed as a percentage of the 2012 and 2016 sizes, in the Bonamia survey area, background strata, and the stock assessment survey area. Increases are shown in pale green and decreases in salmon highlights.

Bonamia survey area						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1	54.6	39.2	23.7	1.1	51.5	43.5	60.6
B3	158.9	27.2	49.9	5.8	105.3	31.4	183.5
B6	34.4	19.6	41.0	22.9	66.4	119.3	209.4
C1a	18.2	26.2	21.3	2.5	45.2	116.9	81.2
C2	21.2	23.7	12.1	5.2	21.7	57.3	51.2
C3	47.1	47.9	20.8	5.8	40.7	44.2	43.5
C5	74.5	29.8	29.5	9.9	55.1	39.5	98.8
C5a	31.6	1.1	7.1	1.7	14.2	22.3	641.3
C7	36.4	45.6	44.0	14.3	83.2	121.0	96.6
C7a	42	4.5	4.9	1.8	9.0	11.6	108.5
C8	44.3	33.3	23.9	4.7	48.1	54.0	71.8
C9	35.8	37.9	54.8	28.4	91.9	153.2	144.7
E2	80.3	24.9	20.8	1.7	44.4	25.9	83.6
E4	8.8	24.1	9.6	0.0	25.0	109.6	40.0
Subtotal	**688.1**	**385.2**	**363.6**	**233.9**	**559.1**	**52.8**	**94.4**

Table 2: Continued.

Background strata						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1a	5.9		0.1	0.0	0.3	2.2	-
B1b	7.7		24.6	5.5	48.3	319.0	-
B2	7.7		5.3	1.9	9.7	68.2	-
B2a	24.1		8.2	0.0	25.7	34.2	-
B2b	33.7		21.5	0.0	47.8	63.7	-
B4	4.5		6.4	0.2	14.1	142.8	-
B5	39.9		10.7	0.0	32.1	26.9	-
B6b	0.8		0.1	0.0	0.3	16.0	-
B7	69.9		49.5	19.1	90.5	70.8	-
C4	6.3		4.1	1.2	7.9	65.1	-
C6	5.3		20.8	6.4	39.9	392.3	-
C6a	24.7		12.5	0.0	35.2	50.7	-
Subtotal	**230.5**	**176**	**163.9**	**95.4**	**260.4**	**65.2**	**85.3**
Stock assessment survey area						**Percentage Change**	**Percentage Change**
	2012	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
Total	**918.4**	**561.1**	**527.4**	**343.1**	**797.7**	**57.4**	**94.0**

Table 3: Changes in the population size of pre-recruit oysters (millions of oysters) between 2012, 2016 and 2017 by stratum (where available); 95% confidence intervals for the 2017 estimates, and the 2017 population size expressed as a percentage of the 2012 and 2016 sizes, in the Bonamia survey area, background strata, and the stock assessment survey area. Increases are shown in pale green and decreases in salmon highlights.

Bonamia survey area						**Percentage**	**Percentage**
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1	46.6	17.2	10.4	5.2	17.6	22.3	60.4
B3	41.8	5.1	9.9	2.9	18.8	23.6	193.7
B6	15.5	3.5	10.9	6.4	17.3	70.4	311.8
C1a	6	12	9.0	1.6	18.3	149.5	74.8
C2	14.3	13.2	8.1	3.1	14.8	56.5	61.2
C3	17.5	10.6	3.6	1.8	6.2	20.8	34.3
C5	21.8	9.6	6.0	1.8	11.6	27.4	62.3
C5a	8.4	0.2	1.0	0.2	2.1	12.3	517.7
C7	31.6	19.7	23.6	4.6	48.1	74.6	119.6
C7a	32.9	2.4	2.7	0.4	5.6	8.2	112.9
C8	13.7	7.8	12.8	0.8	27.3	93.2	163.6
C9	9	9.7	12.6	4.4	23.6	139.9	129.8
E2	36.2	6.1	9.2	0.0	20.5	25.3	150.4
E4	2.1	3.7	3.4	0.0	8.3	164.2	93.2
Subtotal	**297.4**	**120.5**	**123.1**	**77.5**	**191.7**	**41.4**	**102.2**

Table 3: Continued.

Background strata						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1a	4.1		0.0	0.0	0.0	0.0	-
B1b	17.5		14.3	5.1	26.4	81.5	-
B2	2.8		2.4	0.2	5.1	85.5	-
B2a	6.3		1.9	0.0	6.2	30.6	-
B2b	10.3		5.4	1.2	10.8	52.7	-
B4	11		3.3	0.9	6.4	30.0	-
B5	23.9		3.0	0.0	9.2	12.5	-
B6b	0.4		0.0	0.0	0.0	0.0	-
B7	18.2		9.2	2.3	17.9	50.4	-
C4	3.9		0.7	0.0	1.6	17.3	-
C6	1.8		3.3	0.1	7.1	182.2	-
C6a	16.5		1.6	0.0	4.6	9.9	-
Subtotal	**116.7**	**70.7**	**45.0**	**26.0**	**71.5**	**38.6**	**63.7**
Stock assessment survey area						**Percentage**	**Percentage**
	2012	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
Total	**414.3**	**191.2**	**168.2**	**108.9**	**253.4**	**40.6**	**88.0**

Table 4: Changes in the population size of small oysters (millions of oysters) between 2012, 2016 and 2017 by stratum (where available); 95% confidence intervals for the 2017 estimates and the 2017 population size expressed as a percentage of the 2012 and 2016 sizes, in the Bonamia survey area, background strata, and the stock assessment survey area. Increases are shown in pale green and decreases in salmon highlights.

Bonamia survey area						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1	88	48.9	19.3	11.2	30.9	21.9	39.4
B3	32.8	10.6	26.0	10.4	47.0	79.4	245.6
B6	22.1	9.7	26.4	16.8	39.9	119.3	271.8
C1a	9.8	12.3	16.9	8.2	29.0	172.7	137.6
C2	30.4	25.8	21.8	10.1	38.1	71.8	84.6
C3	16.1	19.4	9.8	3.5	18.4	60.7	50.4
C5	20.9	22.7	10.9	3.0	21.1	52.1	48.0
C5a	12.2	0.9	5.1	1.4	10.0	41.5	562.7
C7	49.2	47.5	47.0	7.0	96.7	95.6	99.0
C7a	95.5	4.3	5.1	0.7	10.6	5.3	117.8
C8	11.7	19.8	27.4	7.5	52.9	233.8	138.2
C9	10.3	13.8	21.0	11.3	34.5	203.7	152.0
E2	47.5	14.9	16.1	1.3	34.4	33.9	108.0
E4	4.9	5.6	9.2	0.0	21.8	188.2	164.7
Subtotal	**451.4**	**256.1**	**261.9**	**168.8**	**401.6**	**58.0**	**102.3**

Table 4: Continued.

Background strata						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1a	5.5		0.1	0.0	0.1	0.9	-
B1b	42.0		22.5	3.5	45.7	53.5	-
B2	3.6		6.1	1.5	12.1	169.0	-
B2a	5.9		5.8	0.0	17.9	97.9	-
B2b	13.1		11.8	4.7	21.1	90.0	-
B4	19.5		6.9	0.3	14.9	35.2	-
B5	26.6		12.3	0.0	37.6	46.1	-
B6b	0.2		0.0	0.0	0.1	15.9	-
B7	23.6		16.5	4.5	32.3	70.1	-
C4	3.2		0.6	0.1	1.2	18.5	-
C6	2.4		8.6	2.8	16.3	357.7	-
C6a	15.3		8.6	0.0	22.6	56.5	-
Subtotal	**160.9**	**108.2**	**99.7**	**55.0**	**162.0**	**62.0**	**92.1**
Stock assessment survey area						**Percentage**	**Percentage**
	2012	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
Total	**612.2**	**364.3**	**361.6**	**234.9**	**546.9**	**40.6**	**88.0**

Table 5: Percentage changes in the absolute population estimates from randomly allocated stations only for recruit-sized, pre-recruit, and small oysters in the core strata, and for the whole 2007 stock assessment area (26 strata) sampled in 2012, 2016, and 2017. The mean oyster density per m^2 (Mean density), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), bootstrapped upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the percentage change in population size. Increases are shown in pale green and decreases in salmon highlights.

2012

Core Strata	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI
Recruit	1.4	0.09	688.1	449.2	1046.7
Pre-recruit	0.6	0.1	297.4	192.6	454.4
Small	0.92	0.16	451.3	261.5	731.7
Survey total					
Recruit	0.86	0.08	918.4	600.1	1383.7
Pre-recruit	0.39	0.1	414.3	267.8	629
Small	0.57	0.14	612.2	370.3	967.9

2016

Core Strata	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2012/2016
Recruit	0.78	0.09	385.2	246.9	593.8	-44.0
Pre-recruit	0.25	0.03	120.5	186.7	491.8	-59.5
Small	0.52	0.07	256.1	155	407.3	-43.3
Survey total						
Recruit	0.52	0.07	561.1	341.6	866.7	-38.9
Pre-recruit	0.18	0.03	191.2	109.9	304.8	-53.8
Small	0.34	0.05	364.3	215.9	570.6	-40.5

2017

Core Strata	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2016/2017	% change 2012/2017
Recruit	0.74	0.11	363.6	233.9	559.1	-5.6	-47.2
Pre-recruit	0.25	0.12	123.1	77.5	191.7	2.2	-58.6
Small	0.53	0.10	261.9	168.8	401.6	2.3	-42.0

Table 5: Continued.

Survey total	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2016/2017	% change 2012/2017
Recruit	0.49	0.09	527.4	343.1	797.7	-6.0	-42.6
Pre-recruit	0.16	0.10	168.2	108.9	253.4	-12.0	-59.4
Small	0.34	0.09	361.6	234.9	546.9	-0.7	-40.9

Population estimates from stock assessment surveys 1999 to 2017

Estimates of population size for recruit-sized, pre-recruit, and small oysters from stock assessment surveys 1999 to 2017 are shown in Table 6 and Figure 12.

The estimates of mean population size of recruit-sized oysters have cycled up and down since the 1999 survey. Population size declined from 1461 million oysters in October 1999 to 408 million oysters in January 2005, increased to 913 million oysters in February 2012, and then declined again to 527 million oysters in February 2017 (Table 6). Pre-recruit oysters declined from 899 million oysters in 1999 to 414 million oysters in 2005, remained generally static until 2012, and declined further to 168 million in 2017. Small oysters remained similar between 1373 million in 1999 and 1344 million in 2005, and then declined to 362 million in 2017.

Table 6: Absolute population estimates for recruit-sized, pre-recruit, and small oysters from stock assessment surveys 1999–2017. Mean population size (millions of oysters) with upper and lower 95% confidence intervals in parenthesis. Estimates exclude stratum B1a.

Survey	Recruits		Pre-recruits		Small	
1999 (October)	1461	(872–2334)	899	(570–1387)	1373	(874–2115)
2001 (October)	995	(632–1511	871	(548–1330	1410	(884–2156)
2002 (October)	502	(310–785)	520	(333–795)	1243	(806–1884)
2005 (January)	408	(253–628)	414	(247–652)	1344	(845–2056
2007 (February)	622	(398–947)	463	(293–708)	842	(546–1273)
2009 (February)	720	(470–1085)	354	(228–538)	889	(574–1351)
2012 (February)	913	(603–1376)	410	(268–623)	607	(369–952)
2017 (February)	527	(343–798)	168	(109–253)	362	(235–547)

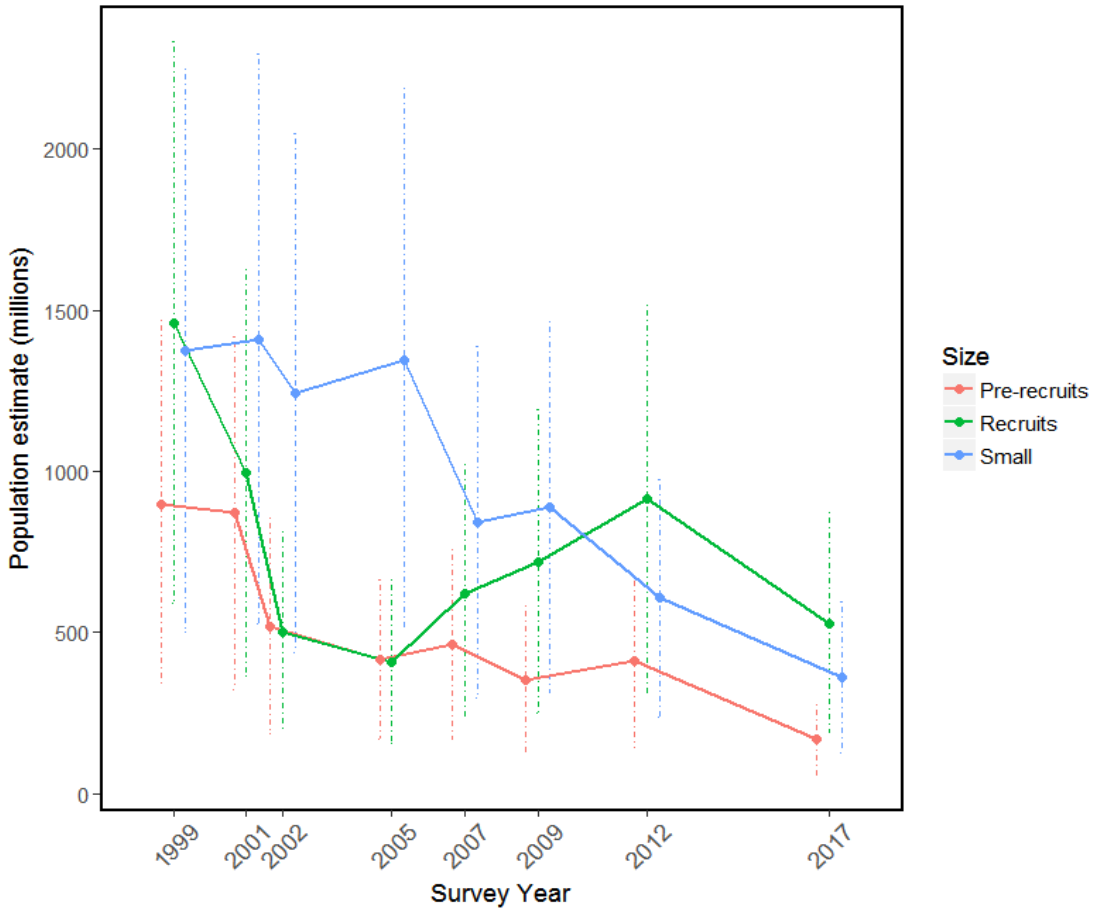

Figure 12: Mean population estimates for recruit-sized (green), pre-recruit (salmon), and small oysters (blue) from stock assessment surveys between 1999 and 2017. 95% confidence intervals shown in dashed lines of the same colour.

Estimates of "commercial" population size 2012, 2016 and 2017

In 1995 and 1997, the commercial population used to estimate yield was estimated as the percentage of the entire population above a density of 400 oysters per tow (equivalent to about 6–8 sacks per hour during commercial dredging). This threshold was based on an historical, economic catch rate, and when the catch rate dropped below 6 sacks per hour, it was assumed that 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 stock assessment reports.

Table 7 shows estimates of commercial population size, using the catch of recruit-sized oysters at each station minus 400 oysters, for the 2012 and 2017 stock assessment area and for the 2016 Bonamia survey area. Three Bonamia survey strata (B3, C7, and C9) supported commercial densities in 2017 compared to four in 2016 and nine in 2012. One background stratum (B1b) supported commercial densities in 2017 compared to two (B2a and B7) in 2012.

The commercial population size declined 80.3% from 473.9 million oysters in 2012 to 88.8 million oysters in 2016 and increased slightly by 5.3% to 93.5 million oysters in 2017 (Table 7). The declines in the oyster populations are consistent with the levels of Bonamia mortality observed in the fishery and the prolonged period of relatively low recruitment.

Table 7: Changes in the population size of recruit-sized oysters (millions of oysters) above a density of 400 oysters per survey tow (equivalent to about 6–8 sacks per hour in commercial dredging) between 2012, 2016 and 2017 by stratum (where available); and the 2017 population size expressed as a percentage of the 2012 and 2016 sizes, in the Bonamia survey area, background strata, and the stock assessment survey area. Increases are shown in pale green and decreases in salmon highlights.

Bonamia survey area						**Percentage**	**Percentage**
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1	0	0	0.0	0.0	0.0	21.9	39.4
B3	143.7	0	27.8	0.0	87.5	79.4	245.6
B6	26.4	0	0.0	0.0	0.0	119.3	271.8
C1a	0	0	0.0	0.0	0.0	172.7	137.6
C2	0	16	0.0	0.0	0.0	71.8	84.6
C3	29.2	0	0.0	0.0	0.0	60.7	50.4
C5	50.1	0	0.0	0.0	0.0	52.1	48.0
C5a	22.3	0	0.0	0.0	0.0	41.5	562.7
C7	0	20.3	28.2	0.0	69.0	95.6	99.0
C7a	23.6	0	0.0	0.0	0.0	5.3	117.8
C8	31.2	19.1	0.0	0.0	0.0	233.8	138.2
C9	19.9	15.9	28.7	0.0	71.5	203.7	152.0
E2	66.4	0	0.0	0.0	0.0	33.9	108.0
E4	0	17.5	0.0	0.0	0.0	188.2	164.7
Subtotal	**412.8**	**88.8**	**85.6**	**9.7**	**175.7**	**58.0**	**102.3**

Table 7: Continued.

Background strata						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1a	0.0		0.0	0.0	0.0	-	-
B1b	0.0		8.9	0.0	28.6	-	-
B2	0.0		0.0	0.0	0.0	-	-
B2a	19.1		0.0	0.0	0.0	0.0	-
B2b	0.0		0.0	0.0	0.0	-	-
B4	0.0		0.0	0.0	0.0	-	-
B5	0.0		0.0	0.0	0.0	-	-
B6b	0.0		0.0	0.0	0.0	-	-
B7	42.1		0.0	0.0	0.0	0.0	-
C4	0.0		0.0	0.0	0.0	-	-
C6	0.0		0.0	0.0	0.0	-	-
C6a	0.0		0.0	0.0	0.0	-	-
Subtotal	**61.2**	**0**	**8.9**	**0.0**	**28.4**	**14.6**	**-**
Stock assessment survey area						**Percentage**	**Percentage**
	2012	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
Total	**473.9**	**88.8**	**93.5**	**16.1**	**191.1**	**19.7**	**105.3**

3.3 Changes in the distribution of live oysters

Stratified random surveys are generally not as good as grid design surveys at estimating the distribution of oysters in Foveaux Strait, especially because of the patchy oyster distribution. Moreover, the numbers of stations, and therefore spatial coverage of the area differed between the 2012 and 2017 stock assessment surveys and the 2016 Bonamia survey. All stations were generated with a 0.75 nautical mile exclusion zone to spread sampling effort. These survey data are not likely to have estimated the distributions of oyster density well.

