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Tini a Tangaroa

A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU 5) in commercial fishery areas and the status of Bonamia (*Bonamia exitiosa*) in February 2018.

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The February 2018 Foveaux Strait oyster and Bonamia surveys are the fourth in a new time-series of surveys. The current programme of five-yearly stock assessments (the last in 2017) has placed greater onus on these surveys to monitor changes in the oyster population in commercial fishery areas as well as to monitor the status of Bonamia. February Bonamia surveys provide a "weather forecast" immediately before the oyster season begins. These surveys update information on oyster densities in the important commercial fishery areas. This forecast also updates the status of infection and estimates of disease mortality; and together with estimates of recruitment from spat monitoring, catch sampling and survey estimates, are important in predicting the future status of the fishery. Fourteen of the twenty-six 2017 stock assessment survey strata were surveyed as the Bonamia survey area. Limited sampling was also undertaken in a single background stratum (all the remaining stock assessment strata combined) to allow these data to be incorporated into stock assessments.

This survey was undertaken in collaboration with the Bluff Oyster Management Company who provided a vessel, the survey dredge, and crews for the survey. Dredge sampling during the 2018 Bonamia survey was consistent with previous surveys and these data are comparable with others in the time-series. Testing for *Bonamia exitiosa* infection was transferred from the previous qPCR-based method to an improved ddPCR assay with a high level of precision and repeatability, superior levels of sensitivity and detection, and cost-effectiveness.

Population estimates are presented by stratum, and by two fishery areas: the Bonamia survey area (core strata, 491.8 km^2) and the 2007 stock assessment survey area (1070.2 km^2). Only five stations were sampled in the background stratum (578.4 km^2) representing 12 of the 26 stock assessment survey strata; estimates for the background stratum and the stock assessment survey area should therefore be viewed with caution. The population sizes of all three size groups of oysters in core strata increased between 2017 and 2018. Recruit sized oysters were up 35% to 494.1 million oysters, pre-recruit oysters up 45 % to 178.4 million oysters, and small oysters up 53% to 401.8 million oysters in 2018.

Bonamia infection levels have been low since 2016. The use of ddPCR in 2018 has most likely improved the detection of low level infection. Estimates of the mean prevalence of *B. exitiosa* infection in recruit-sized oysters from the Bonamia survey area were 1.7% using heart imprints, and 7.2% using ddPCR. Bonamia mortality was low (2–3%) over the summer of 2017–18. Pre-survey mortality of recruit-sized oysters from new clocks was low, 0.6% of the population in the Bonamia survey area. Post-survey mortality estimated from the numbers of fatally infected oysters was also low (1.4–2.4%). Non-fatal infections declined in 2018 to about 0.1% of the recruit-sized population, suggesting low Bonamia mortality in 2019. The low oyster densities and low non-fatal infections suggest reduced transmission of Bonamia infection.

Fishers target high density patches of "commercial-sized" oysters. In 2017, 66% of the catch was 70 mm in length or larger (recruit size is 58 mm in length or larger). Between 2012 and 2017, Bonamia mortality has greatly reduced the numbers and extent of high-density patches with commercial-sized oysters, and oysters were generally distributed at low densities across the fishery area. Catch rates had fallen from 5.6 sacks per hour (S/H) in 2010 to 2.9 S/H in 2017. Oysters growing to recruit size in 2018 may not grow to "commercial size" for another 1–2 years. Therefore, catch rates in 2018 are expected to remain similar or to fall further.

In the medium-term, all the key indicators for the future rebuilding of the fishery are strongly positive. The three size groups of oysters increased between 35% and 53% between 2017 and 2018. Spat monitoring, catch sampling and the survey data show significant recruitment to the oyster population, and increases in pre-recruit and small oyster will support future increases in recruit-sized oysters. Bonamia mortality was low in 2018, and is it is expected to be low in 2019. At this low mortality, the 2017 stock assessment predicts that by 2020, recruit-sized oysters will increase by about 41%. The medium-term outlook for the OYU 5 fishery is for increasing commercial-sized oyster densities and catch rates.

1. INTRODUCTION

The Foveaux Strait oyster fishery (OYU 5) is a high value, iconic fishery that has been fished for over 150 years. Oysters (*Ostrea chilensis*) are an important customary (taonga), recreational, and commercial species, and are important to the socioeconomics of Bluff and Invercargill. The OYU 5 stock is part of the Group 1 stocks in the Ministry for Primary Industries (MPI) draft National Fisheries Plan for Inshore Shellfish which recognises the relatively high biological vulnerability of Group 1 stocks (including OYU 5) and prescribes a close monitoring approach. Achieving maximum value from Group 1 stocks is best achieved through accurate and frequent monitoring to support responsive management. Additionally, there is a collaborative fisheries plan for the management of the fishery, the Foveaux Strait Oyster Fisheries Plan (Ministry of Fisheries 2009). This plan was collaboratively developed by the Foveaux Strait Oyster Fisheries Plan Management Committee (FSOFPMC) which included representatives from the Bluff Oyster Management Company (BOMC), customary and recreational fishers, and the then Ministry of Fisheries, now Fisheries New Zealand.

The haplosporidian parasite of flat oysters *Bonamia exitiosa* (Bonamia) is thought to be an endemic disease of Foveaux Strait oysters. Two recent Bonamia epizootics in 1985–92 (Doonan et al. 1994, Cranfield et al. 2005) and from 2000 to the present (2018, Michael et al. 2016) have shown that Bonamia mortality is a recurrent feature of the oyster population, and that this mortality is the principal driver of oyster population abundance during epizootics. These recurrent events suggest that Bonamia epizootics can be expected in the future. Management of the fishery recognises that recruit-sized stock abundance and future benefits from the fishery (harvest levels) are mainly determined by the levels of Bonamia mortality, and that the current harvest levels and any effects of fishing on either oyster production or on exacerbating Bonamia mortality are not detectable. A summary of Bonamia and its effects on the fishery is given in Michael et al. (2015a).

Since 2000, research for the fishery has been directed by strategic research plans (Andrew et al. 2000, Michael & Dunn 2005, Michael 2010). In 2010, a strategic research plan (SRP) for OYU 5 was revised for five years from 2010 to 2015 (Michael 2010). This plan was collaboratively developed with the FSOFPMC and the then Ministry of Fisheries. 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). Gaining a better understanding of Bonamia and monitoring its effect in the fishery are rated as the highest priorities in the Foveaux Strait Oyster Fisheries Plan and SRP. The SRP is due to be updated in 2018.

OYU 5 stock assessments, oyster and Bonamia surveys since 1999 are summarised by Michael et al. (2016). These surveys have sampled a consistent survey area, the 1999 survey area (1054 km^2). 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 1999 stratum boundaries have also remained similar, however some of the original strata have been subdivided at various times to better define the areas with commercial densities of oysters. Since 2012, 26 stock assessment survey strata and 15 Bonamia survey strata have been sampled (Michael et al. 2016).

The introduction of five-yearly stock assessments in 2012 has placed greater onus on the annual Bonamia surveys to monitor changes in the oyster population in commercial fishery areas as well as the status of Bonamia. These surveys estimate the densities and population sizes of recruit-sized, pre-recruit, and small live oysters, and recruit-sized and pre-recruit new and old clocks (see below and Michael et al. 2016 for definitions). Oyster density and meat quality in the highest-density patches determine commercial catch rates. These surveys also estimate the prevalence and intensity of Bonamia infection, and short-term (summer) mortality. This information is used by fishers to assess prospects for the following oyster season. The first survey in this new time series was undertaken in February 2014 (Michael et al. 2015a). These surveys incorporate a fully randomised, two-phase sampling design aimed at better estimating oyster densities and population sizes of oysters and new clocks. A standard Bonamia survey area was established to ensure that surveys are comparable from year to year. This area was determined from fishery independent survey data and fishers' logbook data and represents the core commercial fishery that has been consistent through the fluctuations in relative oyster abundance driven by Bonamia mortality. Bonamia survey strata make up 14 of the 26 stock assessment survey strata. The remaining twelve strata are combined into a single background stratum (BK). The Bonamia survey area is 46% of the stock assessment survey area and represented 75% and 69% of the recruit-sized oyster population in 2012 and 2017 respectively. Some limited sampling in the background stratum was also undertaken to allow data from these surveys to be comparable from year to year, and to be incorporated into stock assessments. This survey design and sampling effort predicts a coefficient of variation (CV) for survey estimates of about 11%. The 2014 survey achieved a CV of 11.2% for recruit-sized oysters in the Bonamia survey area, and a CV of 11.7% for the 2007 survey area from an additional 5 stations in the background stratum (Michael et al. 2015a). Surveys since have achieved CVs of 8.0%, 9%, and 11% for recruit-sized oysters in the Bonamia survey area, and CVs of 9.0%, 7%, and 9% for the 2007 survey area (Michael et al. 2017) in 2015, 2016 and 2017 respectively. These surveys achieved low coefficients of variation (CVs) for population estimates, well below the 20% set by MPI for stock assessment surveys.

All three sized groups of oysters declined between 2012 and 2017. Recruit-sized oysters 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) (Michael et al. 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 sized groups of oysters 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 areas and Bonamia survey areas respectively.

Bonamia mortality (summer mortality) declined over the stock area to about 5% of the recruit-sized population in 2016 and 2017. These low levels have not been recorded since 1998. 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 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 infections suggest reduced transmission of disease. In addition to the lower mortality, an increase in settler densities was observed in 2016 and 2017.