The distributions of recruit-sized, pre-recruit, and small oyster density for 2012 and 2016, and 2017 are shown in Figures 13 to 18. The distribution of oyster densities of all sizes is widespread, covering most of the fishery area with the highest densities in core fishery strata. Densities of all three size groups of oysters were lower in 2017 than in 2012, and generally similar to 2016. The numbers and sizes of localised areas of relatively high density of recruit-sized oysters decreased between 2012 and 2017, and between 2016 and 2017 (Figures 13 and 14). The decrease since 2012 is most likely the result of ongoing, low to moderate levels Bonamia mortality and reduced recruitment to the fishery. Densities of recruit-sized oysters decreased across the whole fishery area, including in western areas (B1 and C7a) where there was virtually no fishing.

The distribution of pre-recruit oysters has contracted from being widespread throughout the fishery area in 2012 to mostly within the Bonamia survey area in 2017 (Figure 15). Pre-recruit densities also declined over this period to become low and patchy in 2017, except in stratum C7. Pre-recruit-sized oysters are as vulnerable to Bonamia mortality as recruit-sized oysters, and the low densities also reflect the low settlement of oyster spat and low survival of juveniles (small oysters) in recent years. Pre-recruit densities increased in a few isolated patches between 2016 and 2017 (Figure 16).

The distributions of small oyster densities also contracted from being widespread throughout the fishery area in 2012 to mostly within the Bonamia survey area in 2017 (Figure 17). Relatively high densities of small oysters were widespread throughout the fishery in 2012, except for in the central fishery area, and some localized patches increased in density in 2017 (Figure 17). Densities increased markedly in western, southern and eastern fishery areas between 2016 and 2017 (Figure 18).

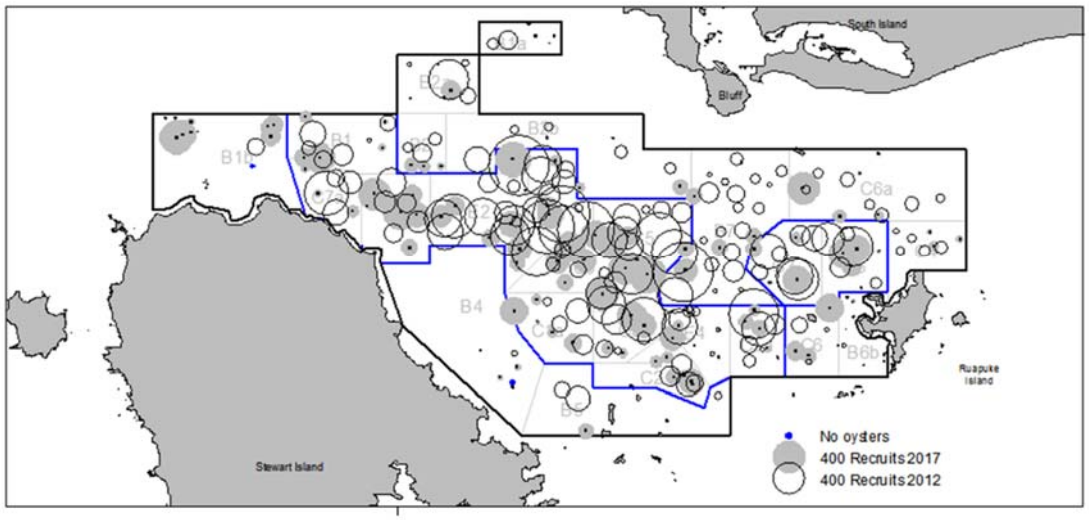

Figure 13: The densities (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled from the stock assessment area during the February surveys in 2017 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

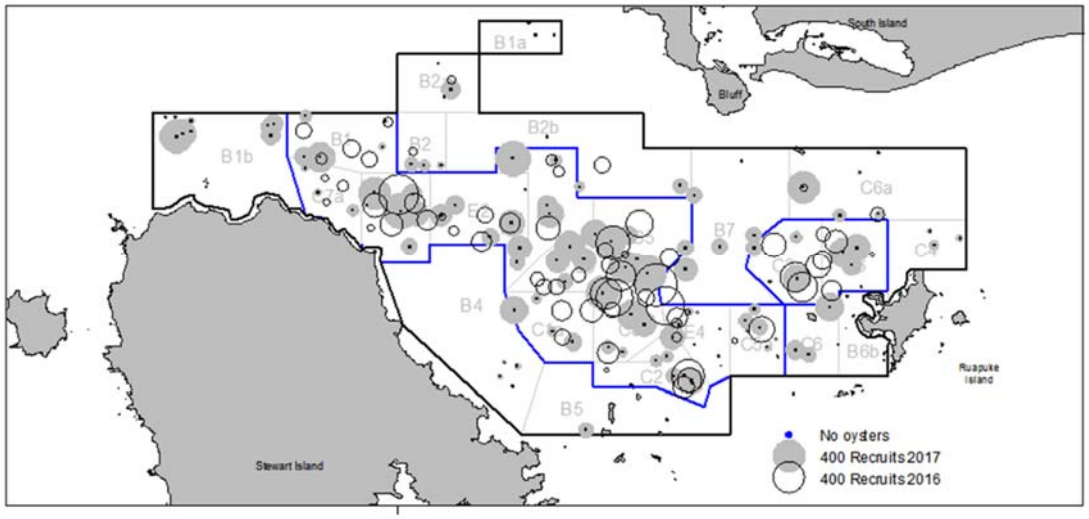

Figure 14: The densities (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled from the Bonamia survey area during the February surveys in 2017 (filled grey circles) and in 2016 (open black circles). Blue filled circles denote no oysters caught.

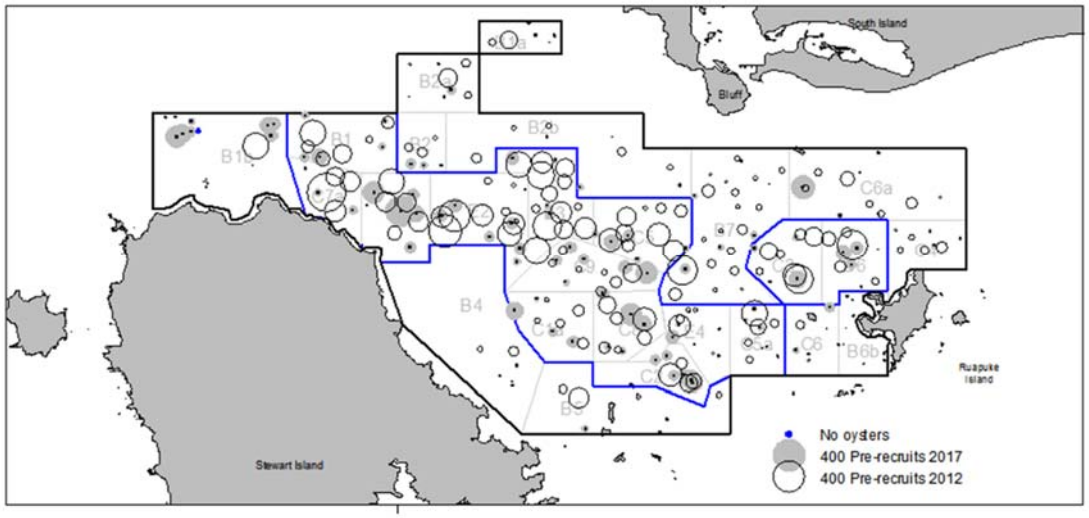

Figure 15: The densities (numbers of oysters per standard tow, 1221 m^2) of pre- recruit sized oysters sampled from the stock assessment area during the February surveys in 2017 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

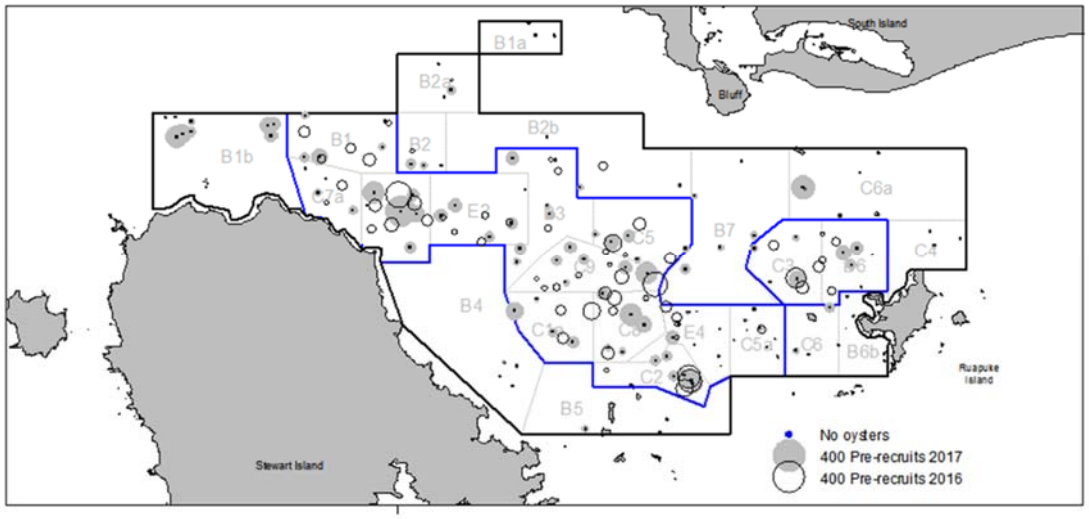

Figure 16: The densities (numbers of oysters per standard tow, 1221 m^2) of pre-recruit sized oysters sampled from the Bonamia survey area during the February surveys in 2017 (filled grey circles) and in 2016 (open black circles). Blue filled circles denote no oysters caught.

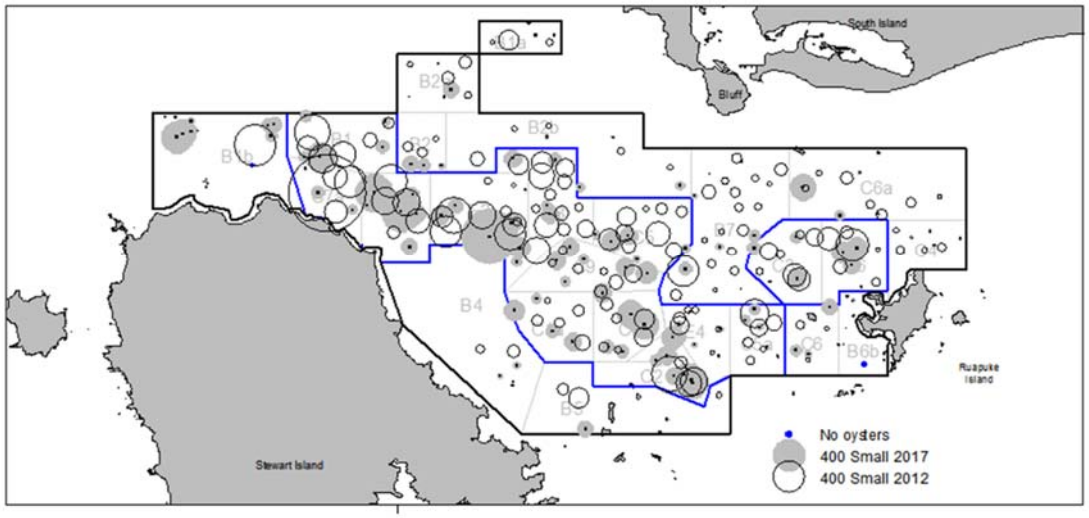

Figure 17: The densities (numbers of oysters per standard tow, 1221 m^2) of small oysters sampled from the stock assessment area during the February surveys in 2017 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

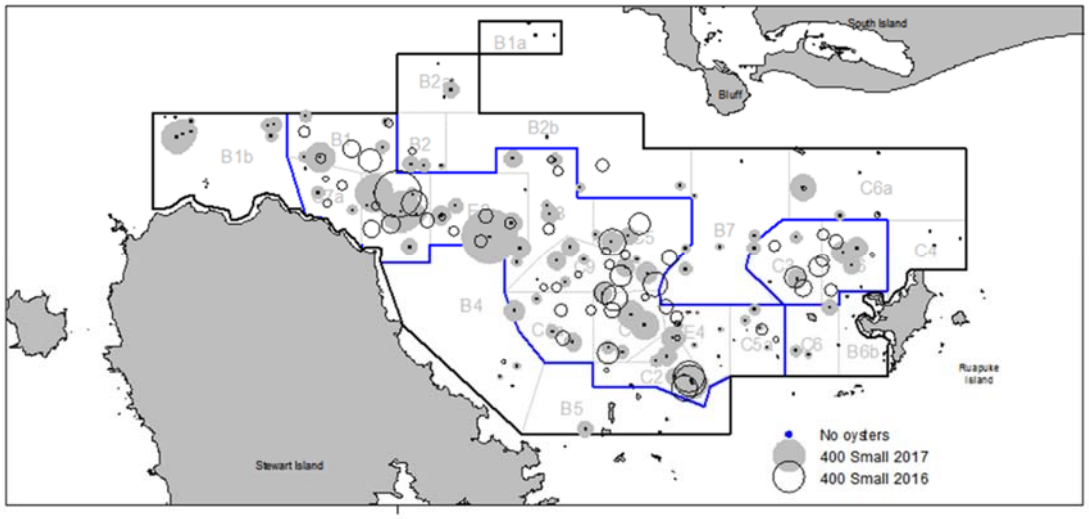

Figure 18: The densities (numbers of oysters per standard tow, 1221 m^2) of small oysters sampled from the Bonamia survey area during the February surveys in 2017 (filled grey circles) and in 2016 (open black circles). Blue filled circles denote no oysters caught.

3.4 Recruitment

Small oysters settle and remain attached to settlement surfaces up to a size of about 40 mm in length. Although oyster spat readily settle on clean shell surfaces, 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 is used as a relative index of replenishment to the population, but not as an absolute estimate of recruitment.

The number of small oysters per recruit shows large fluctuations in a broadly cyclic trend between 1993 and 2017 (Figure 19). The number of small oysters per recruit decreased from 1993 and was generally low between 1995 and 2001, suggesting reduced recruitment to the population at a time when the number of recruit-sized oysters was increasing and relatively high compared to 1993 (Figure 19). The number of small oysters per recruit was relatively high between 2002 and 2005 when the recruit-sized oyster population was declining rapidly due to Bonamia mortality. From 2009, the number of small oysters per recruit declined to low levels and has remained low until 2016 (Figure 19). The number of small oysters per recruit was more variable in 2017, with a slightly higher median and a few stations with higher numbers of spat per recruit.

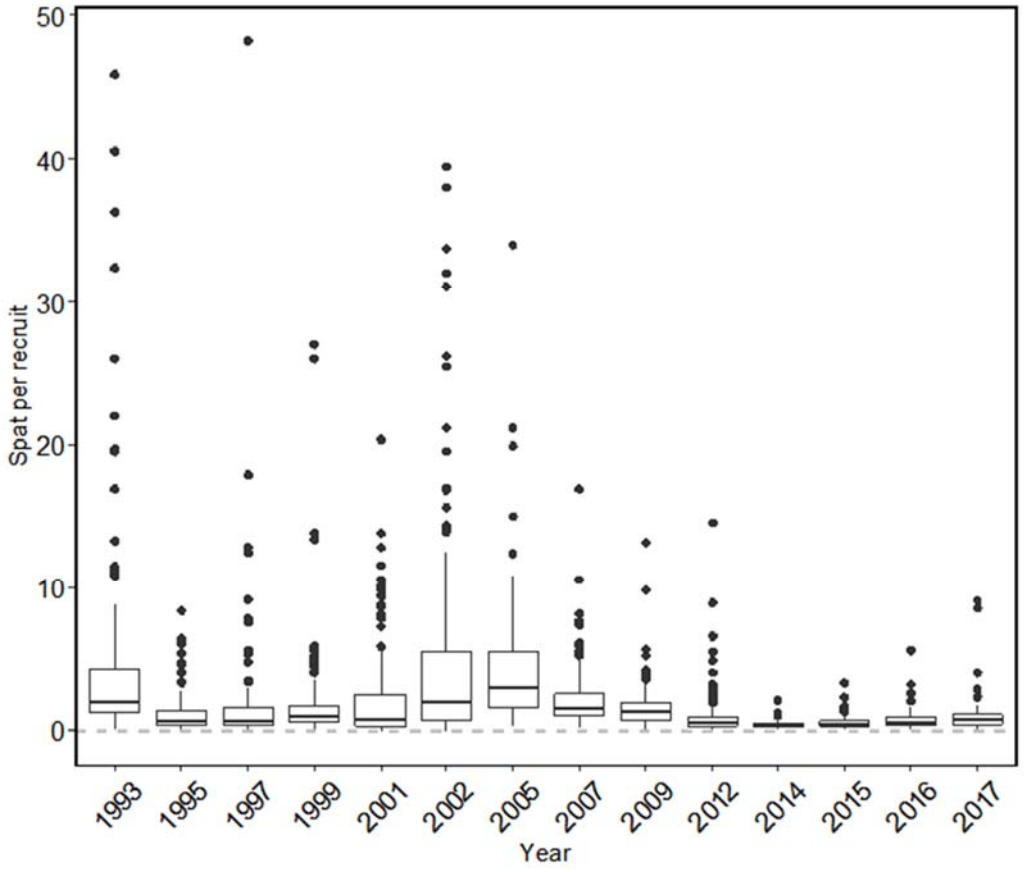

Figure 19: The number of small oysters per recruited oyster sampled between 1993 and 2017. The number of stations sampled each year varies. Medians are shown as solid lines, boxes represent 50th percentiles (25–75%) and whiskers 90th percentiles (5–95%), and outliers smaller than 5% and greater than 95% are shown as filled circles.

3.5 Oyster population size structure

Small oysters settle and remain attached to settlement surfaces up to a size of about 40 mm in length. The lower valves of oyster spat are cemented to settlement surfaces to a size of about 30 mm in length, at which point the shell begins to grow away from the settlement surface and the oyster is separated by mechanical disturbance some time thereafter. We assume that the dredge selectivity of recruit, pre-recruit and small oysters is similar.