This report provides a summary of information from the fourth of the new series of Foveaux Strait oyster surveys in the Bonamia survey area undertaken in February 2018. This survey estimated oyster population size and the status of Bonamia infection and outlines the implications for the future stock status based on the 2017 OYU5 stock assessment. This survey was undertaken as part of the research for MPI programme OYS2017/01 (Objectives 1–4).

2. OBJECTIVES

1. To evaluate the current abundance and biomass of oysters in the OYU 5 fishery and to evaluate current and expected oyster mortality from Bonamia infection for the fishing years 2018, 2019 and 2020.

2. To evaluate the current status of the prevalence and intensity of Bonamia in the OYU 5 fishery for the 2018, 2019 and 2020 years.

Specific Objectives

1. Using a stratified random sampling design estimate the current recruited abundance and biomass in the area of the commercial Foveaux Strait oyster fishery (for the 2018 fishing year), with a target CV of ≤20%.

2. Using a stratified random sampling design estimate the annual mortality from Bonamia in the area of the commercial Foveaux Strait oyster fishery for the 2018 fishing year.

3. Using a stratified random sampling design estimate the prevalence and intensity of Bonamia in the area covering the commercial Foveaux Strait oyster fishery for the 2018 fishing year.

4. Review all qPCR (ddPCR) procedures prior to undertaking any analysis of tissue samples for the 2018 fishing year.

3. METHODS

Detailed methods for annual oyster surveys are given in Michael et al. (2015a). A brief summary and any variation to these standard methods is given below.

3.1 Survey methods

Survey strata for the February 2018 survey were the same as for February Bonamia surveys 2014, 2015, and 2016 (Figure 1). The 2017 stock assessment survey sampled all the 26 strata (Michael et al. 2017). The inclusion of a single large background stratum (Figure 1) for Bonamia surveys ensures that the entire 2007 stock assessment survey area is sampled, and data from these annual surveys can be included in future stock assessments for OYU 5.

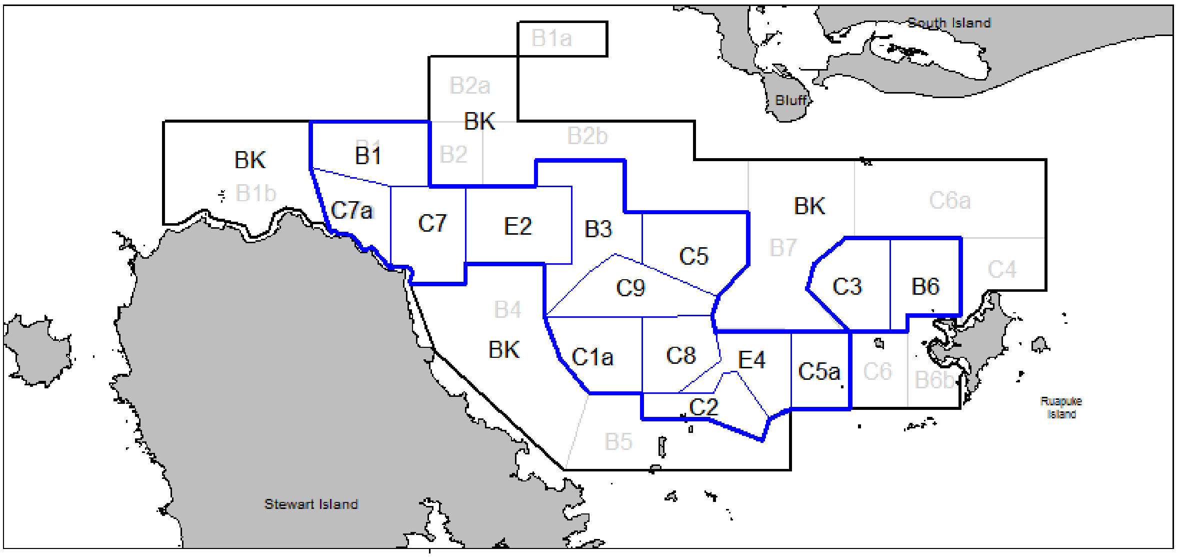

Figure 1: The 2018 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2018 survey strata representing the core commercial fishery area shown as blue lines. Strata are labelled with grey text. The remaining stock assessment survey strata which represent mainly background strata were merged into a single, large background stratum (BK).

Simulations were undertaken in 2014 to determine the optimal stratification and the numbers of stations required to give a survey coefficient of variation (CV) for the recruit-sized population estimate in the range of 8–12% (see Michael et al. 2015a). Simulations predicted that 55 stations in the 14 Bonamia survey strata would produce a CV of about 11%. ALLOCATE (Francis 2006), was used to allocate the numbers of stations to strata in 2018 (see Table 1 below). Rand_Stn (Doonan & Rasmussen 2012) was used to generate the location of 50 random first-phase stations (see Figure 2) and sufficient stations in each stratum to sample 5 second-phase stations in the Bonamia survey strata (hereafter core strata), and 5 stations from the background stratum (see Figure 2). Stations were generated with an exclusion zone of 0.75 nautical miles to spread stations within strata to ensure good spatial coverage and to prevent the overlap of sample tows. The 12 fixed stations were also sampled in February 2018 (see Table 1 and Figure 2) to provide a time series of changes in oyster density and Bonamia status in localised areas. The Ministry for Primary Industries Shellfish Working Group agreed that they add value to the information obtained from these surveys.

Catch sampling

Dredge sampling followed standard procedures for stock assessment and Bonamia surveys between October 2002 and February 2017 (Michael et al. 2017). The commercial oyster vessel *F.V. Golden Quest*) was used for the 2017 and 2018 surveys. The F.V. *Golden Quest* was not available for the 2016 survey and was replaced with a commercial oyster vessel, F.V. *Golden Lea*, used previously for surveys. The same skipper (Stephen Hawke) that has run these surveys since 2011 skippered the survey vessel. Survey stations were sampled with the standard survey dredge (commercial dredge 3.35 m wide and weighing 430 kg) used since 1993 and rebuilt in 2014 to the same specifications. In February 2018, dredges were 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 (station data forms are shown in Appendix 1), and standard methods for sampling oysters to determine the status of Bonamia were used (see Michael et al. 2015a for details).

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

Estimates of oyster densities and population size

Oyster densities and population sizes for the three size groups of live oysters were estimated for the Bonamia survey area (14 core strata), the single background stratum (combining the 12 non-core strata), and all 26 survey strata combined, which comprise the whole stock assessment survey area. Estimates are presented by core strata where three or more randomly selected stations were sampled in February 2018 and these were compared with the estimates from strata sampled in the 2016 and 2017 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, and small oysters, and clocks using the combined population sizes in each stratum as,

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

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

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

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

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

The 95% confidence intervals of the population means for each stratum and the total population are estimated by resampling a normal distribution whose variance is based on a CV and the error of the estimated dredge efficiency. The total error of the estimates of the population mean has two sources: one is the sampling error from the survey, 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 2016 and February 2018 surveys.

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

3.3 Methods 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 Michael et al. (2015b). The numbers of infected recruit-sized oysters were estimated using a droplet digital polymerase chain reaction (ddPCR) assay (Bilewitch et al. 2018). A subsample of heart imprints from oysters tested by ddPCR were also examined to estimate prevalence, and we assumed that oysters that tested negative for Bonamia using ddPCR analysis were also negative for heart imprints not examined. The numbers of non-fatally and fatally infected oysters were estimated from Bonamia intensity of infection scores derived from heart imprints using the categorical scale of Diggles et al (2003) and scaled-up to the size of the recruit-sized oyster population by strata, and for the commercial fishery area (see Michael et al. 2015b).

Station and sample data were recorded on Bonamia sampling forms (Appendix 2), and the total numbers of live and dead oysters in the samples noted. Samples of up to 25 recruit-sized oysters from each station were taken for ddPCR and heart imprints. 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. 2015a for details). In 2018, 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.

Oysters sampled for ddPCR were also sampled for heart imprints. Heart imprints were made using standard methods. Histological samples were taken from the first five oysters processed for heart imprints (see Michael et al. 2015a for details) as in previous surveys. 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 ddPCR methods and then heart imprints. Subsamples of oysters scored for intensity of infection were determined for all ddPCR-positive samples, all inconclusive samples, and a random selection of negative samples from each station (see Michael et al. 2015b).

A detailed account of the ddPCR method and testing is given in Bilewitch et al. (2018). This method adapts a previous qPCR assay for the duplex amplification of the Bonamia target (ITS region of the ribosomal genes) plus the *Ostrea chilensis* β-actin gene (as an internal control) (Maas et al. 2013). The ddPCR method uses a high-throughput format that is capable of Bonamia detection and quantification through a validated modification of the prior qPCR assay.

3.4 Review of ddPCR procedures prior to testing

Before the samples from the 2018 survey were analysed, quality control of reagents and methods was undertaken. A serial dilution of a synthetic standard for Bonamia (dnature LTD), incorporating the primer and probe sequences, was tested with the Bonamia ddPCR assay and five copies per each 20µl reaction could be reliably detected. Aliquots of a 10^2 copies/µl dilution of synthetic standard were included as positive controls for each run of a 96-well plate. 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 did not exceed the detection limit determined by serial dilution.