The oyster length frequency distribution from the February 2017 survey is shown in Figure 20. The population size structure reflects the low recruitment to the oyster population, and heightened mortality of small and pre-recruit sized oysters. Recruit-sized oysters accounted for 54.0% of the population, pre-recruit oysters 14.4%, and small oysters 31.8%.

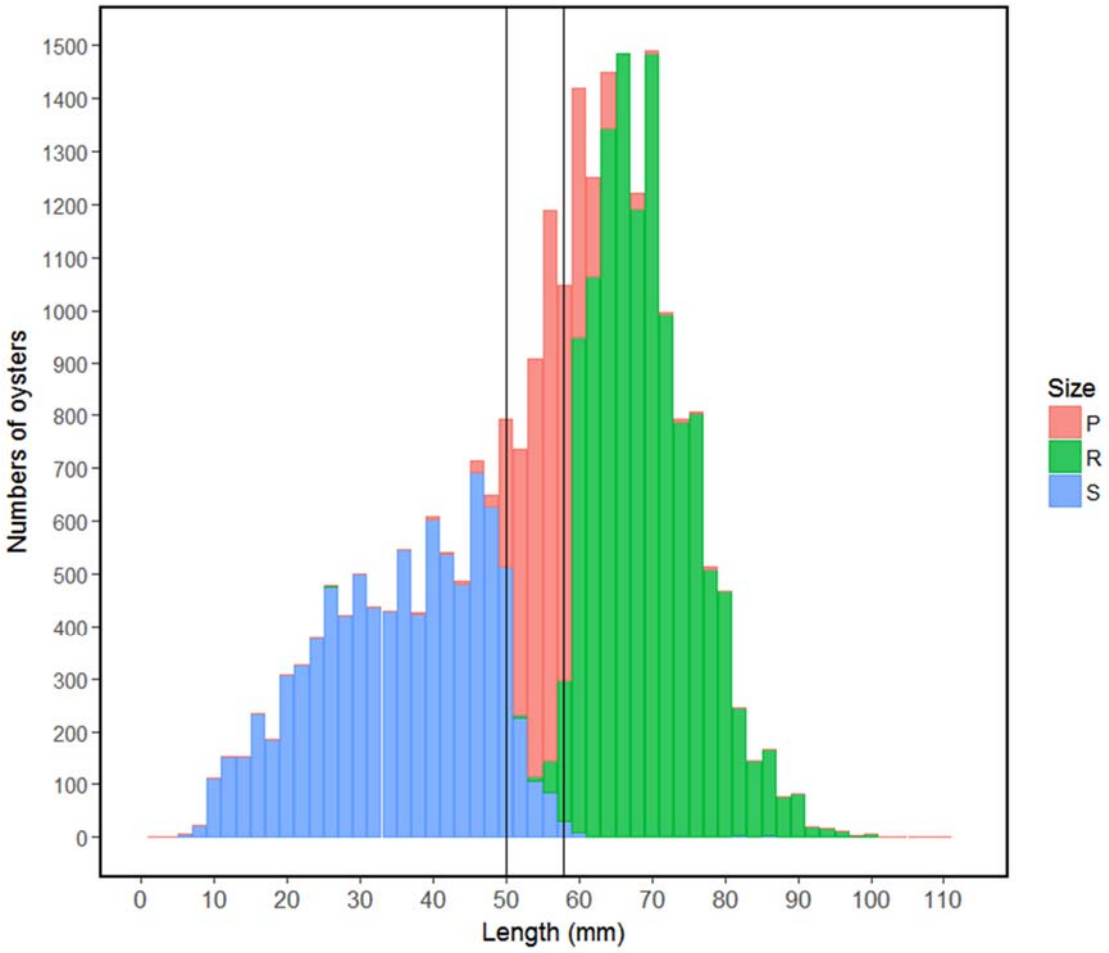

Figure 20: Length frequency distribution of oysters sampled in February 2017 weighted by catch in their respective size group. Recruit-sized oysters (unable to pass through a 58 mm internal diameter ring) shown in green (R), pre-recruits (able to pass through a 58 mm internal diameter ring, but unable to pass through a 50 mm ring) shown in salmon (P), and small oysters (able to pass through a 50 mm internal diameter ring and down to 10 mm in length) shown in blue (S).

3.6 Status of *Bonamia exitiosa*

Estimates of summer mortality

Estimates of oyster mortality before and during the February 201 7 survey

Descriptive statistics for the percentages of recruit-sized and pre-recruit new clocks and gapers sampled between 2012 and 2017 are given in Table 8. Low percentages of recruit sized new clocks and gapers in 2016 and 2017 suggest that pre-survey mortality was markedly lower than in the previous three summers. Pre-survey mortality for pre-recruits showed a similar trend, but the percentages are in part influenced by the low population size compared to that in 2012.

Table 8: Descriptive statistics for the percentage of recruit-sized and pre-recruit new clocks and gapers combined. Percentages are new clocks and gapers to new clocks, gapers and oysters combined, sampled from survey tows with more than 50 live recruit-sized or pre-recruit oysters in 2012, 2014, 2015, 2016, and 2017.

Percentage new clocks and gapers				**Recruit sized**					**Pre-recruits**	
Year	**2012**	**2014**	**2015**	**2016**	**2017**	**2012**	**2014**	**2015**	**2016**	**2017**
No. stations	112	50	54	52	74	78	30	20	26	40
Median	3.3	7.8	4.0	0.4	1.2	2.6	2.5	1.6	0	0
Minimum	0	1.7	0	0	0	0	0	0	0	0
Maximum	28.9	15.1	14.3	3	12.7	12.5	8.1	5.2	1.2	5.8
Lower 5th percentile	0.3	2.5	0	0.2	0	0	0	0	0	0
Upper 95th percentile	7.2	14.0	11.4	0.6	4.97	10.1	7.8	4.3	0.1	3.27
No. stations zero NC+G	5	0	5	22	13	11	8	9	22	21
Percentage zero stations	4.5	0	9.3	42.3	17.6	14.1	26.7	45	84.6	52.5

We recorded more gapers in 2017, 25 stations (28.1%) had one or more recruit-sized gaper, than in previous years 2016 (3.3%), 2015 (6.9%), 2014 (14%) and 2012 (26%). Markedly fewer recruit-sized new clocks were sampled from survey tows with more than 50 live oysters in 2017 than in 2012 (Figure 21), but more than in 2016 (Figure 22). However, changes in the numbers of gapers and recruit-sized new clocks do not show changes in disease mortality, and probably just reflect the timing of peak mortality.

The distributions of recruit-sized new clocks in 2012 and 2017 (Figure 23) show that pre-survey mortality was widespread and locally variable in 2012, and new clock densities were higher at stations where recruit-sized oyster densities were high, mostly in strata designated as commercial (E2, B3, C3, CB6, C7, and C7a). Recruit-sized new clock densities were generally low in southern strata in 2012. By 2017, recruit-sized new clock densities were low across the fishery area, probably reflecting the low oyster densities and reduced transmission of disease (Figure 23). Recruit-sized new clock densities were also low throughout the fishery area in 2016 (Figure 24) with many sites showing no pre-survey mortality (Figure 24 and Table 8). Pre-survey mortality increased slightly at localised sites in the central, southern and eastern fishery area in 2017 (Figure 24 and Table 8) but was still relatively low.

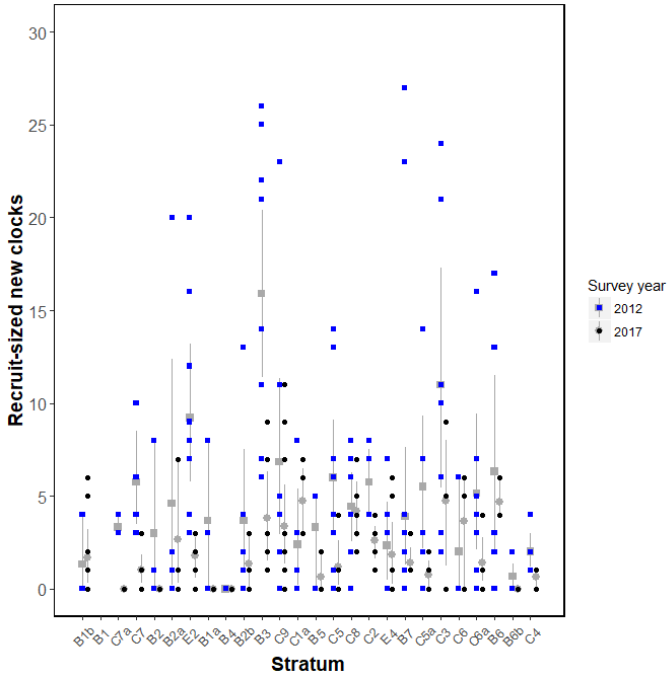

Figure 21: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized new clocks, their means and 95% confidence intervals (grey) by stratum sampled during the 2012 (blue) and 2017 (black) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

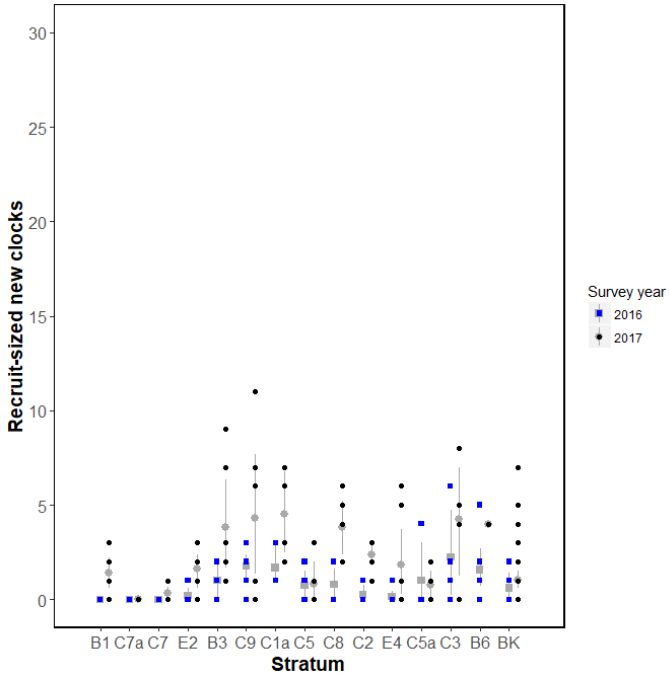

Figure 22: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized new clocks, their means and 95% confidence intervals (grey) by stratum sampled during the 2016 (blue) and 2017 (black) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

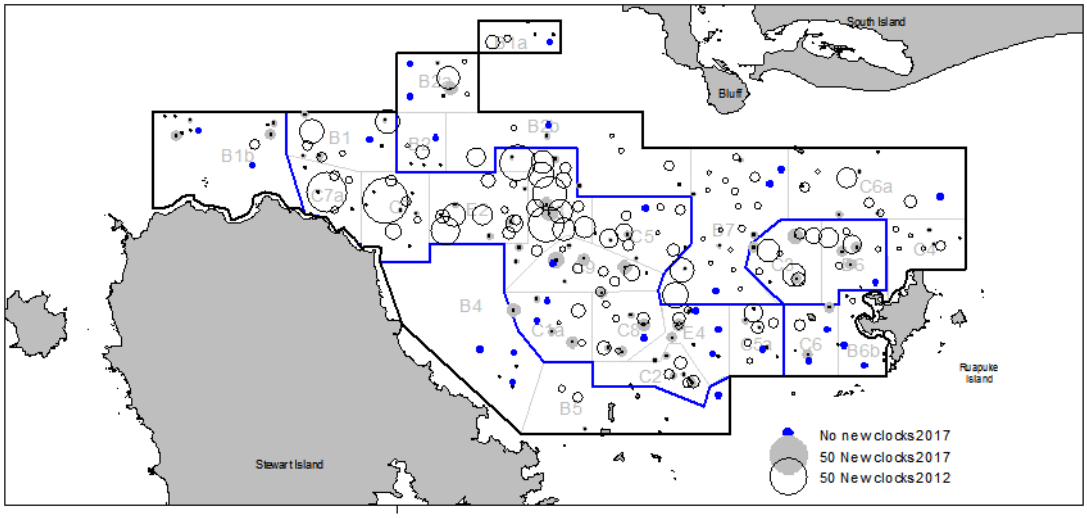

Figure 23: The distribution of recruit-sized new clocks and gapers ("New clocks") densities combined. Pre-survey mortality in February 2012 shown as open black circles and February 2017 shown as filled grey circles. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

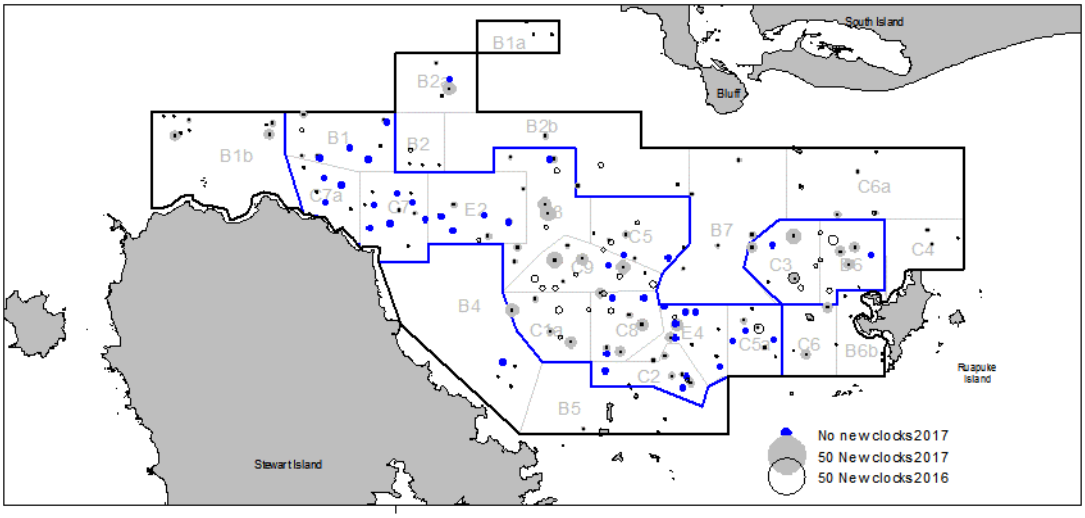

Figure 24: The distribution of recruit-sized new clocks and gapers ("New clocks") densities combined. Pre-survey mortality in February 2016 is shown as open black circles and February 2017 shown as filled grey circles. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles. New clock densities estimate pre-survey mortality. Estimates of the population sizes for recruit-sized and pre-recruit new clocks in core strata, the background stratum, and for the whole 2007 stock assessment survey area sampled at random stations in 2017 are shown in Tables 9 and 10 respectively.

The population size of recruit-sized new clocks decreased 76.4% in the Bonamia survey area and decreased 74.0% in the stock assessment area between 2012 (22.5 million) and 2017 (5.3 million), (Table 9). The numbers of new clocks were similar or increased in key commercial strata (C1a, C8, C9, and E4), and in background stratum (C6). New clocks decreased in other strata (Table 9). Between 2016 and 2017, the population size of recruit-sized new clocks increased nearly four-fold in the Bonamia survey area and over twice in the stock assessment survey area, however, the numbers of recruit-sized new clocks were still relatively low in 2017 (Table 9).

Pre-recruit-sized new clocks decreased 99.7% in both the Bonamia survey and stock assessment areas between 2012 and 2017 (Table 10). The numbers of new clocks decreased in all strata over the same period (Table 10). Between 2016 and 2017, the population size of pre-recruit-sized new clocks remained relatively low, however, it increased three-fold in the Bonamia survey area and decreased by a third in the stock assessment survey area (Table 10).

Pre-survey mortality of recruit-sized oysters in the Bonamia survey area increased between 2012 and 2014 from 3.2% to 6.8%, declined to 0.4% in 2016, and increased slightly to 1.4% in 2017 (Table 11). Pre-survey mortality in the stock assessment survey area showed similar trends, peaking at 7.6% in 2014, decreasing to a low of 0.6% in 2016, and increasing slightly to 1.5% in 2017 (Table 11). Pre-survey mortality of pre-recruit-sized oysters in the Bonamia survey area showed a consistent decline between 2012 (2.9%) and 2016 (0.2%), increasing in 2017 (0.7%). Pre-survey mortality of pre-recruits in the stock assessment survey area showed a similar trend, but peaked at 3.6 % in 2015, and then declined to 0.8% in 2017 (Table 11).

Table 9: Changes in the population size of recruit-sized new clocks (millions of clocks) between 2012, 2016 and 2017 by stratum (where available), 95% confidence intervals for the 2017 estimate, and the percentage change of population size between 2012 and 2017, and 2016 and 2017 for the Bonamia survey area, background strata, and the stock assessment survey area. Increases are shown in pale green and decreases in salmon highlights.

Bonamia survey area						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1	3.9	0	0.4	0.0	1.0	10.3	-
B3	4.7	0.2	0.4	0.2	0.7	8.5	200.0
B6	0.9	0.3	0.6	0.4	0.8	66.7	200.0
C1a	0.4	0.3	0.7	0.3	1.2	175.0	233.3
C2	0.5	0	0.2	0.1	0.4	40.0	-
C3	1.6	0.2	0.9	0.4	1.6	56.3	450.0
C5	0.8	0.1	0.2	0.0	0.5	25.0	200.0
C5a	0.5	0	0.1	0.0	0.2	20.0	-
C7	3.5	0	0.1	0.0	0.1	2.9	-
C7a	2	0	0.0	0.0	0.0	0.0	-
C8	0.4	0.1	0.5	0.2	0.9	125.0	500.0
C9	0.7	0.3	0.7	0.1	1.5	100.0	233.3
E2	2.4	0.1	0.3	0.2	0.6	12.5	300.0
E4	0.2	0	0.2	0.0	0.5	100.0	-
Subtotal	**22.5**	**1.4**	**5.3**	**3.4**	**8.1**	**23.6**	**378.6**

Table 9: Continued.

Background strata						Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
B1a	0.3		0.0	0.0	0.0	-	-
B1b	0.2		0.2	0.0	0.5	100.0	-
B2	0.3		0.0	0.0	0.0	0.0	-
B2a	0.6		0.4	0.0	1.1	66.7	-
B2b	1.5		0.4	0.0	0.9	26.7	-
B4	0.0		0.0	0.0	0.0	-	-
B5	0.6		0.1	0.0	0.3	16.7	-
B6b	0.1		0.0	0.0	0.0	0.0	-
B7	1.5		0.5	0.3	0.8	33.3	-
C4	0.2		0.1	0.0	0.2	50.0	-
C6	0.2		0.3	0.0	0.8	150.0	-
C6a	2.0		0.5	0.0	1.3	25.0	-
Subtotal	7.5	2.2	2.7	1.3	4.4	36.0	122.7
Stock assessment survey area						**Percentage**	**Percentage**
	2012	**2016**	**2017**	**L95%CI**	**U95%CI**	**2017/2012**	**2017/2016**
Total	**30.0**	**3.6**	**7.8**	**5.1**	**11.9**	**26.0**	**216.7**

Table 10: Changes in the population size of pre-recruit new clocks (millions of clocks) between 2012, 2016 and 2017 by stratum (where available), 95% confidence intervals for the 2017 estimate, and the percentage change of population size between 2012 and 2017, and 2016 and 2017 for the Bonamia survey area, background strata, and the stock assessment survey area. Increases are shown in pale green and decreases in salmon highlights.

Bonamia survey area							Percentage	Percentage
Stratum	**2012**	**2016**	**2017**	**L95%CI**	**U95%CI**		**2017/2012**	**2017/2016**
B1	46.6	0	0	0	0		-	-
B3	41.8	0	0	0	0		-	-
B6	15.5	0.1	0.1	0	0.2		0.6	100.0
C1a	6.0	0.1	0.2	0	0.4		3.3	200.0
C2	14.3	0	0	0	0.1		-	-
C3	17.5	0	0.1	0	0.3		0.6	-
C5	21.8	0	0	0	0.1		-	-
C5a	8.4	0	0	0	0		-	-
C7	31.6	0	0	0	0		-	-
C7a	32.9	0	0	0	0.1		-	-
C8	13.7	0.1	0.1	0	0.3		0.7	100.0
C9	9.0	0	0.3	0.1	0.6		3.3	-
E2	36.2	0	0	0	0		-	-
E4	2.1	0	0	0	0		-	-
Subtotal	297.4	0.3	0.9	0.5	1.5		0.3	300.0

Table 10: Continued.

Background strata							Percentage	Percentage
Stratum	**2012.0**	**2016**	**2017**	**L95%CI**	**U95%CI**		**2017/2012**	**2017/2016**
B1a	4.1		0	0	0		-	-
B1b	17.5		0.1	0	0.2		0.6	-
B2	2.8		0	0	0		-	-
B2a	6.3		0	0	0		-	-
B2b	10.3		0.1	0	0.4		1.0	-
B4	11.0		0.2	0	0.5		1.8	-
B5	23.9		0	0	0		-	-
B6b	0.4		0	0	0		-	-
B7	18.2		0	0	0		-	-
C4	3.9		0	0	0		-	-
C6	1.8		0	0	0.1		-	-
C6a	16.5		0	0	0		-	-
Subtotal	116.7	0.6	0.4	0	0.9		0.3	66.7
Stock assessment survey area							**Percentage**	**Percentage**
	2012	**2016**	**2017**	**L95%CI**	**U95%CI**		**2017/2012**	**2017/2016**
Total	414.1	0.9	1.3	0.6	2.2		0.3	144.4

Table 11: Estimates of pre-survey mortality for the Bonamia survey area and the 2007 stock assessment area for recruit-sized and pre-recruit new clocks for the 2012, and 2014 – 2017 surveys. Estimates are from randomly selected stations only. Pre-survey mortality (% mort) calculated as the percentage of new clocks (millions) over new clocks and oysters combined (millions).

Bonamia survey area		Recruit-sized			Pre-recruit	
Year	Oysters	New clocks	% mort	Oysters	New clocks	% mort
2012	688.1	22.4	3.2	297.7	8.9	2.9
2014	538.0	39.4	6.8	148.4	3.6	2.4
2015	351.4	13.5	3.7	89.2	2.2	2.4
2016	385.2	1.4	0.4	120.5	0.2	0.2
2017	363.6	5.3	1.4	123.1	0.4	0.3
Stock assessment survey area						
Year	Oysters	New clocks	% mort	Oysters	New clocks	% mort
2012	918.4	30	3.2	414.3	12	2.8
2014	1020.9	84.1	7.6	226.2	5.3	2.3
2015	509.9	23.7	4.4	122.1	4.5	3.6
2016	561.1	3.6	0.6	191.2	0.8	0.4
2017	527.4	7.8	1.5	168.2	1.3	0.8

A summary of checks made to ensure consistency amongst qPCR assays between surveys

Quality control of reagents and procedures was undertaken before the analysis of samples in March 2017. The synthetic standard for *Bonamia exitiosa* standard was tested by dnature Ltd and on the NIWA BioRad CFX96 to ensure that the internal control was not affecting the sensitivity of Bonamia detection. The standard was diluted in water and in diluent (1:15 dilution) of pooled negative oyster tissue which had the internal control amplifying and competing for reagents. Both dilution schemes went out to the same dilution before flatlining showing that the internal control is not affecting the sensitivity of detection. The synthetic Bonamia standard (tested by dnature Ltd) run in a duplex assay could reliably detect Bonamia in 2 µl of the lowest dilutions representing an average of two gene copies.

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

A FAM (6-carboxyfluorescein) fluorophore was used to detect Bonamia and a TR (Texas-red, sulforhodamine 101 acid chloride) fluorophore was used to detect DNA from oyster tissue (β-actin) as a cross check to ensure that the qPCR reaction occurred. Cq values of 37 and below were considered positive for FAM and TR. The differences in Cq values between the Bonamia positive control and β-actin positive control in each sample are due to the internal control (IC) block that allows the FAM fluorophore to fluoresce before the TR fluorophore. The IC block causes some variation in the fluorescence levels of the TR fluorophore at low dilutions of the Bonamia standard. Rarely, low level inhibition of the qPCR reaction may result in a positive sample returning a Cq slightly above 37.

Fifty tests of positive controls for both Bonamia and β-actin, and corresponding negative controls were undertaken during the qPCR assay runs to analyse the 2017 samples. None of the negative controls tested positive and none of the positive controls tested negative. The Bonamia interplate positive control (synthetic standard of 10^3 copies/µl) produced a mean Cq 26.7 (95%CI 26.6–26.8), which is very similar to previous years.

Estimates of the prevalence and intensity of Bonamia in commercial fishery areas

Sampling effectiveness for the prevalence and intensity of infection by Bonamia

Samples of 25 recruit and pre-recruit sized oysters were collected from all but nine stations in 2017. In all, 2134 heart imprint slides were sampled and archived. This sample comprised 2056 recruit-sized oysters, 46 pre-recruits, and 32 small oysters. Almost all of the samples (96.3%) were of recruit-sized oysters, similar to 2016 (96.6%), 2015 (97.2%), and to previous surveys. Only a subsample of these were screened (N = 398, 18.9%). Stations with fewer than 15 recruit and pre-recruit sized oysters (station 47, N = 12; 69, N = 13; 29, N = 11; and 80, N = 6) were not used in the analysis of infection.

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

qPCR detection of Bonamia in oyster heart tissues

A summary of qPCR samples tested is given in Table 12. All hearts that showed anomalies in the qPCR data were rerun. The repeat scores were used in the analysis for presence/absence. Samples that failed a second assay were omitted from the qPCR data analysis, and the corresponding heart imprint slides examined (Table 12).

Twelve heart tissues (0.6%) didn't amplify and were scored by heart imprint only in 2017 (Table 12). One hundred and twenty-four heart tissues (7.3%) didn't amplify in 2016, which is more than in 2015 (2.3%, Michael et al. 2015a) and 2014 (5.7%, Michael et al. 2015b). No heart tissue samples amplified early in 2017. One false negative was reported, a sample tested positive by histology (category 1) and negative for *B. exitiosa* and negative for *B. ostreae* using qPCR with Cqs just above the threshold (FAM 37.92, TR 31.81) for *B. exitio*sa, possibly due to some inhibition in the qPCR reaction or quenching by the internal control. No other Bonamia infection was detected by heart imprints in the 265 random samples selected from qPCR negative samples.

Table 12: The numbers of heart tissue samples screened for Bonamia using qPCR, and the numbers of heart imprints in 2017. The summary of qPCR samples gives the total number of samples tested (Sample (N)), the numbers of samples omitted because they failed inclusion criteria after repeat sampling (Omitted), the numbers of valid samples (qPCR.N), and those that tested positive (Positive (≤35Cq)) and those where no Bonamia DNA was detected (Negative (>35 Cq)). Histology samples gives the number of heart imprint slides screened which included qPCR positives, randomly selected negatives, and qPCR anomalies. The summary statistics for qPCR infection give the numbers of qPCR positive and negative samples (heart tissue only) and the numbers of corresponding heart imprint samples that scored positive for Bonamia infection. One anomaly was found in the randomly selected samples which tested qPCR negative (*). This sample tested negative for *Bonamia ostreae*, and Cqs just above the threshold (FAM 37.92, TR 31.81) for *B. exitiosa*, possibly due to some inhibition in the qPCR reaction.

qPCR samples						
Bonamia infection		**Sample (N)**	**Omitted**	**qPCR.N**	**Positive(≤35Cq)**	**Negative (>35 Cq)**
Heart		2134	12	2122	120	2002
Histology samples						
Number of slides read		398				
qPCR infection		**Sample (N)**	**Histo+ve**	**Histo%detec**		
Heart qPCR +ve		120	96	80.0		
Selection qPCR -ve		265	1*			
qPCR anomalies		12	0			

Comparison of qPCR and heart imprint methods.

The qPCR method shows higher sensitivity in the detection of Bonamia than heart imprints (Maas et al. 2013). Bonamia could not be detected in a large number of histological samples which scored positive using qPCR (Figure 25).

The quantification of Bonamia cannot be directly compared between qPCR and histology as the qPCR Cq values estimate numbers of Bonamia ITS region copies while histology scores categorise the average numbers of Bonamia cells in oyster haemocytes. A small number of samples with anomalous qPCR results were included in the subset of heart imprint samples examined for infection.

qPCR tests where samples did not amplify FAM (Bonamia) and amplified Texas Red (β-actin) are Bonamia negative. These samples do not have Cq values for FAM (because there was no amplification). Cqs for these samples were set to 43 for graphs to reflect the actual numbers of samples that tested negative for *B. exitiosa*. qPCR tests where samples did not amplify either FAM or Texas Red (flatliners) are anomalous results, i.e., cannot be considered positive or negative. Heart imprint slides for all flatliners were scored for Bonamia. Cqs from qPCR were plotted against heart imprint score (Figure 25). Twenty percent of the samples that were heart imprint negative, were positive by qPCR. All qPCR negatives not examined by heart imprints were assigned as negatives. Boxplots of Cq values for heart tissues show a decreasing trend with increasing intensity of Bonamia infection estimated from heart imprints i.e., Bonamia scores increasing from 1 to 5 (Figure 25).

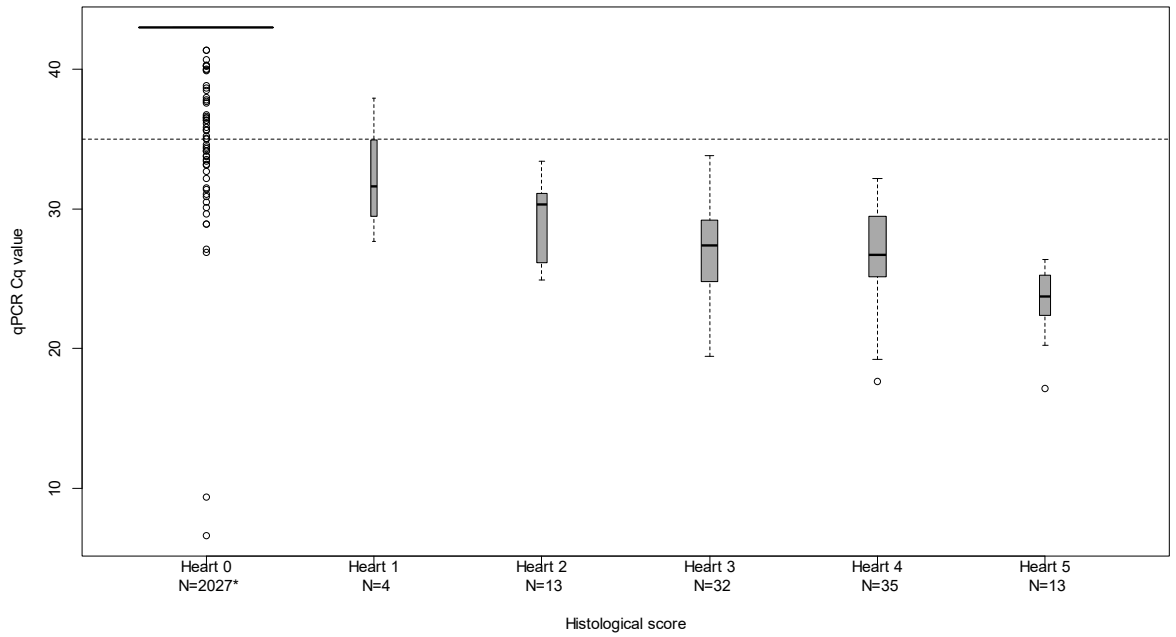

Figure 25: Boxplots of Cq values from qPCR analysis of the Bonamia ITS region for samples of heart tissues by histological score (heart imprints) from the February 2017 survey. Cut-off levels set at 35 Cq (dashed line). Outliers for heart tissues denote generally weak reactions most likely caused by inhibitors in the crude samples. Box plots show medians (solid lines), boxes 25th and 75th percentiles, whiskers at 95th percentiles, and outliers shown as black circles above and below whiskers. Samples that didn't amplify FAM (Bonamia) and amplified Texas Red (β-actin) were assigned Cq 43 to better account for the numbers of oysters that tested negative.

Prevalence and intensity of infection in oysters by Bonamia

We assumed that all heart imprint slides corresponding to samples that were qPCR negative, but not scored for Bonamia were negative. Stations with too few oysters and target stations were excluded from the analysis of prevalence and intensity of infection (Table 13). Infection intensity was estimated from heart imprint slides using the categorical score of Diggles et al. (2003).

Heart imprints underestimate the true prevalence of Bonamia infection and are lower than the qPCR estimates (Table 12). The mean prevalence from heart imprints in 2017 was lower (4.3%) than in 2016 (7.5%), 2015 (15.3%) and 2014 (15.2%), and recent surveys (2009–2012, 8–12%). qPCR analysis of heart tissues was more sensitive than heart imprints, (Table 12). Mean prevalence from qPCR was lower in 2017 (5.4%) than in 2016 (11.8%), 2015 (22.4%), 2014 (25.0%), and 2013 (19.6 %). Details of recruit-sized oysters and densities by station, and Bonamia infection status from histology and qPCR are shown in Table A5.1, Appendix 5.

Of the 2115 slides taken from random stations with more than 15 recruit and pre-recruit sized oysters in 2017, a subset of 398 heart imprint slides were examined for Bonamia. The remaining 1723 slides were from oysters screened using qPCR and were not infected. In 2017, 95.4% of oysters had no detectable infection, which is more than in 2016 (87.3%) 2015 (84.7%), 2014 (85.8%), or for 2010 to 2013 (90%, 88%, 89%, and 88% respectively).

Of the 4.6% of oysters with detectable infections in 2017, 0.8 % had category 1 and 2 light infections (3–5% in 2010–2016), and 3.8% had category 3 and higher infections (4–11% in 2010–2016) which are normally fatal. The prevalence of infection ranged from 0% to 24% in 2017; with no detectable infection at 36 of the 86 stations, a marked increase from 3 in 2015. The median prevalence in 2016 was 4.5%, which is similar to 2016 (4%) and less than in 2015 (12%).

Intensity of infection was determined from heart imprints to maintain the time series of Bonamia survey data. The median level of infection in 2016 (4.0, Table 13) was more than in 2016 (2.5), 2015 (3.1) and 2014 (3.0). Infection levels were generally high with 82% or more of infected oysters in 2017 expected to die within a few weeks of sampling compared with 59% in 2016, 52% in 2015 and 50% in 2014. The mean intensity of infection in 2017 (4.3) was also more than in 2016 (2.8), 2015 (3.2) and years 2009–2014.

The percentage of stations in 2017 with category 3 and higher infections was 50% in 2017, which is less than in 2016 (58%) 2015 (90%), 2014 (81%), and for the years 2009–2014 (67%–94%, the coverage of sampling and numbers of stations sampled differed between years). The intensity of infection was highly variable within stations, and patterns of variation were similar across the fishery area, in all years.

Table 13: Comparisons of infection levels (prevalence and intensity) between 2016 and 2017. Number of samples each method (N), mean and median prevalence (Prev (%)) and intensity estimated by histology (heart imprints), and prevalence from qPCR. Standard deviation (s.d.) and upper and lower 95% confidence intervals (L95%CI and U95%CI). Data from random stations sampled for Bonamia with more than 15 recruit and pre-recruit oysters in the sample.

2016					**2017**			
	Histology	**Histology**	**qPCR.heart**			**Histology**	**Histology**	**qPCR.heart**
	Prev (%)	**Intensity**	**Prev.H (%)**			**Prev (%)**	**Intensity**	**Prev.H (%)**
N	55	42	55		N	76	43	76.0
mean	7.5	2.8	11.8		mean	4.3	3.4	5.4
median	4.0	2.5	8.0		median	4.0	3.5	4.0
s.d.	7.1	1.1	9.5		s.d.	4.5	0.9	5.5
L95%CI	5.7	2.5	9.3		L95%CI	0.0	2.0	4.0
U95%CI	9.4	3.1	14.3		U95%CI	13.0	4.9	16.0

The median prevalence of infection at all sample stations in 2017 was less than in 2016 and is similar to levels in 2008 and 2009 (Figure 26). qPCR samples generally showed similar prevalence to histology, except at a few sites where both methods showed high prevalence, but qPCR showed higher prevalence (Figure 26). Prevalence from qPCR was also much lower than for 2014 and 2015 (Figure 26). Prevalence appears to have dropped below the long-term average of 8–12%. The median intensity of infection was higher in 2017 than in 2016, with most infected oysters fatally infected. Median intensity of infection was lowest in 2016 for the period 2007 to 2017 (Figure 27).