The ddPCR data from tested survey samples were analysed using QuantaSoft Analysis Pro™ software (Version 1.0.596), and if needed, ddPCR assays were repeated based on similar criteria for qPCR, that is, failed β-actin or positive control reactions; insufficient number of tested droplets (minimum 10^3); insufficient number of negative droplets (reaction saturation) (see Mass et al. 2013 for qPCR repeat criteria and Bilewitch et al. 2018 for testing standards for ddPCR).

In 2018, 25 heart tissues from each station were analysed for infection using ddPCR. All heart imprint slides for those samples that tested positive for Bonamia infection were examined. At least three samples that were ddPCR negative were also randomly selected from the remaining samples from each station. Repeated samples that gave inconclusive results such as instances where no β-actin reaction was detected were also scored from heart imprints.

Heart imprints were examined based on the methods of Michael et al. (2015a), and imprints scored based on a categorical scale (Diggles et al. 2003). In 2018, 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.

The oyster samples were tested for the presence of Bonamia infection using a ddPCR aassay modified from a qPCR protocol established in 2013 (Maas et al. 2013; Bilewitch et al. 2018). Samples were tested on a 96-well plate format. 4µl of 1:20-diluted tissue digests were combined with BioRad ddPCR SuperMix, primers and probes in a total volume of 23µl. A BioRad AutoDG was used to automate droplet generation and ddPCR was conducted on a thermocycler prior to droplet reading on a BioRad QX200. All plates were run with two positive controls: the synthetic Bonamia standard (which lacks oyster DNA) and a pooled oyster diluent that is negative for Bonamia. A single well of deionised distilled water was used as a negative template control. Samples that showed anomalies in the ddPCR data were retested. The repeated ddPCR scorings were used in the analysis for presence/absence and quantification.

Data was analysed using QuantaSoft Pro and positive/negative thresholds for both FAM and HEX channels were set at 2000 relative fluorescence units. Each ddPCR reaction was assessed to ensure that it contained a minimum of 10^3 droplets and that at least one droplet was negative for each target, as required for Poisson-based calculations of sample concentration. Reactions with less than 10^3 total droplets were repeated. In some cases, highly concentrated samples (particularly β-actin) displayed zero negative droplets and were repeated using a further 1:1 dilution of the same 1:20 tissue digest dilution.

Samples displaying a minimum of five positive droplets were classed as positive for either target (Bonamia or oyster β-actin). Any sample with fewer than five positive droplets for the β-actin internal control was repeated by creating a new 1:20 dilution of tissue digest from both heart and gill samples and using both in a repeated ddPCR reaction.

Quantification of Bonamia levels in infected oysters used the concentration of β-actin as a normalisation factor, to account for variations in the amount of starting DNA template added to each ddPCR reaction. A benefit of ddPCR is that it is capable of absolute quantification without an exogenous reference (*e.g.* standard curve), but our final quantification value was relative, since it was calculated as the ratio of the concentration of Bonamia targets to the concentration of β-actin targets in each sample. Thus, for each oyster sample we determined 1) whether Bonamia was present (within the limit of detection for ddPCR) and 2, the relative level of infection– the latter being directly comparable to heart imprint scores determined via histology.

3.5 Method to evaluate the best future stock projection from the 2017 OYU 5 assessment

Under the new management plan for OYU 5, stock assessments will be carried out five-yearly with annual population and Bonamia surveys between assessments. The last assessment was completed in 2017 (Large et al. 2017a) updating the stock assessment models with data on recruitment, harvest, catch rates, population size, and mortality (mostly mortality from Bonamia during epizootics). Three projections of future stock status were based on 0%, 10%, and 20% disease mortality.

Projections from the 2009 stock assessment based on a TACC of 15 million oysters and with no mortality of oysters from Bonamia, predicted an increase in recruit-sized stock abundance of 29% by 2012; however, with a Bonamia mortality of 10%, the population size was expected to increase by only 11% over the same period (Fu & Dunn 2009; Fu 2013). Bonamia mortality was about 10% between 2009 and 2012; and the estimated mortality of recruit-sized oysters between the 2009 survey and the 2012 survey was about 198 million oysters. The population size of recruit-sized oysters increased by 21.1% between the 2009 and 2012 surveys. If the estimated post-survey mortality in 2012 (81 million oysters) is taken into account, the population size of recruit-sized oysters increased by 13.5%, consistent with the 2009 stock assessment.

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

4. RESULTS

Sea conditions and tides were good for sampling during the survey. Observations from the survey suggest little pre-survey mortality (few new clocks), including in the eastern fishery areas where mortality has been historically high. The distribution of oysters was widespread, with 9/72 (12%) of stations recording recruit-sized oyster counts of 500 or more oysters per standard survey tow. There was good growth in some areas, facilitating an increase in recruit-sized oysters. Many of these oysters are legal-sized, but not commercial-sized. Many more spat and wings were observed on oysters in some areas in 2018 than in previous surveys.

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 and very few oysters were caught in stratum E4 (stations 137 and 53), and stratum C5a (station 33). Oyster density was most likely underestimated at these stations.

The efficiency of dredge sampling during the 2018 survey was consistent with previous surveys. Dredge tow lengths were almost all about 0.2 nautical miles (371 m) in length. Wind and resulting sea conditions were less than 10 knots and dredge saturation was similar to previous surveys (Appendix 3, Figures A3.1–A3.3).

4.1 Survey operational detail

NIWA and the Bluff Oyster Management Company Ltd staff began the survey on the 7th of February 2018 and finished on the 11th of February, sampling on all four days during this period. The oyster vessel F.V. *Golden Quest* successfully sampled all 72 stations (Table 1). The locations of survey tows are shown in Figure 2, and the numbers of stations sampled in each stratum are shown in Table 1. A few allocated stations couldn't be sampled because of rough ground, first-phase stations 21, 22, and 54, and second-phase station 127 were replaced by stations 77, 89, 137, and 128 respectively.

Thirty recruit-sized oysters were randomly sampled from each station, to provide tissue samples for ddPCR and heart imprints (n=25), and histology (n=5). Target sample size was achieved from 66 of the 72 stations. At sites where fewer than 30 recruit-sized oysters were caught, samples included pre-recruit and small oysters. At stations 32 (n=15), 53 (n=13), 33 (n=10), 15 (n=7), 137 (n=6), and 16 (n=5) fewer than 30 oysters were caught. Samples of 30 pre-recruit oysters were also sampled at stations 5, 6, 7, 50, 126, and 128. Oyster samples were couriered to NIWA, Greta Point (Wellington) where they were processed for heart imprints and ddPCR. Oyster tissues were also taken for histology and these were archived for future research.

Table 1: The numbers of first-phase, second-phase, and fixed stations sampled in each stratum during the February 2018 Bonamia survey, and the area of each stratum. A single, large background stratum (BK) represents the merged stock assessment survey strata outside the Bonamia survey area (see Figure 1).

Stratum	First-phase	Second-phase	Fixed	Area (km^2)
B1	4		1	78.2
B3	6			44.7
B6	3			30.1
B6a			2	*
BK	5			578.3
C1a	3			31.3
C2	3		1	21.9
C3	3		1	32.7
C5	3		1	37.7
C5a	4		1	23.5
C7	5			36.1
C7a	3			23.6
C8	3	2	1	26.8
C9	4	2		34.5
E2	3		2	42.8
E4	3	1	2	28.0
Totals	**55**	**5**	**12**	**1070.2**

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

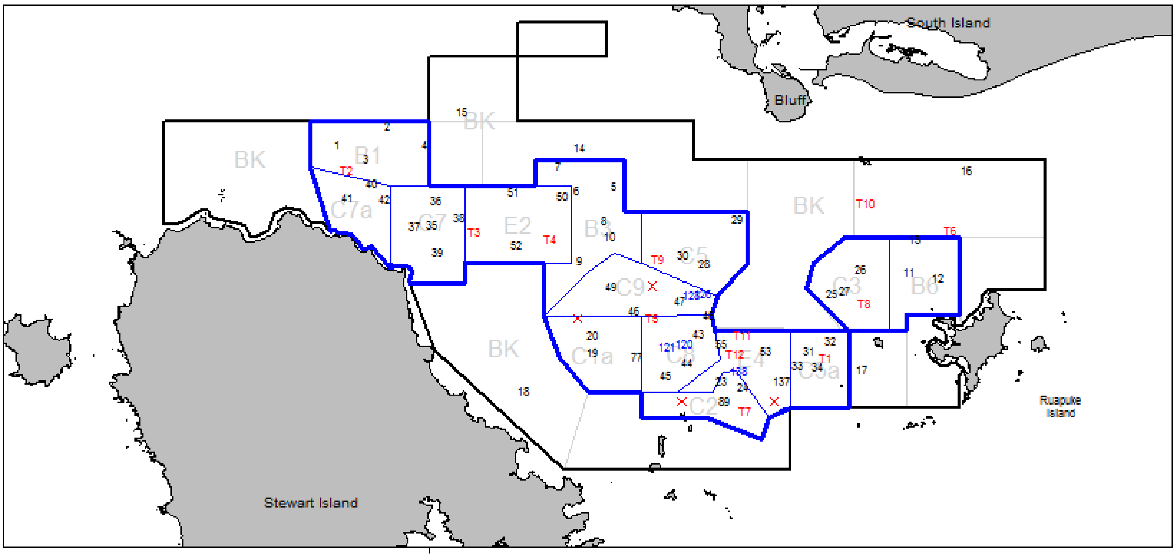

Figure 2: The 2018 survey area with the 2007 survey boundary shown as a heavy, black outer line, the Bonamia survey area as a heavy blue line, and the 2018 Bonamia survey strata shown as blue lines. The remaining stock assessment survey strata (light grey lines) in the large background stratum were merged into a single stratum (BK). First-phase station numbers shown in black text, second-phase in blue, and fixed stations in blue text. Red crosses denote stations that couldn't be towed because of foul ground.