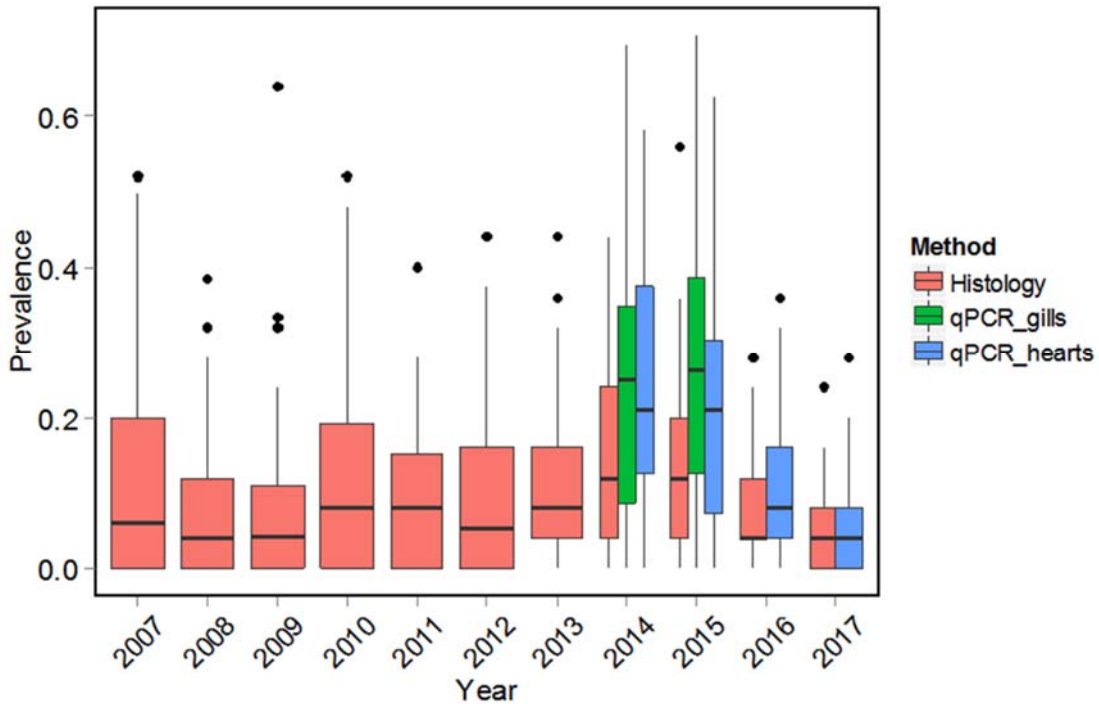

Figure 26: Boxplots of the median prevalence of Bonamia infection 2007–2017. The median prevalence of infection at all stations determined from histology (heart imprints) 2007–2013, and for qPCR heart tissues (qPCR_hearts) and gill tissues (qPCR_gills) in 2014 and 2015, but only heart tissues in 2016 and 2017. Medians shown as solid lines, boxes represent 50th percentiles and whiskers 95th percentiles, and outliers are shown as filled black circles.

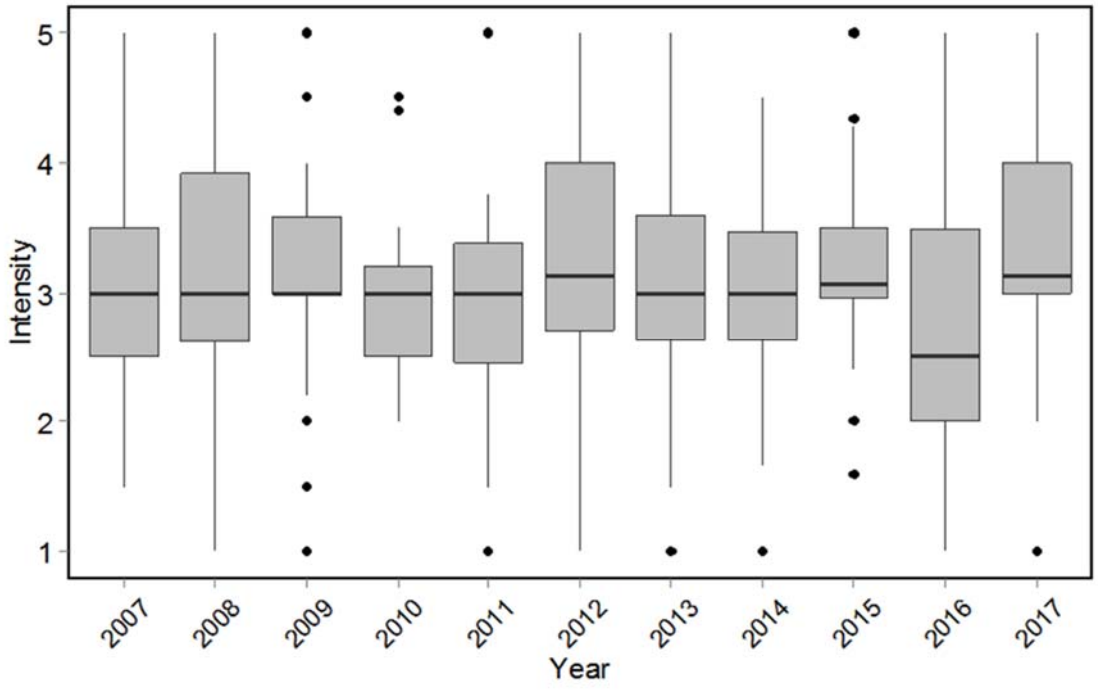

Figure 27: Boxplots of the mean intensity of Bonamia infection 2007–2017. The mean intensity of infection at all stations determined from histology. Medians shown as solid lines, boxes represent 50th percentiles and whiskers 95th percentiles, and outliers are shown as filled black circles.

The distributions of prevalence and intensity of Bonamia infection

The distribution of stations with Bonamia infection estimated from heart imprints and from qPCR analysis shows that the prevalence of infection was limited to the central and eastern fishery in 2016 and was more widespread across the survey area in 2017 (Figures 28 and 29), however this may be an artefact of sampling given the differences in the numbers of stations sampled between 2016 and 2017. The distributions of stations with no detectable infection were similar between 2016 and 2017 (Figures 28 and 29), but there were more stations with no detectable infection in 2017 than in 2016, possibly due to the larger number of stations sampled in 2017. The distributions of prevalence of infection from qPCR and heart imprints were similar in both 2016 and 2017, however, qPCR showed higher sensitivity than heart imprints in 2016 and was generally similar in 2017 (Figures 28 and 29).

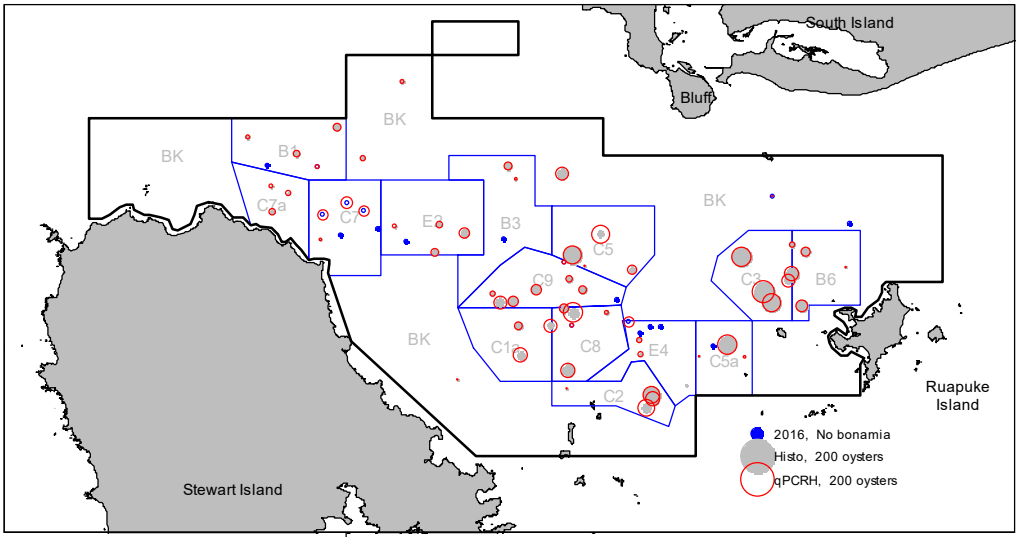

Figure 28: The distribution of Bonamia infection in February 2016 estimated from heart imprints, and qPCR analysis of heart tissues only. The numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and qPCR heart tissues (qPCRH, open red circles), and stations with no Bonamia (filled blue circles) are shown. The 2007 survey area (black outer line), the February 2016 survey strata (blue lines), and the stratum labels (grey) are indicated.

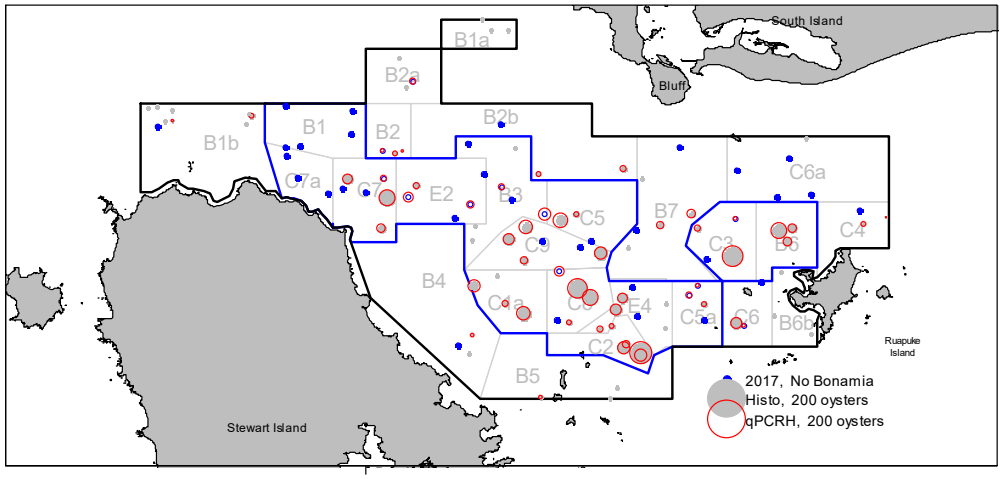

Figure 29: The distribution of Bonamia infection in February 2017 estimated from heart imprints, and qPCR analysis of heart tissues only. The numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and qPCR heart tissues (qPCRH, open red circles) and stations with no Bonamia (filled blue circles) are shown. The 2007 survey area (black outer line), the February 2017 survey strata (blue lines), and the stratum labels (grey) are indicated. In February 2012, the prevalence of infection was highest in eastern, southern, and western fishery areas, with little

infection in the central fishery areas east of a line between Bluff Hill and Port William where oyster density was high (Figure 30). In areas with relatively high infection, Bonamia infection was widespread and patchy, the prevalence and intensity of infection was highly variable at small spatial scales. Stations with high prevalence and high intensity of infection in 2012 were interspersed amongst stations with no detectable infection. By 2016 Bonamia mortality had markedly reduced oyster density (Figure 31), and fatal infections were also reduced markedly and confined to the fishery areas east of a line between Saddle Point and Bluff Hill. Recruit-sized oyster densities were similarly low in 2017, and fatal infections widespread (Figure 32). However, infection was low and patchy in 2017 (Figure 32).

Patterns in the distribution of prevalence and intensity of infection between 2012 and 2017 were not consistent with patterns in the distribution of oyster dredging from fishers' logbook data or with oyster density from survey data; there were areas of high oyster density with a relatively high prevalence and intensity of infection in areas that had not been fished since 2008 because of the low meat quality there.

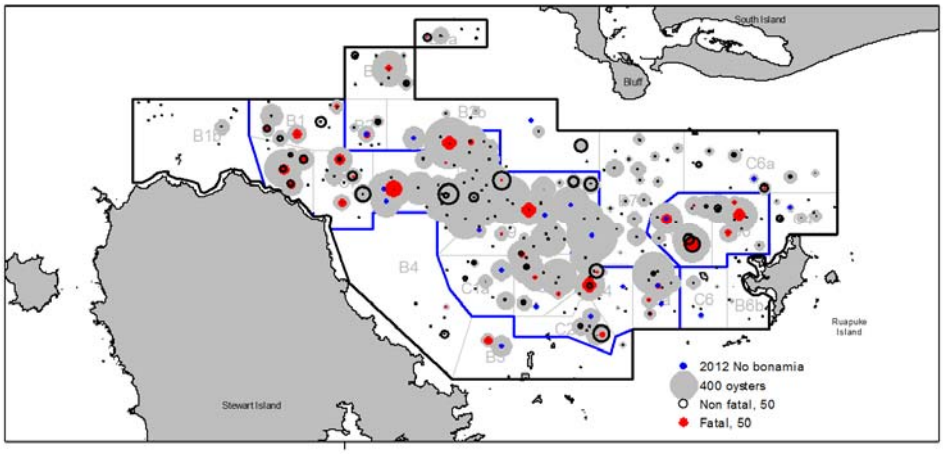

Figure 30: The distribution of oysters and Bonamia infection in February 2012. Numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles) and stations with no Bonamia (filled blue circles) are shown. The 2007 survey area (black outer line), the February 2012 survey strata (blue lines), and the stratum labels (grey) are indicated.

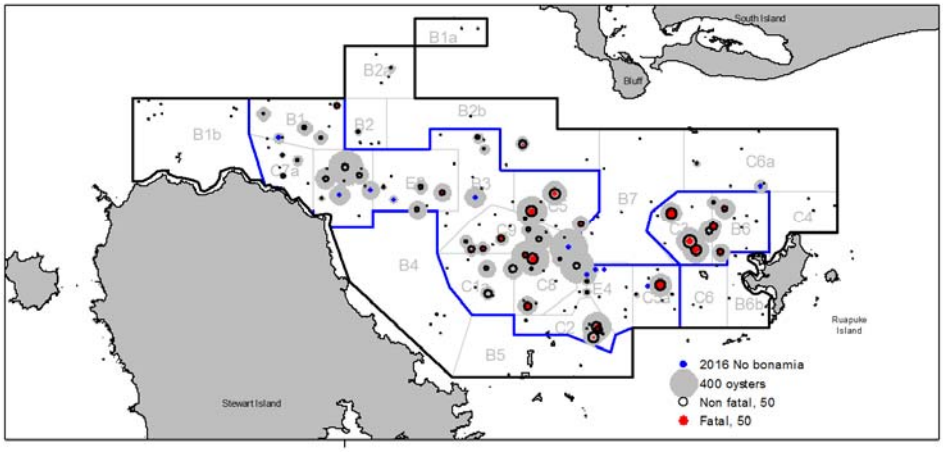

Figure 31: The distribution of oysters and Bonamia infection in February 2016. Numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles); fatal infections (intensity categories 3–5 combined, filled red circles) and stations with no Bonamia (filled blue circles) are shown. The 2007 survey area (black outer line), the February 2016 survey strata (blue lines), and the stratum labels (grey) are indicated.

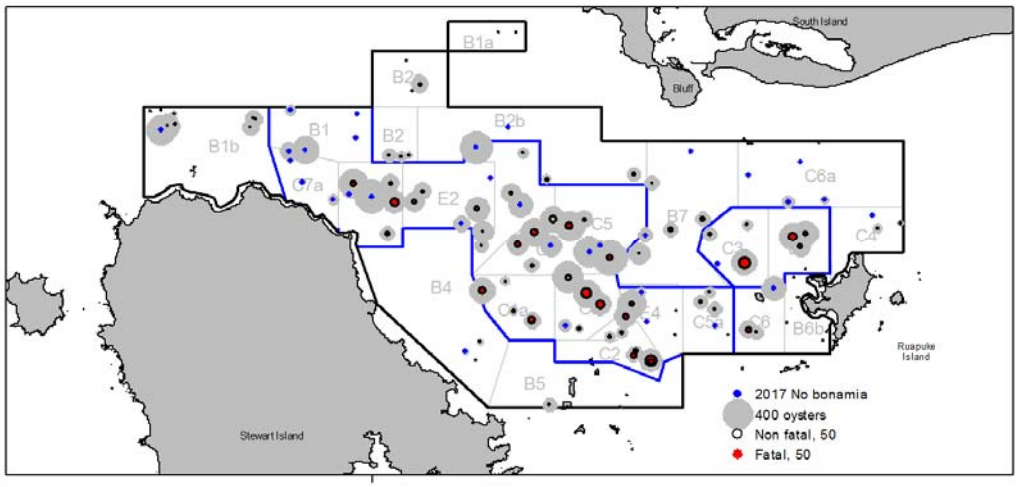

Figure 32: The distribution of oysters and Bonamia infection in February 2017. Numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles); fatal infections (intensity categories 3–5 combined, filled red circles) and stations with no Bonamia (filled blue circles) are shown. The 2007 survey area (black outer line), the February 2017 survey strata (blue lines), and the stratum labels (grey) are indicated.

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

The distribution of non-fatal infections was widespread and variable across the fishery in 2014 and 2015. The prevalence of non-fatal, categories 1 and 2 infections varied at small spatial scales; stations with relatively high prevalence were often close to stations with low prevalence or no infection. Stations with high non-fatal prevalence were likely to be subjected to Bonamia mortality in the future. The numbers of stations with heightened non-fatal infections in 2016 were considerably fewer (Figure 33), and mainly in central (C5 and C9) and eastern (C3 and B6) fishery areas. Non-fatal infections were further reduced in 2017 (Figure 34), with single stations of low prevalence in strata B3, B6, C2, and C8, suggesting low Bonamia mortality in 2018.