4.2 Oyster abundance

Changes in oyster densities between 2016 and 2018

Plots of catches adjusted to the standard tow length (0.2 nautical miles) from the 2016, 2017 and 2018 surveys for recruit-sized, pre-recruit and small oysters, their means and 95% confidence intervals by stratum are shown in Figures 3–5 respectively. Strata are arranged west to east with northern strata at similar longitudes shown before those to the south. Catches of all size classes have been spatially patchy.

At stratum level, catches of recruit-sized oysters were generally similar or higher in 2018 than in 2016 and 2017 across the entire fishery area (Figure 3). The highest catches were in central areas (strata B3, C9, C5, and C8), and many of these were localised individual tows. There were no large declines recorded in 2018, consistent with the low summer mortality recorded in 2016 and 2017 (Figure 3). Low catches in strata C5a and E4 may be due to reduced dredge efficiency from kaeos.

Numbers of pre-recruit-sized oysters in 2018 were generally similar to or have increased since 2016 and 2017 (Figure 4), especially in western and central strata (B1, E2, B3, C5, and C9). The numbers of pre-recruits in strata C1a, E4, C5a, B6, and BK have remained similarly low between 2016 and 2018 (Figure 4).

Relatively high catches of small oysters were patchy in 2018. At the stratum level, catches were generally higher across the fishery in 2018 compared to 2016 and 2017, except in strata C5a and BK (Figure 5). The number of small oysters reflects the trend of increasing recruitment to the population from spat monitoring.

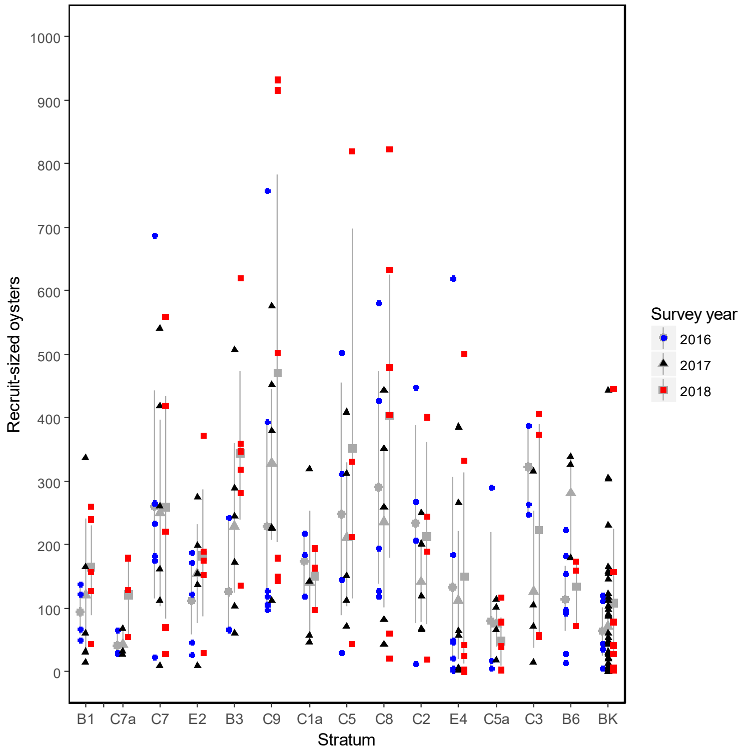

Figure 3: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized oysters, their means (grey filled circles) and 95% confidence intervals (grey lines) by Bonamia survey stratum and background strata combined (BK) sampled during the 2016 (blue filled circles), 2017 (black filled circles), and 2018 (red filled circles) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

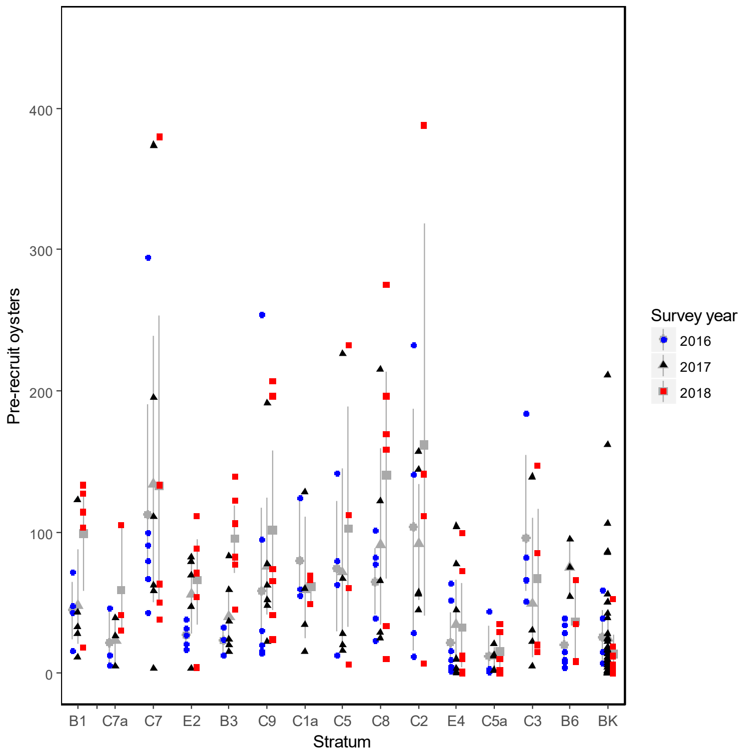

Figure 4: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for pre-recruit oysters, their means (grey filled circles) and 95% confidence intervals (grey lines) by Bonamia survey stratum and background strata combined (BK) sampled during the 2016 (blue filled circles), 2017 (black filled circles), and 2018 (red filled circles) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

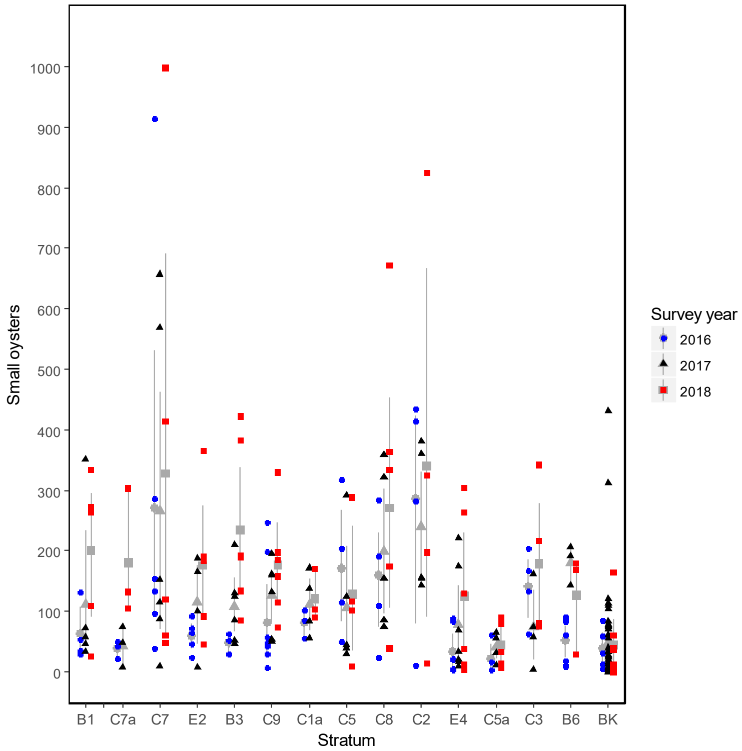

Figure 5: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for small oysters, their means (grey filled circles) and 95% confidence intervals (grey lines) by Bonamia survey stratum and background strata combined (BK) sampled during the 2016 (blue filled circles), 2017 (black filled circles), and 2018 (red filled circles) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

Survey estimates of population size

Estimates of absolute population size for recruit-sized, pre-recruit, and small oysters from the February 2018 survey are shown by stratum in Tables 2–4. 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 (BK), *n*= 12: B1a, B1b, B2, B2a, B2b, B4, B5, B6b, B7, C4, C6, and C6a), and the whole 2007 stock assessment survey area (Survey total). Comparisons between the population estimates for the background stratum should be made with caution as there were only 5 stations sampled in total. Bootstrapped estimates of 95% confidence intervals (B.lower and B.upper) were made by 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 estimates from this patchily distributed population. The population estimates for recruit-sized, pre-recruit, and small oysters from the 2012, 2016, 2017, and 2018 surveys are shown in Table 5 and in Figure 6.

The density and population size of recruit-sized oysters in core strata (commercial fishery areas) increased 35.9% between 2017 and 2018 (Table 2). The mean density in core strata declined from 1.40 oysters m^{-2} in 2012 to 1.09 oysters m^{-2} in 2014, further declined to 0.71 oysters m^{-2} in 2015 and remained at a similar level until 2017 (0.78 oysters m^{-2} in 2016 and 0.74 oysters m^{-2} in 2017) before increasing in 2018 to 1.00 oysters m^{-2}. Usually an increase in recruit-sized oyster density results in an increased catch rate, however, the good growth observed in some areas in 2018 resulted in large numbers of fast growing and thin legal-sized oysters that are not of commercial size.