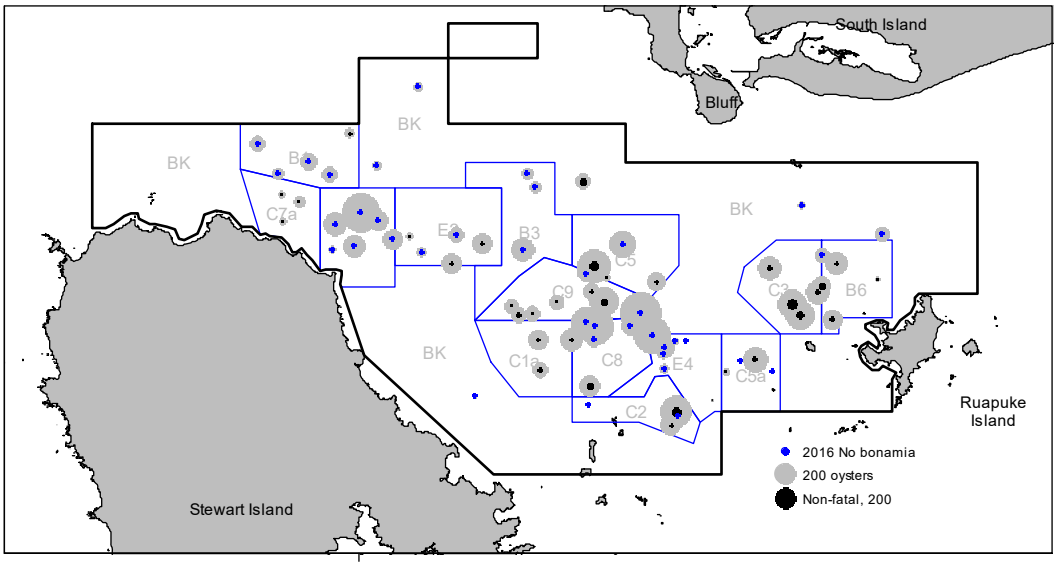

Figure 33: The distribution of recruit-sized oysters (filled grey circles showing numbers per standard tow) and oysters with non-fatal infections (filled black circles, the numbers of oysters scaled to the size of the catch with intensity of infection category 1 and 2) in February 2016. Stations with no Bonamia infection are shown by blue circles.

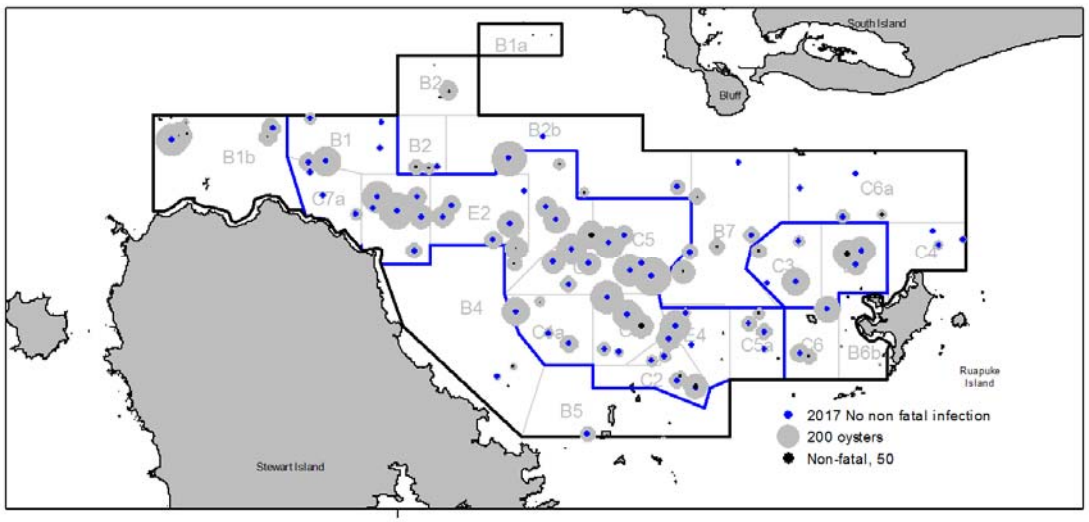

Figure 34: The distribution of recruit-sized oysters (filled grey circles showing numbers per standard tow) and oysters with non-fatal infections (filled black circles, the numbers of oysters scaled to the size of the catch with intensity of infection category 1 and 2) in February 2017. Stations with no Bonamia infection are shown by blue circles.

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

Pre-survey mortality was estimated from the population size of recruit-sized new clocks and gapers in Section 4.5.1. In 2017, pre-survey mortality in the Bonamia survey area combined was estimated to be 5.3 million recruit-sized oysters (95% CI 3.4–8.1), 1.5% of the recruited population, higher than the 1.4 million recruit-sized oysters (95% CI 1.3–1.4 in 2016, 0.4% of the recruited population). Projections of post-survey mortality (within about two months of sampling) from the proportion of oysters with categories three and higher (fatal) infections scaled-up to the size of the total recruit-sized oyster population are given in Table 14 below. We used two methods to crosscheck the scaled-up estimates of fatal infections: 1, by applying a correction factor to the population estimates derived from the average proportion of infected oysters in the stratum; and 2, post-survey mortality was estimated from the numbers of infected oysters at each station scaled to the catch, then to stratum, and to the survey area level.

Projected short-term mortality from Bonamia infections

Post-survey mortality of recruit-sized oysters was estimated for the Bonamia survey area with three or more randomly selected stations. Because the abundance of kaeos reduced sampling efficiency in some strata (B6b and E4), insufficient numbers of oysters were caught for Bonamia samples, and these were omitted from the analysis. The small numbers of oysters resulted in several strata with fewer than three Bonamia stations for analysis. Details of projected short-term mortality are therefore only presented using method 1 (Table 14).

Post survey mortality in February/March 2017 was expected to be relatively high (above 10%) in the Bonamia survey area in strata C2 and C8, and the largest numbers of oysters likely to be lost were from strata B6 (2.6 million oysters), C8 (2.6 million oysters), and C7 (2.0 million oysters), (Table 4–13). Post survey mortality was expected to be relatively low in background strata (Table 14). Estimates of post survey mortality in the Bonamia survey area are 13.5 million oysters, and 18.3 million oysters in the stock assessment survey area.

Post survey mortality for the stock assessment survey area and Bonamia survey area for years 2012, 2015, 2016, and 2017 are given in Table 15. Post survey mortality was markedly lower in 2016 and 2017 than in 2012–2015. How quickly low level, categories 1 and 2 infections progress to category 3+ infections, and the variance amongst individual oysters is not known.

Summer mortality, estimated as the percentage of deaths of recruit-sized oysters from the time mortality began at the beginning of summer to the end of the seasonal mortality (about mid-March), was calculated as the percentage of all deaths (pre-survey mortality and post survey mortality combined) of the recruit-sized population at the beginning of summer (population size of recruit-sized new clocks and population size of recruit-sized oysters at the time of survey combined). Summer mortality in the Bonamia survey area is expected to be slightly higher in 2017 (5.1%) than in 2016 (4.2%), and lower than in 2012, 2014, and 2015 (Table 15). Summer mortality in the stock assessment survey area is similar between 2016 and 2017 (4.9%), and also lower than in 2012, 2014, and 2015 (Table 4–14).

Table 14: Absolute population estimates for recruit-sized oysters (millions of oysters) after projected mortality from Bonamia based on category 3 and higher infections in the Bonamia survey area, background stratum (BK), and for the 2007 stock assessment survey area sampled in February 2017. The table gives the correction factor applied to each stratum (Correction factor), mean population size at the time of survey (Pop.1), mean post mortality population size (Pop.2) in millions of oysters, upper and lower 95% confidence intervals (CI) for the post-mortality estimate, the area of each stratum (Area.km^2), the mean post mortality population size scaled from catches (Sc_pop), upper and lower 95% confidence intervals (CI) the percentage mortality and the difference between the correction factor and scaled estimates, by stratum for the February 2017 survey.

Stratum	Factor	Pop.1	Pop.2	Mortality	Pop2L.CI	Pop2U.CI	Area.km^2	%mortality		Sc_pop	PopSL.CI	PopSU.CI	%mortality	Diff
Bonamia survey area														
B1	1.00	23.7	23.7	0.0	1.1	51.5	78.2	0.0		0.0	0.0	0.0	0.0	0.0
B3	1.00	49.9	49.9	0.0	6.4	103.9	44.7	0.0		NA	NA	NA	NA	NA
B6	0.94	41.0	38.4	2.6	21.4	62.1	30.0	6.5		2.6	1.2	4.5	6.4	0.1
C1a	0.92	21.3	19.5	1.7	2.3	41.2	31.3	8.2		NA	NA	NA	NA	NA
C2	0.90	12.1	10.9	1.2	4.6	19.5	21.9	10.1		1.2	0.4	2.2	10.0	0.1
C3	0.99	20.8	20.7	0.1	5.6	40.3	32.7	0.6		0.1	0.0	0.4	0.6	0.0
C5	0.99	29.5	29.2	0.3	10.1	55.2	37.7	0.9		0.3	0.0	0.9	0.9	0.0
C5a	1.00	7.1	7.1	0.0	1.5	14.0	23.5	0.0		0.0	0.0	0.0	0.0	0.0
C7	0.96	44.0	42.1	2.0	14.5	79.2	36.1	4.5		2.0	0.0	4.7	4.4	0.0
C7a	1.00	4.9	4.9	0.0	1.8	9.0	23.6	0.0		0.0	0.0	0.0	0.0	0.0
C8	0.89	23.9	21.4	2.6	4.1	43.2	26.8	10.7		2.5	0.0	5.6	10.6	0.1
C9	0.97	54.8	53.0	1.8	27.7	88.3	34.5	3.3		1.8	0.5	3.4	3.2	0.1

Table 14: Continued.

Stratum	Factor	Pop.1	Pop.2	Mortality	Pop2L.CI	Pop2U.CI	Area.km^2	%mortality		Sc_pop	PopSL.CI	PopSU.CI	%mortality	Diff
Bonamia survey area														
E2	0.98	20.8	20.4	0.4	1.9	43.2	42.8	2.0		0.4	0.0	1.3	2.0	0.0
E4	0.93	9.6	9.0	0.7	0.0	23.4	28.0	7.0		NA	NA	NA	NA	NA
Subtotal		**363.6**	**350.1**	**13.5**				**3.7**		**10.9**			**3.0**	**0.7**

Stratum	Factor	Pop.1	Pop.2	Mortality	Pop2L.CI	Pop2U.CI	Area.km^2	%mortality		Sc_pop	PopSL.CI	PopSU.CI	%mortality	Diff
Background strata														
B1a	NA	0.1	NA	NA	0.0	0.0	16.0	NA		NA	NA	NA	NA	NA
B1b	0.99	24.6	24.3	0.2	5.9	47.8	36.2	0.9		NA	NA	NA	NA	NA
B2	0.98	5.3	5.1	0.1	1.8	9.4	17.9	2.0		0.1	0.0	0.3	2.0	0.0
B2a	1.00	8.2	8.2	0.0	0.0	26.0	29.8	0.0		NA	NA	NA	NA	NA
B2b	0.95	21.5	20.4	1.1	0.0	45.7	83.3	5.0		1.1	0.0	2.3	5.0	0.0
B4	1.00	6.4	6.4	0.0	0.0	14.3	98.7	0.0		NA	NA	NA	NA	NA
B5	0.96	10.7	10.3	0.4	0.0	30.7	63.6	4.0		NA	NA	NA	NA	NA
B6b	NA	0.1	NA	NA	0.0	0.0	19.8	NA		NA	NA	NA	NA	NA
B7	0.96	49.5	47.6	1.9	18.4	86.3	86.1	3.9		NA	NA	NA	NA	NA

Table 14: Continued.

Stratum	Factor	Pop.1	Pop.2	Mortality	Pop2L.CI	Pop2U.CI	Area.km^2	%mortality		Sc_pop	PopSL.CI	PopSU.CI	%mortality	Diff
Background strata														
C4	0.97	4.1	4.0	0.1	1.2	7.6	26.3	3.5		0.1	0.0	0.3	3.4	0.1
C6	0.97	20.8	20.1	0.7	6.1	38.2	23.5	3.4		0.7	0.0	2.2	3.4	0.0
C6a	1.00	12.5	12.5	0.0	0.0	36.0	77.1	0.0		NA	NA	NA	NA	NA
Subtotal		**163.9**	**159.0**	**4.9**				**3.0**		**2.0**			**1.2**	**1.7**
Stock assessment survey area														
Total		**527.4**	**509.1**	**18.3**	**136.2**	**1016.2**	**1070.3**	**3.5**		**12.9**			**2.5**	**1.0**

Table 15: Summer mortality estimated as the percentage of recruit-sized oyster deaths from the time mortality began at the beginning of summer to the end of the seasonal mortality (about mid-March), calculated as the percentage of all deaths (pre-survey mortality and post survey mortality combined) of the recruit-sized population at the beginning of summer (population size of recruit-sized new clocks and population size of recruit-sized oysters at the time of survey combined).

	Stock area				Bonamia area		
Pre-survey mortality	**2012**	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**
Recruit-sized new clocks (NC)	30.0	23.7	3.6	7.8	13.5	1.4	5.3
Post-survey mortality							
Correction factor	81.1	49.0	24.3	18.3	34.4	14.8	13.4
Scaled catch	56.9	50.9	24.8	*12.9	31.6	14.8	10.9
Combined summer mortality							
Correction factor +NC	111.1	72.7	27.9	26.1	47.9	16.2	18.7
Scaled catch +NC	86.9	74.6	28.4	*20.7	45.1	16.2	16.2
Population before summer mortality							
Recruit-sized oysters +NC	948.4	533.6	564.7	535.2	364.9	386.6	368.9
Percent summer mortality							
Correction factor +NC	11.7	13.6	4.9	4.9	13.1	4.2	5.1
Scaled catch +NC	9.2	14.0	5.1	*3.9	12.4	4.2	4.4

*** Scaled post-survey mortality (Method 2) not estimated from all survey strata, and therefore an underestimate.**

4. DISCUSSION

Stock assessments are scheduled five-yearly, principally to provide updated model projections of future stock size under three levels of Bonamia mortality and three levels of harvest (0, 7.5, and 15.0 million oysters). The three projections of future stock status are based on 0%, 10%, and 20% disease mortality (Dunn 2007). Five-yearly population surveys are undertaken to provide key inputs into the OYU 5 stock assessment model: estimates of oyster density and population for the three size groups of oysters, estimates of summer mortality from Bonamia, and the population size structure of oysters for the length-based model. Annual Bonamia surveys provide estimates of summer mortality to inform changes in future recruit-sized oyster abundance for management, and as a "weather forecast" for the next oyster season for the oyster industry.

A new time-series of annual Bonamia and oyster surveys established in 2014, incorporates a fully randomised, two-phase sampling design and a standard Bonamia survey area to make these surveys comparable from year to year. The Bonamia survey area represents the range of the core commercial fishery over the recent history of the fishery, during which oyster abundance has varied due to Bonamia mortality. This area comprised 14 of the 26 stock assessment survey strata from 2012, representing 75% of the recruit-sized oyster population and 46% of the stock assessment survey area. Some limited sampling was also undertaken in the remaining 12 strata combined into a single background stratum so that the Bonamia survey data could provide oyster density estimates for the whole stock assessment survey area and therefore allow these data to be incorporated into stock assessments. These surveys estimate changes in oyster density and population size for the three size groups of oysters and the status of Bonamia (prevalence and intensity of infection, and summer mortality). Estimates of summer mortality require estimates of new clocks and fatal infections scaled to the size of the oyster population. The 2017 stock assessment survey incorporated the fourth Bonamia survey in this new time-series.

4.1 Survey performance

Sea conditions for the 2017 survey were rough and although some days were lost, the survey was completed to a high standard consistent with previous surveys. Rough weather is known to reduce dredge efficiency and make sampling more difficult. Sampling rules established for these surveys, where sampling is not undertaken when forecast wind speeds exceed 20 knots, helps maintain dredge and sampling efficiency. Moreover, the rapid recolonization and growth of epifauna, especially in areas with large numbers of kaeos (*Pyura pachydermatina*) can severely reduce sampling efficiency and underestimate oyster densities at some sites. The level of sampling disruption was similar to previous surveys. Sampling conditions and dredge efficiency were consistent with previous surveys.

Low CVs (9–12%) were achieved on the survey with relatively few stations sampled, probably because of the consistent, low oyster densities resulting from heightened Bonamia mortality between 2013 and 2015. These are about half of the target CV set by Fisheries New Zealand for stock assessment surveys.

Full samples of oysters for Bonamia testing were not achieved at a few sites, because of the greatly reduced oyster densities due to Bonamia mortality. The missing samples did not affect the coverage of sampling or the estimates of disease status and mortality but were problematic for analyses that require three or more stations to be sampled. Estimates of the prevalence of infections scaled to population levels require three Bonamia samples in each stratum, but these were not available for some of the survey strata. The performance of qPCR testing was good with a very low sample rejection rate (0.6%).

4.2 Changes in the OYU 5 stock between 2012 and 2017

In 2017, oyster densities and population sizes of recruit (legal-sized), pre-recruit (medium) and small oysters were all about half the size of 2012 levels. Recruit sized oysters in commercial areas (core strata representing the Bonamia survey area) have declined 47.2% since 2012 and 5.6% since 2016. The decline in the whole survey area is similar, 42.6% from that in 2012 and 6.0% from 2016. The declining trend in the recruit sized population appears to have levelled off.

These declines are consistent with the levels of Bonamia mortality, which has greatly reduced oyster densities and the numbers of high-density oyster patches supporting good catch rates in the fishery. The low oyster densities affected catch rates in the 2017 season, and catch rates are expected to continue to decline, mainly depending on future levels of Bonamia mortality.

Recruitment to the oyster population has increased slightly, but the numbers of settlers are still low and unlikely to support significant rebuilding of the commercial-sized oyster population. The population sizes and densities of pre-recruits and small oysters are low, and recruitment to the fishery and rebuilding of areas of high oyster densities are expected to be slow in the medium-term (4–6 years).

Bonamia mortality has declined to low levels not recorded since 2005. Summer mortality over the stock area was about 5% in 2016 and 2017. The densities of new clocks were low in 2016 and 2017 reflecting lower pre-survey Bonamia mortalities and reduced oyster densities. Pre-survey mortality over the stock survey area was 1.5% of recruit-sized oyster population in 2017, slightly up from 0.5% in 2016. Prevalence of infection was low (5.4%) in 2017, most of these were fatal infections. Moreover, non-fatal infections have declined to less than 1%, suggesting low Bonamia mortality in 2018. The low oyster densities and low non-fatal infection suggest reduced transmission of disease.

Overall, the slight increase in settler densities and low expected Bonamia mortality suggest that the fishery will be relatively stable in the short term and catch rates may not decline significantly.

5. MANAGEMENT IMPLICATIONS

Disease mortality of recruit-sized oysters and recruitment (to the population and to the fishery) are the main drivers of future stock size in the OYU 5 fishery. The stock assessment for OYU 5 suggests that an annual commercial harvest of up to 20 million oysters is not likely to have a significant effect on the future (1–3 years) status of the stock (Figure 35).

Between 1993 and 1999, the fishery rebuilt rapidly from a historically low size, mainly due to low and non-detectable Bonamia mortality and high recruitment to the fishery. After the second low point in the fishery in 2005, the fishery again rebuilt rapidly with good spat-fall and juvenile survival driving recruitment, with a Bonamia mortality of about 10% of the recruit-sized population. Since 2009, the population size of recruit-sized oysters has continued to increase, and this high number of recruits should have led to increased recruitment to the fishery. However, recruitment to the fishery has been low since 2009. The low recruitment and high Bonamia mortality between 2013 and 2015 flattened the stock trajectory between 2010 and 2012. Significant summer mortality from Bonamia, 15.9% in 2013, 18.3% in 2014, and 13.6 % in 2015, along with the low recruitment to the fishery has led to a decline in the recruit-sized population since 2012. In 2017, population sizes of recruit-sized oysters, pre-recruit and small oysters were all about half that of 2012. However, the population size of recruit-sized oysters is higher (527 million) than the last low in the fishery in 2005 (408 million oysters). The stock size is at 19% B_0 compared with 15% B_0 in 2005, but down from 35% B_0 in 2012. Moreover, recruitment has increased slightly, but the numbers of small and pre-recruit oysters are still low and unlikely to support significant rebuilding of the commercial-sized oyster population. Bonamia mortality is low (about 5%). The low densities of oysters and low numbers of oysters with non-fatal infections suggest that Bonamia

mortality may be low again in 2018. The short-term outlook for the fishery is a continued decline in commercial sized oyster densities determined by levels of Bonamia mortality, and declining catch rates.

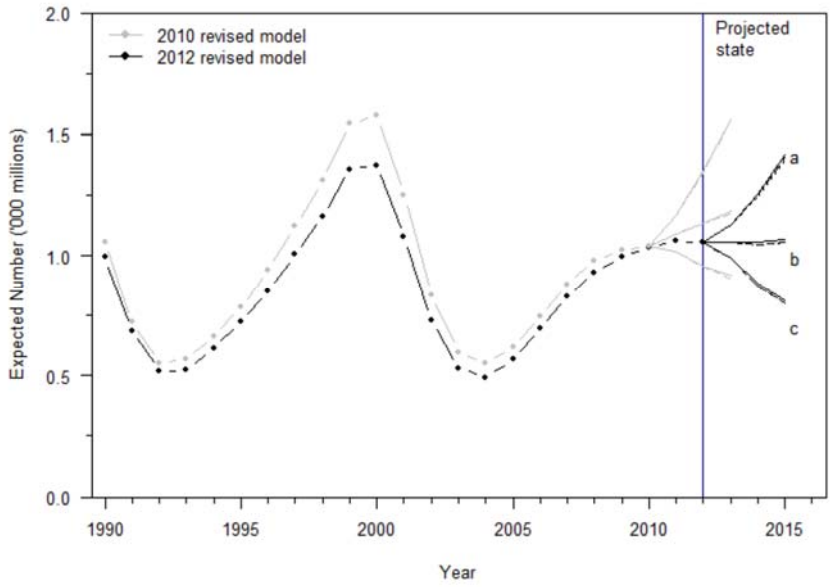

Figure 35: Model estimates of recruit-sized stock abundance (2012) and projected recruit-sized stock abundance for 2013–15 with a catch of 7.5 (solid line), 15 (dash dot), and 20 million oysters (dashed line) under assumptions of (a) no disease mortality, (b) disease mortality of 0.10 per year, and (c) disease mortality of 0.20 per year, for the 2010 and 2012 revised models (figure reproduced from Fu 2013). Note that the projections for the three catch levels overlay, and are therefore difficult to see, showing that levels of catch up to 20 million oysters have little discernible effect on future stock status.

5.1 Future survey considerations

Stock assessments use a length-based model. The relative importance of population length frequency data and optimal frequencies for sampling these data should be assessed. Sampling for population length frequency could be included in the annual Bonamia surveys.

We estimate Bonamia mortality for recruit-sized oysters only. Survey data suggest that pre-recruit oysters may be equally as vulnerable to disease mortality. Occasional estimates of the prevalence of fatal and non-fatal Bonamia infections across the size range of the population should be undertaken. Moreover, surveillance for *Bonamia ostreae* infection in Foveaux Strait oysters could be concurrently undertaken with the *B. exitiosa* surveys to increase efficiency and minimise costs. The development of a ddPCR duplex assay for both pathogens could have broad biosecurity applications and could therefore be publicly funded along with the surveillance component of future Bonamia surveys.

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8. APPENDICES

8.1 Appendix 1: Data forms and sampling methods

Station data form

FOVEAUX STRAIT OYSTER SURVEY, STATION DATA RECORD

Vessel name | Recorder

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

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

Finish position: Latitude | S | Longitude | E

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

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

% fullness of dredge including sediment | Live Bryozoa | Bycatch photo numbers

Wind force, beaufort | Did the dredge fish well? Y=1 or N=2 | *Bonamia* sample? | Comments?

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

Sediment type

Circle the main type (one only)

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

Comments:

1 Nautical mile = 1.853 km

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

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

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

Bonamia sampling form

FOVEAUX STRAIT OYSTER BONAMIA DATA RECORD

Page ___ of ___

Date

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

Recorder .....

Comments .....

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

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

8.2 Appendix 2: Sampling methods

Navigation

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

Survey tows

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

When it was possible in 2017, fixed tows were repeated over the same tow line and in the same tow direction as in previous surveys and started on station position where possible.

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

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

Sorting the catch

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

The catch of oysters and bycatch from each survey tow were photographed with a digital camera before the catch was sorted into live oysters, gapers (live, but moribund oysters containing the whole oyster and valves remaining apart after the adductor muscle has lost its ability to contract), and clocks (the articulated shells of recently dead oysters with the ligament attaching the two valves intact) to estimate mortality.

In this February survey, new clocks were defined as those that had clean inner valves that had retained their lustre but may have had some minor speckling of fouling organisms (Figure A2.1). For this analysis, we assumed that new clocks were only those oysters that had died since summer mortality from Bonamia began, and oysters that died before this were categorised as old clocks.

The shells of oysters that are fouled or in which the inner valves have lost their lustre are termed old clocks (Figures A2.2 and A3.3). Old clocks can be covered in fouling organisms on both external and internal surfaces, and as the ligaments of oysters are thought to break down over about a three-year period, old clocks represent oysters that died between 1 and 3 years previously (Cranfield et al. 1991). The classification of old clocks may vary depending on habitat. Old clocks from sand habitats may be

older as they may be filled with sand preventing the settlement of fouling organisms and reducing physical forces on the hinge and prolonging the time that both valves remain attached beyond three years. Gravel habitats are usually shallower with stronger tidal currents and higher swell energy and the valves of old clocks there may be disconnected much more quickly than three years or the clocks (new and old) may be transported out of the fishery area by the strong tides.

The catch was further sorted into two size groups: recruit-sized (unable to pass through a 58 mm internal diameter ring), and pre-recruits (able to pass through a 58 mm internal diameter ring, but unable to pass through a 50 mm ring). Live oysters were sorted into a third size group, small oysters (able to pass through a 50 mm internal diameter ring and down to 10 mm in length). Reference rings (58 mm and 50 mm internal diameter) were used to ensure accurate allocation to each size group.

The data recorded at each station included start and finish location of the tow, depth, speed of tow; numbers of oysters, new clocks, and gapers caught; percentage fullness of the dredge; wind force (Beaufort scale); stations where live bryozoans (*Cinctipora elegans*) were observed; and sediment type. The presence/absence of bycatch species was also recorded directly from the dredge contents. The data form used to record these data is shown in Appendix 1–1.

Figure A2.1: New clock (with hinge intact), glossy inner valve with no fouling except a few white coralline specks.

Figure A2.2: Recent old clock (with hinge intact), glossy inner valve with light fouling.

Figure A2.3: Old clock with hinge intact. No gloss on inner valve and heavy fouling.

8.3 Appendix 3: Analysis

Estimates of oyster densities and population size

We estimated abundance as the mean oyster density of oysters, but in three size groups (recruit-sized, pre-recruit, and small oysters), and biomass as the population size in millions of oysters for the same size groups by stratum, for the Bonamia survey area, and for the 2007 stock assessment area. Oyster densities and population sizes were estimated for strata where three or more randomly selected stations were sampled in February 2017 and these were compared with the estimates from the same strata sampled in the 2012 stock assessment survey and the 2016 Bonamia survey. These estimates are presented separately. The absolute population size of each size group of oysters in 2017 was estimated using the combined population sizes in each stratum.

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

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

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

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

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

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

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

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

1. The sampling 95% confidence intervals:

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

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

2. The 95% confidence intervals of the population means for each stratum and the total population are estimated by resampling a normal distribution whose variance is based on a CV and the error of the estimated dredge efficiency. The total error of the estimates of the population mean has two sources: one is the sampling error from the survey. The survey estimate of population size follows a normal distribution and this is based on standard survey sampling theory. The other source is error associated with dredge efficiency, which are assumed to be normally distributed (there are only three data points). If the two sources of error are independent, then the error can be estimated by simply adding the two variance components.

Patterns of recruitment

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

Recruitment to the fishery was summarized using plots of changes in the population estimates of pre-recruit and small oysters, and from changes in the patterns of distribution of small oyster densities, between the February 2012, and the February 2016 and 2017 surveys. The numbers of small oysters per recruit-sized oyster were estimated to investigate trends in recruitment between 2012, 2016 and 2017.

Size frequencies of the population

About two hundred oysters were randomly selected from the dredge catch sampled at each of the first three first-phase stations. Each oyster was assigned a size group (recruit-sized, pre-recruit, and small oysters) using the reference rings and measured for length (along the anterior-posterior axis) and height (along the dorsal ventral axis), see Figure A3.1.

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

Estimates of annual mortality from Bonamia

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

Most of the recent Bonamia mortality has occurred in the summer months, however significant winter mortality from Bonamia has occurred previously (Hine 1991). We estimated summer mortality from Bonamia only, and for recruit-sized oysters only. Summer mortality comprises the aggregate of two different estimates:

1. Pre-survey mortality estimated from the population size of recruit-sized new clocks and gapers, and

2. Post-survey mortality (within about two months) from the proportion of oysters with categories three and higher (fatal) infections scaled-up to the size of the total recruit-sized oyster population (objective 5).

Although pre and post survey mortality measure different variables and pre-survey mortality may include heightened natural (non-disease related) mortality, the sum of pre and post survey totals gives the best estimate of summer mortality.

Pre-survey mortality was estimated as the absolute population size of recruit-sized new clocks and gapers using the same methods as for live oysters (see Section 3.3). In the absence of dredge efficiency data for clocks, we assume that the dredge efficiency is the same for clocks as it is for live oysters. The catchability (dredge efficiency) and persistence of new clocks at the location of death varies spatially for new clocks, and their classification as new (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 sometime 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. Dredge efficiency for clocks is likely to be much lower and therefore underestimate clock densities.

Post-survey mortality is estimated from the numbers of fatally infected, recruit-sized oysters in the samples. Bonamia studies (Diggles et al. 2003) suggest that category 3 Bonamia infections (see Table A3.1) are elevated and systemic and are assumed to quickly progress to category 4 and 5 infections, quickly leading to death (soon after the survey). The mean proportion of oysters with category 3−5 infections in each stratum is used as a correction factor for the population estimates, i.e. 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. A second estimate of post-survey mortality uses the combined prevalence of oysters with category 3–5 infections, that is the actual numbers of fatally infected oysters in the catch, and stratum and population estimates of fatally infected oysters are made using the same analytical methods as for live oysters, scaled up to the size of the population.

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

Estimate of prevalence and intensity of Bonamia infection

The numbers of infected recruit-sized oysters in the commercial population (defined by the core survey strata) and by stratum were estimated from the numbers of infected oysters determined from qPCR assays. The numbers of non-fatally and fatally infected oysters were estimated from Bonamia intensity scores derived from heart imprints and scaled-up to the size of the recruit-sized oyster population by strata and for the commercial fishery area.

Samples of up to 30 randomly selected recruit-sized oysters from each station were collected for heart imprints, histology, and molecular (qPCR) analysis to estimate levels of Bonamia infection. When there were insufficient recruit-sized oysters in the catch, pre-recruit and small oysters were used to fill the sample size, or the whole catch was retained for processing. Samples were bagged, labelled with station number, date, and time on waterproof labels and the sacks tied securely. The oysters for Bonamia samples were kept cool and damp in oyster sacks, transferred to poly bins, and flown to NIWA, Wellington, for processing. Oyster samples generally arrived in Wellington within 36 hours of capture and were processed that day. The samples were held in poly bins under cool conditions (about 8–12 °C) in the aquarium. If they could not be processed the day they arrived, they were held in tanks of flowing seawater and processed at the first opportunity.

Heart imprints and qPCR sampling methods

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.

Station and sample data were recorded on Bonamia sampling forms (Appendix 1), and the total numbers of live and dead oysters in the samples noted. A subsample of up to 25 recruit-sized oysters from each station was taken for heart imprints and qPCR to estimate the prevalence and intensity of Bonamia. Each oyster in the sample was assigned a unique number from 1 to 25, and assigned a size category using oyster size rings, and oysters were measured for length and height (Figure A3.1) using callipers, and the measurement truncated to the lower whole millimetre. If samples contained insufficient recruit-sized oysters, pre-recruits were used in preference to small oysters. Recruit-size oysters were denoted with an R, pre-recruit oysters with P, and small oysters with an O. Gaping oysters with valves of the shell apart, but which closed when tapped, were marked with an asterisk alongside the corresponding oyster number. These samples also provide estimates of the numbers of oysters incubating larvae at each station to provide information on reproduction in the fishery. Oysters were recorded as either incubating white (early-stage) larvae, grey (late-stage) larvae, yellow (almost ready to settle) larvae; or with no larvae present.

Heart imprints were made by removing the heart (dark organ adjacent to adductor muscle, see Figure A3.2) with fine forceps, draining excess water and fluid on filter paper, and lightly dabbing the heart on a slide to deposit a small amount of haemolymph. Three rows of 8 to 10 imprints were made on labelled slides. Slides were placed in slide racks to air dry for at least 5 minutes. The slides were stained with Hemacolor © and oven dried at 60 °C.

Histological samples were taken from the first five oysters processed for heart imprints (these were noted on the Bonamia data form as Y). A section was taken through the digestive gland (Figure A3.2) and fixed in a quantity of 10% formalin in seawater equal to at least five times the tissue volume of the sample.

Sampling methods for qPCR

Oysters sampled for heart imprints were also sampled for qPCR. Remnants of the oyster hearts sampled for heart imprints and samples of gill tissue were placed into separate, uniquely labelled 96 well qPCR plates. Additional samples of gill tissue from the same oysters were placed into new uniquely labelled 96 well qPCR plates as backup tissues should they be required, and a third series of plates containing gill and mantle tissue provided to the Ministry for Primary Industries Biosecurity (Dr Brian Jones). Laboratory work sheets recorded sampling data including: date, name of sampler, plate number and station number and the date and time the sample was collected.

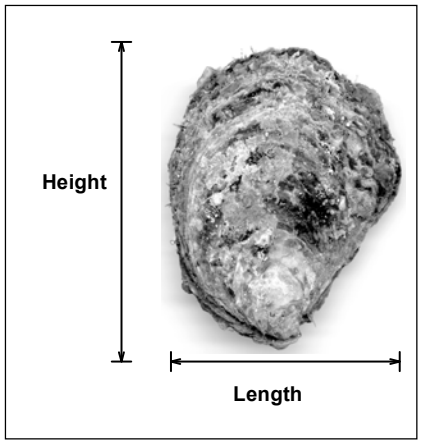

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Figure A3.1: An oyster showing length (anterior-posterior axis) and height (dorsal-ventral axis) dimensions.

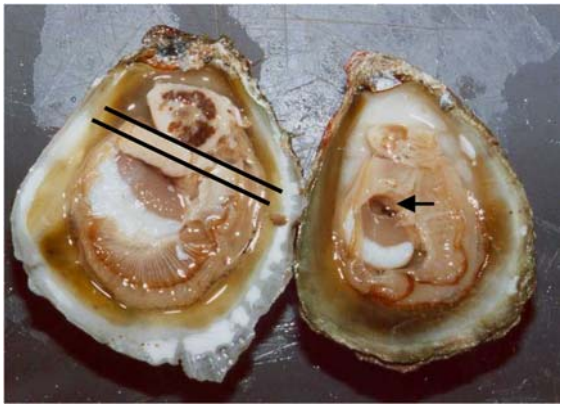

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Figure A3.2: Lines on left oyster show location of 5 mm thick standard section taken for histology. The arrow on the oyster on the right shows the heart, a black organ adjacent to the adductor muscle.

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

Analysis of qPCR samples

Methods to review qPCR procedures prior to testing

The qPCR method requires strict adherence to aseptic and molecular biology techniques due to the sensitivity of the method. New reagents were tested against serial dilutions of a synthetic standard to ensure consistency of reaction and Cq values between survey years. Reagents were checked using both positive and negative controls and Cq values were checked to ensure that they were within an acceptable range. Further testing was carried out to ensure that serial dilution of the positive control produced the standard cut off value for negatives (Cq35).

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

qPCR analysis

A detailed account of the qPCR method and testing is given in (Maas et al. 2013). This novel qPCR method has been successfully developed to detect and quantify *Bonamia exitiosa* in oysters from Foveaux Strait. This method relies on two key innovations: a duplex qPCR assay and a shortened bench top method. The characteristics of the qPCR assay include the co-amplification of the *Bonamia* target (ITS region of the ribosomal genes) and *Ostrea chilensis* β-actin gene as an internal control. In addition, the assay uses a new master mix containing a robust taq polymerase mix (thermostable DNA polymerase used in polymerase chain reaction (PCR) to amplifying short segments of DNA) that is able to cope with inhibitors often found in crude extracts and extracts from environmental samples. A novel system is also employed to delay the amplification of the internal control to prevent a low level Bonamia ITS amplification being outcompeted by the stronger internal control (β-actin) reaction.

This method has also successfully incorporated a shortened bench top method to minimise handling and was transferred to a 96 well plate format to allow the simultaneous screening of up to four Bonamia stations per hour compared to 3−4 stations per day using histological methods (heart imprints). Oyster heart and gill tissue were analysed using the same method. Tissues were digested and diluted, and aliquots of extract added to qPCR reagents that were then analysed with a BIORAD-CFX96 qPCR (quantitative polymerase chain reaction instrument).