The density of recruit-sized oysters in the background stratum is not likely to be well estimated by recent surveys (since 2012) due to the low numbers of stations sampled (n=5), over a large area (578.4

km^2). Mean recruit-sized oyster density in the background stratum was 67% (0.67 oysters m^{-2}) of that in the Bonamia survey area in 2018 (Table 2), and higher than in 2017, 0.28 oysters m^{-2} in 2017.

The population size of recruit-sized oysters in core strata declined from 688.1 million oysters in 2012 to 363.6 million oysters in 2017, and increased to 494.1 million oysters in 2018. Between 2012 and 2018, the coefficients of variation (CV) for all core strata combined ranged from 8.0% CV to 11.2% sampling 55 stations. The population size in BK increased from 230.3 million oysters in 2012 to 482.9 million oysters in 2014, declined with a varying trend to 163.9 million oysters in 2017, and increased to 389.2 million oysters in 2018. The CVs have increased from 19.7% in 2012 (stations sampled, n=62), to 59.0% in 2018 (n=5). It is not likely that the five stations are a good representation of the size of the oyster population in the background stratum given the size of the CV, and we advise caution when population estimates from the whole 2007 survey area are compared. Recruit-sized oyster population sizes (and mean densities) increased in all strata except B6 and C5a (Table 2) where these estimates may have been affected by the abundance of kaeos (which reduced the efficiency of sampling). Increases in population size ranged from about 3% to 182%.

Pre-recruit mean oyster densities in all core strata combined declined from 0.60 m^{-2} in 2012, to 0.25 oysters m^{-2} in 2017 in a fluctuating trend, and increased to 0.36 oysters m^{-2} in 2018 (Table 3). The population size declined from 297.4 million oysters in 2012 to 89.2 million oysters in 2015, and increased to 178.4 million oysters in 2018. Pre-recruit population size increased 44.9 % between 2017 and 2018 (Table 3). The population size in the background stratum was similar between 2017 and 2018, 45.0 million oysters and 47.4 million oysters respectively (Table 3). The population size in the 2007 stock assessment survey area increased 34.3% from 168.2 million oysters in 2017 to 225.8 million oysters in 2018. Pre-recruit oyster population sizes (and mean densities) increased in all strata except B6 and C5a (Table 3), the same as for recruits. Increases in population size ranged from about 5% to 250%.

The mean densities and population sizes of small oysters for all the core strata combined declined markedly (65%) from 451.3 million oysters in 2012 to 156.3 million oysters in 2014, and increased to 261.9 million oysters in 2017, and 53.4 % to 401.8 million oysters in 2018 (Table 4). The population in the background stratum remained similar between 2012 (160.9 million oysters) and 2014 (156.3 million oysters), but declined to 99.7 million oysters in 2017, and increased 51.2% in 2018 to 150.8 million oysters (Table 4). Overall, the population size of small oysters in the 2007 survey area declined from 612.2 million oysters in 2012 to 249.0 million oysters in 2015, but increased to 552.5 million oysters in 2018 (Table 4). Small oyster population sizes (and mean densities) increased in all strata except B6, C5a, and C8 (Table 4), similar to recruits and pre-recruits. Increases in population size ranged from about 9% to 303%.

In 1995 and 1997, the commercial population used to estimate yield was estimated as the percentage of the entire population above a density of 400 oysters per tow (equivalent to about 6–8 sacks per hour during commercial dredging). This threshold was based on an historical, economic catch rate, and when the catch rate dropped below 6 sacks per hour, fishers would move to new fishery areas. Although this method is no longer used for stock assessments, estimates of commercial population size allow some comparison with previous years so the Shellfish Working Group requested that these estimates be included in this report. Table 6 shows estimates of commercial population size, using the catch of recruit-sized oysters at each station minus 400 oysters, for the 2018 core strata (n= 14), all core strata combined, all background strata combined (n= 12), and for the whole 2007 stock assessment survey area sampled. Ten core strata supported commercial densities in 2012, six in 2014, only two in 2015, six in 2016, three in 2017 and five in 2018 (B3, C7, C8, C9 and E4). The mean commercial density in the core strata was 0.17 oysters.m^{-2} in 2017, increasing to 0.37 oysters.m^{-2} in 2018. In the background stratum, oyster density was 0.02 oysters.m^{-2} in 2017, increasing to 0.43 oysters.m^{-2} in 2018.

Table 2: **Absolute population estimates for recruit-sized oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2018 by stratum. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded light blue), upper and lower 95% confidence intervals (95% CI) in millions of oysters where a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres. Also given are the 2017 mean population estimates and percentage of the 2017 population size by stratum. The percentage of the 2017 estimate (% of 2017) is shaded green for increases in population size and tan for decreases.**

Stratum	No. stns	Mean density	Density s.d.	CV	2018 Pop.n	B.lower 95%CI	B.upper 95%CI	Area km^2	2017 Pop.n	% of 2017
B1	4	0.72	0.22	0.30	56.1	21.0	102.4	78.2	23.7	236.8
B3	6	1.66	0.31	0.19	74.3	41.6	120.0	44.7	49.9	148.9
B6	3	0.66	0.15	0.23	19.7	9.6	33.4	30.0	41.0	47.9
C1a	3	0.73	0.14	0.19	22.9	12.6	37.5	31.3	21.3	107.7
C2	3	0.73	0.33	0.45	16.1	2.0	33.8	21.9	12.1	133.0
C3	3	0.79	0.52	0.66	25.8	0.0	64.4	32.7	20.8	124.2
C5	3	0.90	0.37	0.41	33.9	6.1	69.4	37.7	29.5	115.0
C5a	4	0.19	0.13	0.68	4.5	0.0	11.5	23.5	7.1	63.8
C7	5	1.25	0.49	0.39	45.2	10.5	90.2	36.1	44.0	102.8
C7a	3	0.58	0.18	0.30	13.8	5.2	25.1	23.6	4.9	281.7
C8	5	1.74	0.72	0.41	46.7	8.4	95.2	26.8	23.9	195.3
C9	6	2.26	0.73	0.32	78.0	26.9	146.2	34.5	54.8	142.2
E2	3	0.90	0.49	0.54	38.6	0.0	89.4	42.8	20.8	185.7
E4	4	0.66	0.59	0.89	18.4	0.0	55.4	28.0	9.6	191.8
Core total	**55**	**1.00**	**0.11**	**0.11**	**494.1**	**315.0**	**764.9**	**491.8**	**363.6**	**135.9**
BK	5	0.67	0.40	0.59	389.2	0	935.1	578.4	163.9	237.5
Survey total	**60**	**0.83**	**0.22**	**0.27**	**883.3**	**380.4**	**1566.5**	**1070.2**	**527.4**	**167.5**

Table 3: Absolute population estimates for pre-recruit oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2018 by stratum. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded light blue), upper and lower 95% confidence intervals (95% CI) in millions of oysters where a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres. Also given are the 2017 mean population estimates and percentage of the 2017 population size by stratum. The percentage of the 2017 estimate (% of 2017) is shaded green for increases in population size and tan for decreases.

Stratum	No. stns	Mean density	Density s.d.	CV	2018 Pop.n	B.lower 95%CI	B.upper 95%CI	Area km^2	2017 Pop.n	% of 2017
B1	4	0.47	0.13	0.28	36.5	15.1	65.1	78.2	10.4	350.5
B3	6	0.46	0.07	0.15	20.6	12.5	32.2	44.7	9.9	208.4
B6	3	0.18	0.08	0.46	5.3	0.5	11.2	30.0	10.9	48.8
C1a	3	0.30	0.03	0.11	9.3	6.0	14.2	31.3	9.0	103.8
C2	3	0.42	0.20	0.47	9.3	0.8	19.9	21.9	8.1	114.5
C3	3	0.20	0.11	0.57	6.4	0.0	14.8	32.7	3.6	177.8
C5	3	0.27	0.14	0.50	10.3	0.1	22.7	37.7	6.0	170.9
C5a	4	0.06	0.04	0.68	1.3	0.0	3.4	23.5	1.0	133.8
C7	5	0.64	0.31	0.48	23.1	1.5	49.8	36.1	23.6	98.1
C7a	3	0.28	0.11	0.40	6.7	1.3	13.4	23.6	2.7	248.5
C8	5	0.55	0.19	0.34	14.8	4.7	28.0	26.8	12.8	115.6
C9	6	0.49	0.15	0.32	16.8	6.0	31.2	34.5	12.6	133.0
E2	3	0.33	0.16	0.48	14.2	0.7	31.1	42.8	9.2	154.4
E4	4	0.14	0.12	0.85	3.8	0.0	11.1	28.0	3.4	111.5
Core total	**55**	**0.36**	**0.04**	**0.11**	**178.4**	**113.5**	**276.5**	**491.8**	**123.1**	**144.9**
BK	5	0.08	0.05	0.56	47.4	0	111.1	578.4	45.0	105.3
Survey total	**60**	**0.21**	**0.03**	**0.15**	**225.8**	**134.0**	**353.3**	**1070.2**	**168.2**	**134.3**

Table 4: **Absolute population estimates for small oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2018 by stratum. The number of stations sampled (No. stns), the mean oyster density per m² (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded light blue), upper and lower 95% confidence intervals (95% CI) in millions of oysters where a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km²) in square kilometres. Also given are the 2017 mean population estimates and percentage of the 2017 population size by stratum. The percentage of the 2017 estimate (% of 2017) is shaded green for increases in population size and tan for decreases.**