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

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

qPCR data from oyster heart and gill samples were assessed based on the information for each plate contained within the sample sheets to ensure that there were no sampling issues such as tissue size, contamination, or missing samples that may have affected the assay or interpretation of the results; plots of relative fluorescence units (RFU) against Cq values to look for problems in the reaction; and Cq values for the positive (Bonamia ITS and β-actin gene) and the negative internal control reactions to ensure that the reactions occurred to standard.

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

- Out of range Cq values for the Bonamia positive and negative control wells (at about a Cq of 28) occurred,
- The Bonamia ITS and internal control Cq values were both NAs (there were no values to show that the reaction had worked),
- Either the Bonamia ITS or internal control (β- actin) amplified very early in the cycles (Cq <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. Repeated samples that gave anomalous results such as flatliners where no reaction was detected or early ampers (very low Cq values) were also screened with heart imprints.

Analysis of oyster heart imprint data

Examination of heart imprints is at least as sensitive as histology, but whereas histology is time consuming and expensive, heart imprints can be screened rapidly and are comparatively inexpensive. 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 stations, at least 3 randomly selected samples from each station that tested negative with qPCR, and flatliners and early ampers after samples underwent repeat qPCR assays. Oyster heart imprints were examined under a microscope using a times 50 objective lens under oil and scored for intensity of infection using the criteria listed in Table A3.1. Three good heart imprints containing oyster haemocytes were located and examined on each slide, and the number of Bonamia cells counted for each. If no Bonamia cells were found, further imprints were examined to confirm the absence of Bonamia. In 2014, heart imprints were examined by a single experienced reader. A review of scoring protocols was undertaken before screening samples.

Table A3.1: 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

Before qPCR assays were available, we assumed 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 the proportion of the total sample number with Bonamia infection from the qPCR results of heart tissues where Cq is 35 or less, and for the 25th slide 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 from heart imprints samples only as the mean frequency of stages 1–5 oysters (i.e., the mean stage of all oysters examined that had at least one Bonamia cell observed). The inclusion of the additional smaller oysters at stations where few recruit-sized oysters were caught is likely to introduce a bias to estimates of prevalence and intensity of infection because oysters are increasingly less vulnerable to infections and mortality as oyster size decreases. Exact 95% confidence intervals are given for prevalence and for the proportion of new clocks, determined from the F-distribution, i.e., for a proportion π, where π = r/n (where r is the number of oysters infected with Bonamia and n the number of oysters in the sample), the 95% confidence interval is determined by:

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

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

Population estimates of Bonamia infection

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

Method 1 used a correction factor from strata with three or more randomly selected stations only i.e., we did not include target stations. Method 2 used the total numbers of oysters in each Bonamia infection category (1–5) based on the estimated proportion of oysters in each infection category in the sample and scaled to the total catch for each station. The overall intensity was calculated as the average

Bonamia level in the population. Variance for prevalence and intensity was estimated using standard methods as for population estimates.

8.4 Appendix 4: Survey comparability

Dredge tow lengths were almost all about 0.2 nautical miles (371 m) in length (Figure A4.1). 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 wind conditions less than 10 knots (Figure A4.2). The median wind force was 2 on the Beaufort scale (3−6 knots), with 5 and 95 percentiles of Beaufort scale 0 (less than 1 knot) and 3 (7−10 knots) respectively. Maximum wind speed during sampling was about 15 knots. Dredge sampling during the February 2017 surveys was undertaken in similar conditions to recent surveys. The February 2016 survey had a median wind force of 1 on the Beaufort scale (1−2 knots), with 5 and 95 percentiles of Beaufort scale 0 (less than 1 knot) and 4 (11−15 knots) respectively. The February 2015 survey had a median of 3 Beaufort scale (7−10 knots), and 5 and 95 percentiles of Beaufort scale 0 (i.e. less than 1 knot) and 5 (16−20 knots) respectively; and in similar wind conditions to 2014. This wind range and the resulting sea conditions were below the level likely to affect dredge efficiency, but any gains in efficiency may have been moderated by the high tidal flows (spring tides) thought to reduce dredge efficiency (Stephen Hawke, oyster vessel skipper, pers. comm.) over the 2017 sampling period.

Oyster dredges are considered saturated and cease fishing before the end of tow when they are more than 80% full on landing (Cranfield pers. comm.). Dredge saturation may lead to an underestimate of oyster density. No dredge was landed more than 80% full (Figure A4.3). Dredge fullness ranged from 1% to 80% with a median fullness of 40%, the same as in 2016 and 2015, but lower than in 2014 (50%) and higher than in 2013 (30%). Differences in dredge fullness are in part related to levels of pre-survey mortality from Bonamia that increases the quantities of dead shell. Dredge saturation is not likely to have had a large effect on sampling effectiveness in the 2017survey (Figure A4.3). Observations and anecdotal evidence from video data recorded during dredge trials suggest that dredge saturation may occur in dredges landed less than 80% full, however, when this occurs, the dredge contents were unevenly, but symmetrically, spread with contents lower in the middle of the dredge than at the edges of the dredge ring bag. This was not recorded in the 2016 survey data; future surveys will identify stations with this pattern in the distribution of catch. Six stations (2, 3, 12, 28, 50, and 112) were landed over 70% full in 2017 with catches ranging from 31 to 261 recruit-sized oysters. Oyster densities may have been underestimated at these stations.

Dredge efficiency is thought to be greatly reduced in areas densely populated with kaeos (*Pyura pachydermatina*) as the dredge skims above the seabed with little or no contact (personal observation, 2015 to 2017 Bonamia surveys, and 2017 stock assessment survey). Large numbers of kaeos and very few oysters were caught in stratum E4 (stations 51 & 53), stratum C6a (station T10), and, stratum B6b (station 199). Oyster density was most likely underestimated at these stations.

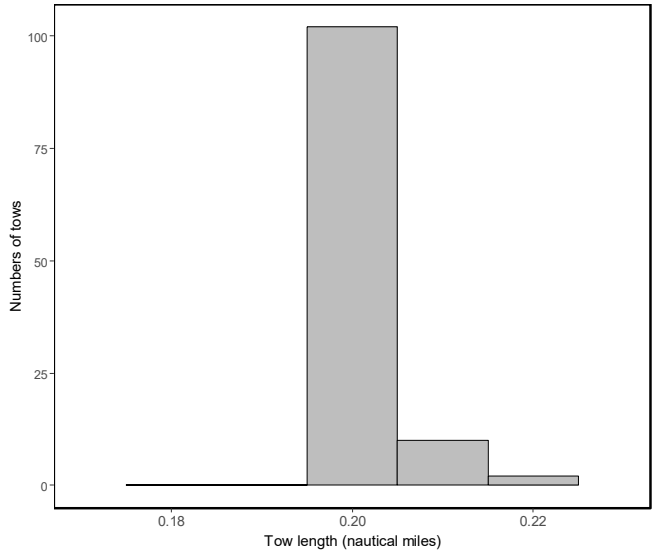

Figure A4.1: Distribution of dredge tow lengths from the February 2017 surveys. The standard tow length was 0.2 nautical mile (371 m).

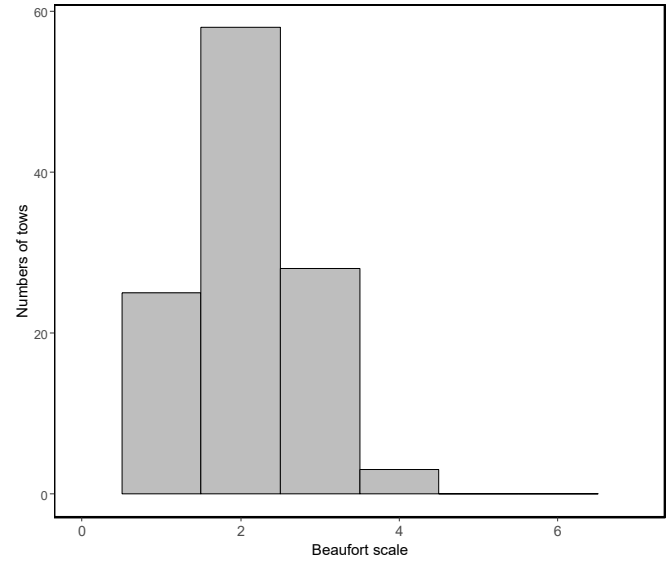

Figure A4.2: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2017 Beaufort scale: 0, < 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

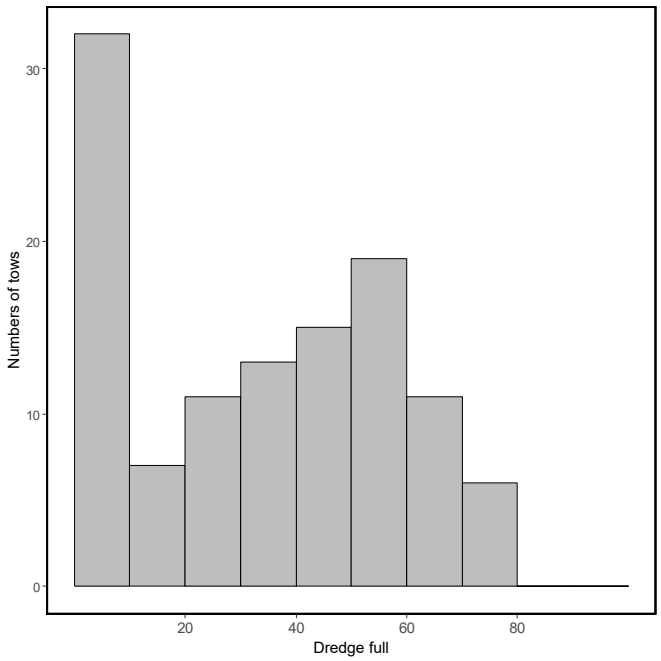

Figure A4.3: Distribution of dredge fullness recorded for survey tows in February 2017 No tows were landed with a dredge fullness of greater than 80%. Unpublished video data suggests that dredge saturation may occur below 80% full.

8.5 Appendix 5: 2017 survey catch and infection details

Table A5.1: Numbers of recruit-sized oysters (Recruits) and absolute densities (Density) by stratum and tow. Total numbers of oysters sampled for Bonamia (Total) with the numbers of non-fatal infections (UN.inf), fatal infections (Fatal.inf), percent prevalence (%Prev), intensity of infection (Intensity) and percent prevalence from heart tissues using qPCR (%Prev.H).

Stratum	Station	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	%Prev	Intensity	%Prev.H
B1	1	14	0.07	25	25	0	0	0	0.0	0
B1	2	31	0.15	25	25	0	0	0	0.0	0
B1	3	163	0.78	25	25	0	0	0	0.0	0
B1	4	59	0.28	25	25	0	0	0	0.0	0
B3	5	287	1.38	25	25	0	0	0	0.0	0
B3	6	171	0.82	25	25	0	0	0	0.0	4
B3	7	505	2.43	25	25	0	0	0	0.0	0
B6	8	324	1.56	25	24	0	1	4	3.0	4
B6	9	334	1.60	25	22	1	2	12	3.0	12

Stratum	Station	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	%Prev	Intensity	%Prev.H
C1A	11	314	1.51	25	24	0	1	4	3.0	8
C1A	12	55	0.26	25	22	0	3	12	3.7	12
C1A	13	139	0.67	25	21	0	4	16	3.8	20
C2	14	118	0.57	25	21	0	4	16	3.5	20
C2	15	68	0.33	25	23	0	2	8	3.5	8
C2	16	199	0.96	25	23	0	2	8	5.0	12
C3	18	71	0.34	25	25	0	0	0	0.0	4
C3	19	104	0.50	25	23	1	1	8	3.0	8
C3	20	15	0.07	22	22	0	0	0	0.0	0
C5	22	148	0.71	25	24	0	1	4	4.0	4
C5	23	109	0.52	24	24	0	0	0	0.0	0
C5	24	71	0.34	25	25	0	0	0	0.0	0
C5A	25	65	0.31	25	24	1	0	4	1.0	4
C5A	27	100	0.48	25	25	0	0	0	0.0	8
C7	28	258	1.24	25	21	0	4	16	3.8	16
C7	29	8	0.04	13	13	0	0	0	0.0	0
C7	30	160	0.77	25	25	0	0	0	0.0	4
C7	31	111	0.53	25	23	0	2	8	4.0	12
C7	32	415	1.99	25	24	0	1	4	3.0	4
C7	33	537	2.58	25	25	0	0	0	0.0	0
C7A	34	68	0.33	25	25	0	0	0	0.0	0
C7A	35	26	0.12	25	25	0	0	0	0.0	0
C7A	36	32	0.15	25	25	0	0	0	0.0	0
C8	37	254	1.22	25	21	1	3	16	3.3	16
C8	38	348	1.67	25	22	0	3	12	4.3	16
C8	39	42	0.20	25	22	0	3	12	4.3	12
C8	40	80	0.38	25	25	0	0	0	0.0	0

Stratum	Station	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	%Prev	Intensity	%Prev.H
C9	42	224	1.07	25	23	0	2	8	4.0	8
C9	43	567	2.72	25	24	0	1	4	4.0	4
C9	44	366	1.76	25	24	0	1	4	4.0	8
C9	45	449	2.16	25	25	0	0	0	0.0	0
C9	46	225	1.08	25	25	0	0	0	0.0	0
E2	47	9	0.04	20	20	0	0	0	0.0	0
E2	48	136	0.65	25	25	0	0	0	0.0	0
E2	49	152	0.73	25	24	0	1	4	4.0	4
E4	50	62	0.30	25	24	0	1	4	4.0	12
E4	51	5	0.02	NA	NA	NA	NA	NA	NA	NA
E4	52	265	1.27	25	23	0	2	8	3.5	8
E4	53	6	0.03	NA	NA	NA	NA	NA	NA	NA
E4	54	12	0.06	17	17	0	0	0	0.0	0
B1A	55	2	0.01	NA	NA	NA	NA	NA	NA	NA
B1A	56	3	0.01	NA	NA	NA	NA	NA	NA	NA
B1A	57	0	0.00	NA	NA	NA	NA	NA	NA	NA
B1B	60	423	2.03	25	25	0	0	0	0.0	0
B2	61	61	0.30	25	23	1	1	8	2.5	8
B2	62	91	0.44	25	24	1	0	4	2.0	4
B2	63	27	0.13	25	24	0	1	4	5.0	8
B2A	64	166	0.80	25	24	1	0	4	2.0	4
B2A	65	3	0.01	NA	NA	NA	NA	NA	NA	NA
B2A	66	0	0.00	NA	NA	NA	NA	NA	NA	NA
B2B	67	48	0.23	25	22	1	2	12	3.0	12
B2B	68	101	0.49	25	24	0	1	4	3.0	8
B2B	69	8	0.04	25	25	0	0	0	0.0	0

Stratum	Station	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	%Prev	Intensity	%Prev.H
B4	70	19	0.09	25	25	0	0	0	0.0	0
B4	71	21	0.10	25	22	2	1	12	3.0	12
B4	72	0	0.00	NA	NA	NA	NA	NA	NA	NA
B5	73	0	0.00	NA	NA	NA	NA	NA	NA	NA
B6B	78	0	0.00	NA	NA	NA	NA	NA	NA	NA
B7	79	121	0.58	25	23	1	1	8	2.5	8
B7	80	7	0.03	7	7	0	0	0	0.0	0
B7	81	111	0.53	25	24	0	1	4	4.0	12
C4	83	31	0.15	25	24	0	1	4	3.0	4
C6	85	154	0.74	25	22	0	3	12	4.3	12
C6	86	298	1.43	25	25	0	0	0	0.0	0
B6A	88	0	0.00	NA	NA	NA	NA	NA	NA	NA
B6A	90	86	0.41	25	25	0	0	0	0.0	0
B3	96	59	0.28	NA	NA	NA	NA	NA	NA	NA
B3	97	241	1.16	NA	NA	NA	NA	NA	NA	NA
B3	98	103	0.49	NA	NA	NA	NA	NA	NA	NA
B6	102	177	0.85	25	23	0	2	8	3.5	8
C1A	106	46	0.22	NA	NA	NA	NA	NA	NA	NA
C2	112	65	0.31	25	22	1	2	12	2.7	16
C5	123	308	1.48	25	24	1	0	4	2.0	8
C5A	126	18	0.09	20	20	0	0	0	0.0	0
C9	147	112	0.54	25	23	0	2	8	3.0	8
B1B	167	103	0.49	25	24	0	1	4	5.0	4
B1B	168	43	0.21	25	23	1	1	8	2.0	4
B1B	170	0	0.00	NA	NA	NA	NA	NA	NA	NA
B5	191	7	0.03	NA	NA	NA	NA	NA	NA	NA
B5	193	96	0.46	25	24	0	1	4	4.0	4
B6B	199	1	0.00	NA	NA	NA	NA	NA	NA	NA

Stratum	Station	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	%Prev	Intensity	%Prev.H
B7	201	228	1.09	NA	NA	NA	NA	NA	NA	NA
B7	202	118	0.57	NA	NA	NA	NA	NA	NA	NA
C4	207	52	0.25	25	24	0	1	4	3.0	8
C4	209	13	0.06	18	18	0	0	0	0.0	0
C6	212	88	0.42	25	24	1	0	4	2.0	4
B6A	217	13	0.06	18	18	0	0	0	0.0	0
B6B	222	3	0.01	NA	NA	NA	NA	NA	NA	NA
B1B	252	0	0.00	NA	NA	NA	NA	NA	NA	NA
B1B	259	135	0.65	NA	NA	NA	NA	NA	NA	NA
B1B	260	140	0.67	NA	NA	NA	NA	NA	NA	NA
B1B	265	289	1.39	NA	NA	NA	NA	NA	NA	NA
B1B	266	28	0.13	NA	NA	NA	NA	NA	NA	NA
C5A	T1	111	0.53	25	24	0	1	4	3.0	4
B1	T2	341	1.64	25	25	0	0	0	0.0	0
E2	T3	198	0.95	25	24	1	0	4	2.0	8
E2	T4	276	1.32	25	24	1	0	4	2.0	4
C8	T5	438	2.11	25	25	0	0	0	0.0	4
B6A	T6	42	0.20	25	25	0	0	0	0.0	0
C2	T7	248	1.19	25	19	0	6	24	3.8	28
C3	T8	310	1.49	25	21	0	4	16	3.8	20
C5	T9	400	1.92	25	24	0	1	4	3.0	8
B6A	T10	30	0.14	25	25	0	0	0	0.0	0
E4	T11	56	0.27	25	25	0	0	0	0.0	0
E4	T12	381	1.83	25	24	0	1	4	3.0	4