Stratum	No. stns	Mean density	Density s.d.	CV	2018 Pop.n	B.lower 95%CI	B.upper 95%CI	Area km²	2017 Pop.n	% of 2017
B1	4	0.90	0.35	0.39	70.1	15.9	138.3	78.2	19.3	363.0
B3	6	1.14	0.27	0.24	50.7	24.8	86.3	44.7	26.0	195.1
B6	3	0.62	0.24	0.38	18.5	4.2	36.2	30.0	26.4	69.9
C1a	3	0.59	0.12	0.21	18.5	9.6	30.8	31.3	16.9	109.2
C2	3	0.88	0.45	0.51	19.2	0.4	42.6	21.9	21.8	88.1
C3	3	0.61	0.23	0.38	19.9	4.9	38.8	32.7	9.8	202.7
C5	3	0.35	0.16	0.45	13.2	1.5	27.8	37.7	10.9	120.7
C5a	4	0.16	0.08	0.51	3.7	0.1	8.3	23.5	5.1	73.2
C7	5	1.58	0.87	0.55	57.1	0.0	130.9	36.1	47.0	121.6
C7a	3	0.87	0.30	0.35	20.6	6.2	39.0	23.6	5.1	403.6
C8	5	0.93	0.34	0.37	24.9	6.6	48.5	26.8	27.4	90.7
C9	6	0.85	0.18	0.21	29.5	15.4	49.3	34.5	21.0	140.4
E2	3	0.99	0.45	0.46	42.2	3.8	90.8	42.8	16.1	262.0
E4	4	0.50	0.29	0.59	13.9	0.0	33.4	28.0	9.2	150.8
Core total	**55**	**0.82**	**0.11**	**0.13**	**401.8**	**249.2**	**631.2**	**491.8**	**261.9**	**153.4**
BK	5	0.26	0.15	0.56	150.8	0.0	353.2	578.4	99.7	151.2
Survey total	**60**	**0.52**	**0.09**	**0.18**	**552.5**	**306.6**	**893.8**	**1070.2**	**361.6**	**152.8**

Table 5: Percentage changes in the population size of recruit-sized, pre-recruit, and small oysters in the Bonamia survey area in 2012, 2016, 2017 and 2018. The mean oyster density per m^2 (Mean density) that determines catch rate (sacks per hour), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n), bootstrapped upper and lower 95% confidence intervals (95%CI) in millions of oysters that reflect the variability in the catches – the closer confidence intervals are to the mean size the better, and the percentage change in population size. Increases in population size are shaded green and decreases tan.

Bonamia survey area

2012	**Mean density**	**CV**	**Pop.n**	**B.lower 95%CI**	**B.upper 95%CI**	
Recruit	1.40	0.09	688.1	449.2	1046.7	
Pre-recruit	0.60	0.10	297.4	192.6	454.4	
Small	0.92	0.16	451.3	261.5	731.7	
2016	**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	155.0	407.3	-43.3
2017	**Mean density**	**CV**	**Pop.n**	**B.lower 95%CI**	**B.upper 95%CI**	**% change 2016-2017**
Recruit	0.74	0.11	363.6	233.9	559.1	-5.6
Pre-recruit	0.25	0.12	123.1	77.5	191.7	2.2
Small	0.53	0.10	261.9	168.8	401.6	2.3
2018	**Mean density**	**CV**	**Pop.n**	**B.lower 95%CI**	**B.upper 95%CI**	**% change 2017-2018**
Recruit	1.00	0.11	494.1	315.0	764.9	35.9
Pre-recruit	0.36	0.11	178.4	113.5	276.5	44.9
Small	0.82	0.13	401.8	249.2	631.2	53.4

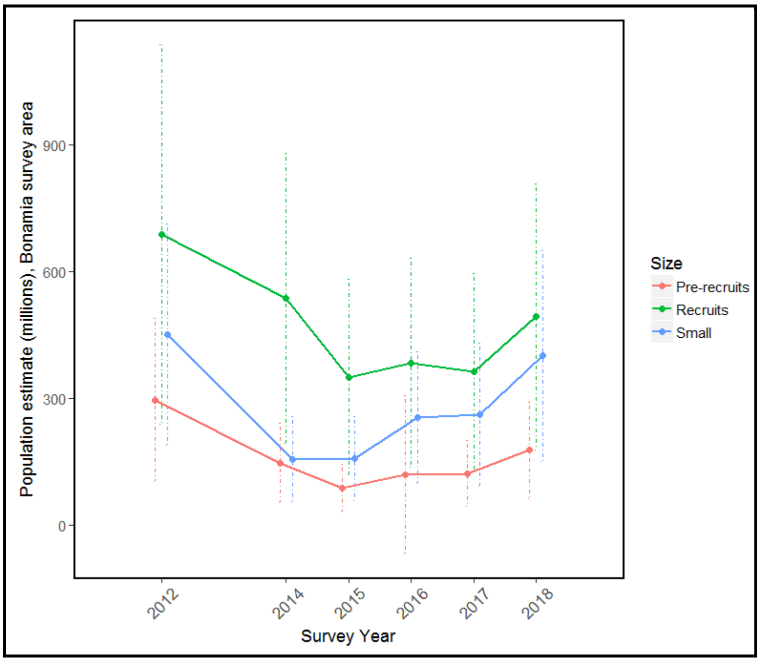

Figure 6: Mean population sizes and 95% confidence intervals for recruit-sized, pre-recruit, and small oysters in the Bonamia survey area between 2012 and 2018.

Table 6: Absolute population estimates for the size of the recruit-sized oyster population above a density of 400 oysters per survey tow (equivalent to about 6–8 sacks per hour in commercial dredging) in core strata, the background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2018 by stratum. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded light blue), upper and lower 95% confidence intervals (95% CI) in millions of oysters where a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres. Also given are the 2017 mean population estimates and percentage of the 2017 population size by stratum. The percentage of the 2017 estimate (% of 2017) is shaded green for increases in population size and tan for decreases, and – for no change.

Stratum	No. stns	Mean density	Density s.d.	CV	2018 Pop.n	B.lower 95%CI	B.upper 95%CI	Area km2	2017 Pop.n	% of 2017
B1	4	0.00	0.00	0.00	0.0	0.0	0.0	78.2	0.0	-
B3	6	0.50	0.50	1.00	22.5	0.0	72.1	44.7	27.8	80.8
B6	3	0.00	0.00	0.00	0.0	0.0	0.0	30.0	0.0	-
C1a	3	0.00	0.00	0.00	0.0	0.0	0.0	31.3	0.0	-
C2	3	0.00	0.00	0.00	0.0	0.0	0.0	21.9	0.0	-
C3	3	0.00	0.00	0.00	0.0	0.0	0.0	32.7	0.0	-
C5	3	0.00	0.00	0.00	0.0	0.0	0.0	37.7	0.0	-
C5a	4	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	5	0.94	0.59	0.62	34.0	0.0	82.3	36.1	28.2	120.7
C7a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.6	0.0	-
C8	5	1.67	0.76	0.46	44.6	4.3	93.8	26.8	0.0	-
C9	6	1.88	0.89	0.47	64.8	4.0	140.0	34.5	28.7	225.8
E2	3	0.00	0.00	0.00	0.0	0.0	0.0	42.8	0.0	-
E4	4	0.60	0.60	1.00	16.9	0.0	53.6	28.0	0.0	-
Core total	**55**	**0.37**	**0.10**	**0.28**	**182.9**	**77.1**	**326.3**	**491.8**	**85.6**	**213.6**
BK	5	0.43	0.43	1.00	250.6	0.0	789.9	578.4	8.9	2815.3
Survey total	**60**	**0.40**	**0.24**	**0.59**	**433.4**	**0.0**	**1022.5**	**1070.2**	**93.5**	**463.5**

Changes in the distribution of live oysters

The February 2018 survey sampled 60 first and second-phase random stations generated with a 0.75 nautical mile exclusion zone that spread sampling effort, and 12 fixed stations. All 72 stations were used to describe oyster distribution. Sampling effort was focused in core strata with background strata receiving only 5 stations for 51.4% of the survey area. The sampling was therefore insufficient to provide a consistent or complete coverage of the fishery area in 2018, and hence the survey is not likely to have estimated the distributions of oyster density well for live recruit, pre-recruit, and small oysters outside of core strata (delimited by a blue line in Figures 7–9). These distributions of oysters are compared with the last stock assessment survey in 2017 which sampled 114 stations in total and provided more complete coverage of the fishery.

Comparisons of the distributions of recruit-sized, pre-recruit, and small oyster density for 2017 and 2018 are shown in Figures 7–9. The distributions of oyster densities of all sizes are widespread, covering most of the fishery area with the highest densities in core fishery strata (Figures 7–9). The numbers and extent of localised areas of relatively high density of recruit-sized oysters increased between 2017 and 2018 (Figure 7), probably the result of relatively low (about 5%) Bonamia mortality.

The densities of pre-recruit oysters are relatively low; however, densities have generally increased throughout the fishery area between 2017 and 2018, but densities are still low in some central fishery areas. The highest pre-recruit oyster densities were in strata C7, C8, and C2. Several factors may be responsible for the relatively low densities of pre-recruit-sized oysters since 2012: low settlement of oyster spat and low survival of juveniles, Bonamia mortality (pre-recruit-sized oysters are as vulnerable to Bonamia mortality as recruit-sized oysters), and small oysters growing to recruit-size in a single summer.

The distribution of small oyster densities (Figure 9) shows similar patterns to recruit and pre-recruit sized oyster densities. Densities increased markedly across the entire fishery between 2017 and 2018 and small oysters are widespread throughout the fishery, but their distribution is patchy as indicated by the slightly higher CV for the estimate of small oysters (13%). The highest densities of small oysters are in the western (C7) and southern strata (C2). Small oysters are not vulnerable to Bonamia mortality. The increasing densities reflect increased recruitment to the oyster population, consistent with increased spat settlement since 2015.

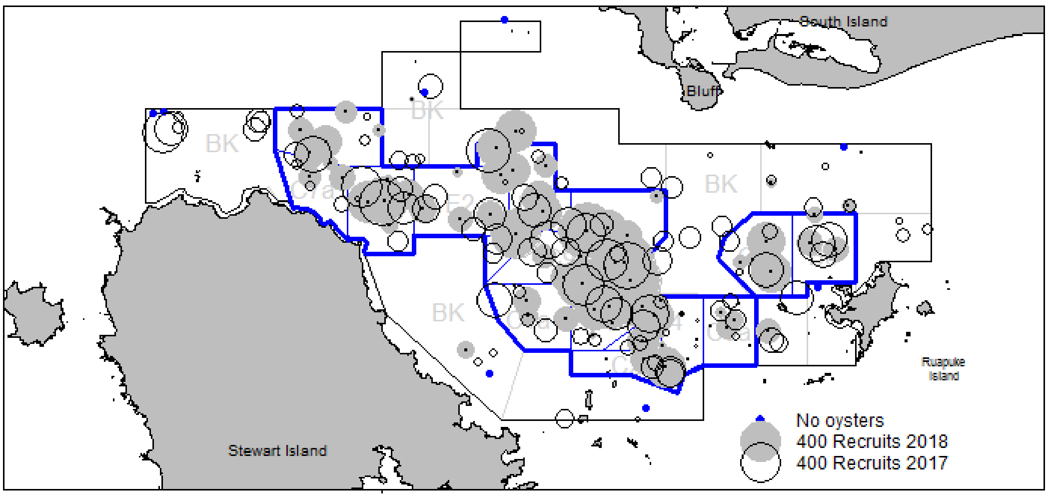

Figure 7: Density (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled during the February surveys in 2018 (filled grey circles) and in 2017 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

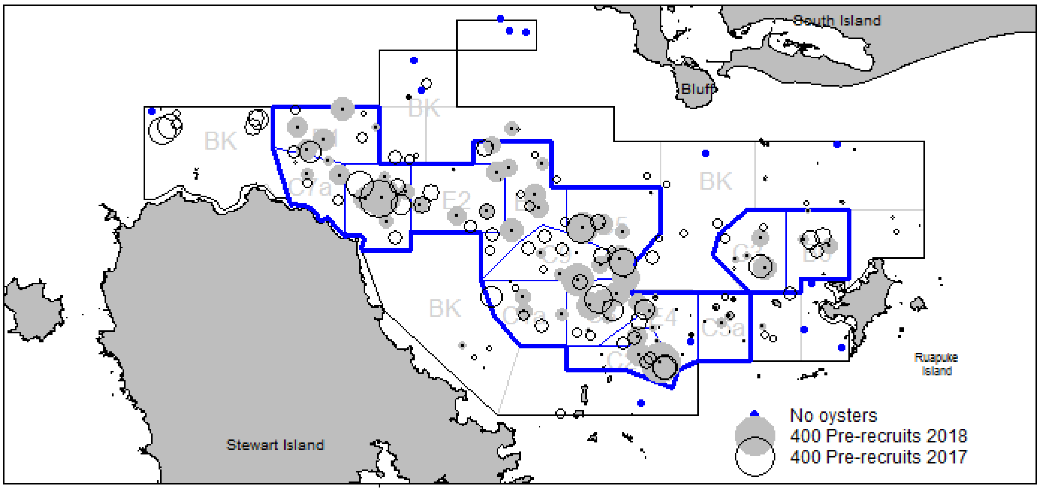

Figure 8: Density (numbers of oysters per standard tow, 1221 m²) of pre-recruit sized oysters sampled during the February surveys in 2018 (filled grey circles) and in 2017 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

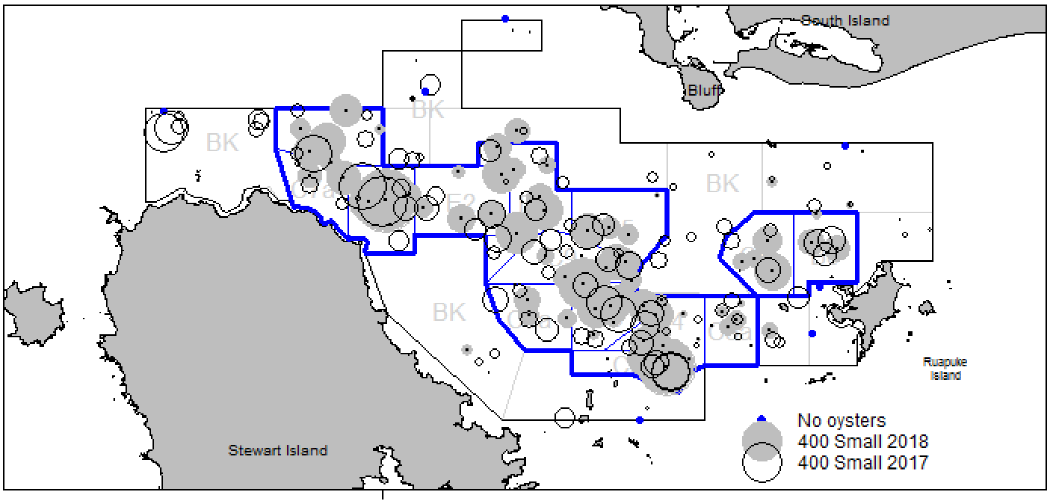

Figure 9: Density (numbers of oysters per standard tow, 1221 m²) of small oysters sampled during the February surveys in 2018 (filled grey circles) and in 2017 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

Recruitment

Small oysters (spat) 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 the 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 an absolute estimate of recruitment.

The number of small oysters per recruit shows large fluctuations in a broadly cyclic trend between 1993 and 2018 (Figure 10). Small oysters per recruit were generally low between 1995 and 2001 (Figure 10), suggesting reduced recruitment to the population at a time when the numbers of recruit-sized oysters were increasing and relatively high compared to 1993 (and earlier) (Figure 10). The number of small oysters per recruit was relatively high between 2002 and 2006 when the recruit-sized oyster population was declining rapidly from Bonamia mortality. From 2009, the number of small oysters per recruit declined to low levels and has remained low (Figure 10) while the recruit-size oyster population was increasing. The trend in spat-per-recruit is consistent with the trends in the numbers of small oysters sampled from the commercial catch between 2009 and 2016 (Large et al. 2017b), and the numbers of settlers recorded on spat collectors (Figure 11). Spat monitoring data and the numbers of 0+ oysters landed in the catch of commercial sized oysters provide indices of early recruitment. These two indices are highly correlated over time, with a Pearson's correlation coefficient of 0.96 ($p < 0.001$) (Keith Michael, NIWA, unpublished data).

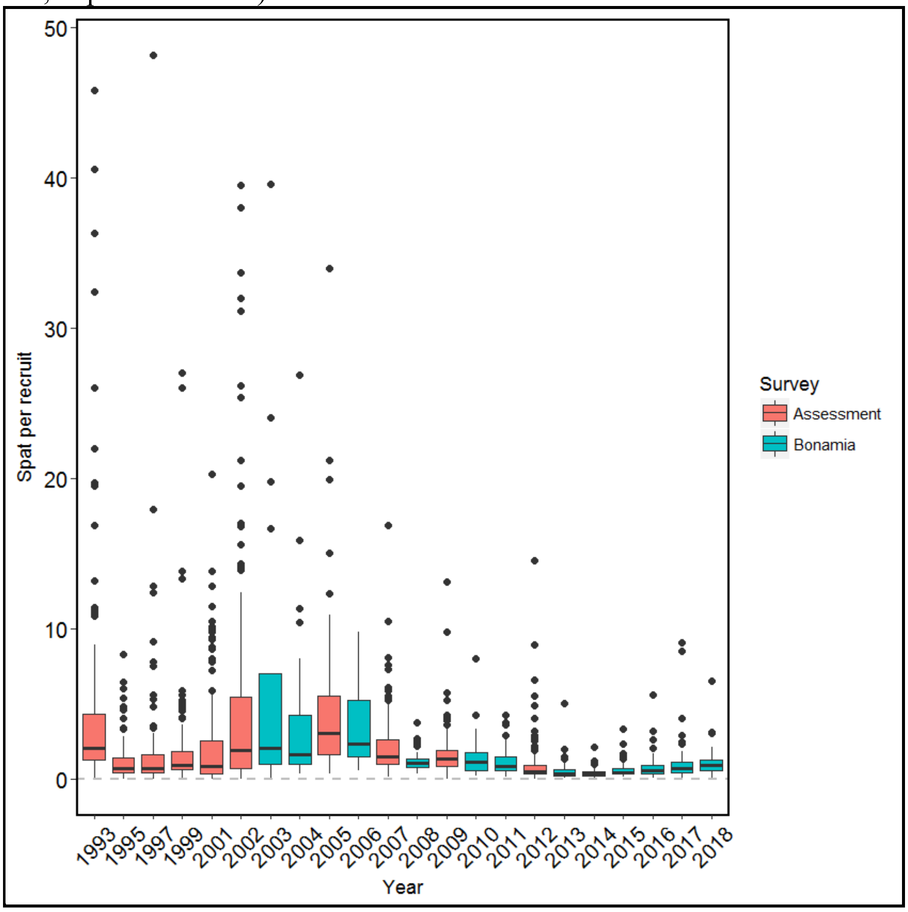

Figure 10: The numbers of small oysters per recruited oyster sampled between 1993 and 2018. Stock assessment surveys (Assessment) shown in salmon, and Bonamia surveys (Bonamia) shown in blue. The numbers of stations sampled each year varies. Medians shown as solid lines, boxes represent 50 percentiles (25–75%) and whiskers 90 percentiles (5–95%), and outliers smaller than 5% and greater than 95% as filled circles.

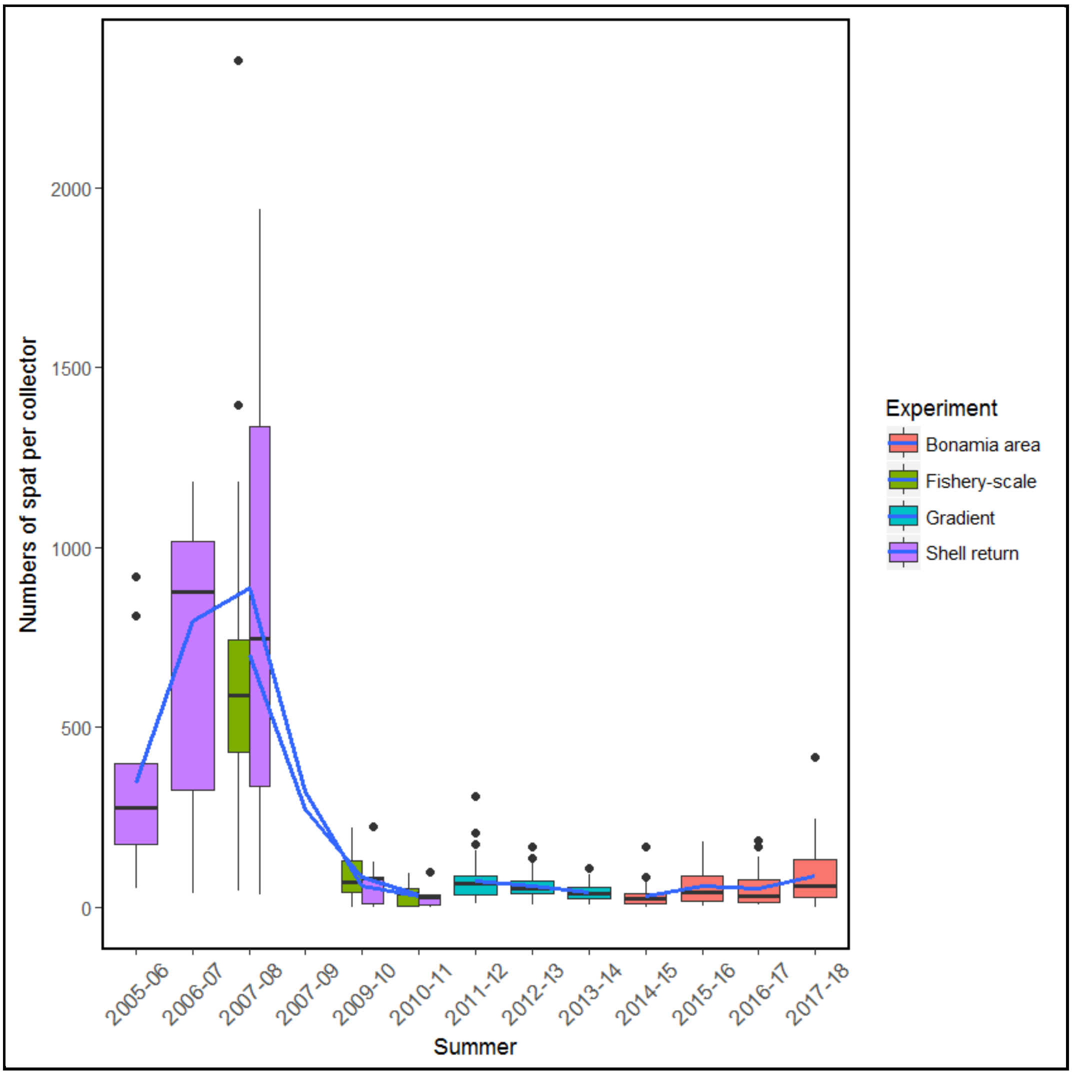

Figure 11: The total numbers of spat per collector sampled over the summers of 2005–06 to 2017–18. Spat settlement shows the success of spawning, and indicates the levels of replenishment to the oyster population. Data represent four different experiments and different areas: the shell return site and fishery scale experiments, the gradient experiment in the central fishery area and fishery scale monitoring that began over the summer of 2014–15.

4.3 Estimates of oyster mortality before and during the February 2018 survey

Descriptive statistics for the percentages of recruit-sized and pre-recruit new clocks and gapers sampled between 2012 and 2016–2018 are given in Table 7. Low percentages of recruit sized new clocks and gapers between 2016 and 2018 suggest that pre-survey mortality was markedly lower than in 2012 and see Michael et al. (2016) for 2014 and 2015. Pre-survey mortality for pre-recruits showed a similar trend, but the percentages are in part influenced by the low population sizes compared to that in 2012.

There were very few gapers observed during the February 2018 survey, six stations (10.9%) had one or two recruit-sized gapers. Only one station (2.5%) had a single pre-recruit-sized gaper in 2018. Fewer stations recorded gapers than in previous surveys; 26% in 2012, 14% in 2014, and 6.9% in 2015.

Table 7: Descriptive statistics for the percentages of new clocks and gapers for two size groups, recruit and pre-recruit. Percentages are new clocks and gapers to new clocks, gapers and oysters combined, sampled from survey tows with more than 50 live recruit-sized or pre-recruit oysters in 2012, 2014, 2015, and 2016.

	Percentage new clocks and gapers							
	Recruit sized				Pre-recruits			
Year	**2012**	**2016**	**2017**	**2018**	**2012**	**2016**	**2017**	**2018**
No. stations	112	52	74	55	78	26	40	40
Median	3.3	0.4	1.2	0.3	2.6	0	0	0
Minimum	0	0	0	0	0	0	0	0
Maximum	28.9	3	12.7	9.1	12.5	1.2	5.8	3.0
5th percentile	0.3	0.2	0	0	0	0	0	0
95th percentile	7.2	0.6	4.97	2.8	10.1	0.1	3.27	2.2
No. zero stations	5	22	13	23	11	22	21	33
Zero stations	4.5	42.3	17.6	41.8	14.1	84.6	52.5	82.5

Comparison of the distributions of recruit-sized new clocks between 2016, 2017, and 2018 (Figure 12) shows that pre-survey mortality was widespread and locally variable in 2018, and new clock densities were higher at stations where recruit-sized oyster densities were high, mostly in strata designated as commercial (B1, C3, C5, C8, C9, and E2). Recruit-sized new clock densities were generally low in 2016, except for some eastern strata. By 2017, recruit-sized new clock densities were generally higher than 2016 and spread across the fishery area (Figure 12). In 2018 recruit-sized new clock densities were lower and generally confined to west of a line between Bluff Hill and Saddle Point (Figure 13) with many sites showing no pre-survey mortality.

The population size of recruit-sized new clocks in core strata was lower in 2018 (2.9 million) than in 2017 (5.3 million) (Table 8). No stratum had recruit-sized new clock densities above 0.01 m^{-2} in 2018 (Table 8). There was a decrease in the numbers of recruit-sized new clocks by strata ranging from 6.6% less to 83.1% less, except in stratum B3 where recruit-sized new clocks increased by 72.3%.

The population size of pre-recruit new clocks in core strata was lower in 2018 (0.4 million) than in 2017 (0.9 million) (Table 9). Only five of the fifteen strata had pre-recruit new clocks in 2018 (Table 9), and the densities in these five strata (B3, B6, C1a, C3 and C8) were very low (Table 9). Between 2017 and 2018 there was a decrease in the numbers of pre-recruit-sized new clocks by strata ranging from 2.6% less to 49.2% less.

The proportion of the total summer mortality occurring before and during the survey is likely to change from year to year, so the levels of pre-survey mortality may, in part, reflect the timing of mortality events and not increases or decreases in total mortality.

Pre-survey mortality of recruit-sized oysters in core strata was low between 2016 and 2018, ranging between 0.4% and 1.4% (Table 10). It was higher in 2012, 2014 and 2014, ranging between 3.2% and 6.8%. Pre-survey mortality of recruit-sized oysters in the 2007 stock assessment survey area was similar to that in core strata, ranging between 0.4% and 1.5% between 2016 and 2018, and between 3.2% and 7.6%. in 2012, 2014 and 2015 (Table 10).

Pre-survey mortality of pre-recruit oysters in core strata was also low between 2016 and 2018, ranging between 0.2% and 0.7% (Table 10). It was higher in 2012, 2014 and 2015 ranging between 2.4% and 2.9%. Pre-survey mortality of pre-recruit oysters in the 2007 stock assessment survey area was similar to core strata between 2016 and 2018, ranging between 0.2% and 0.8%, and between 2.3% and 3.6%. in 2012, 2014 and 2015 (Table 10).

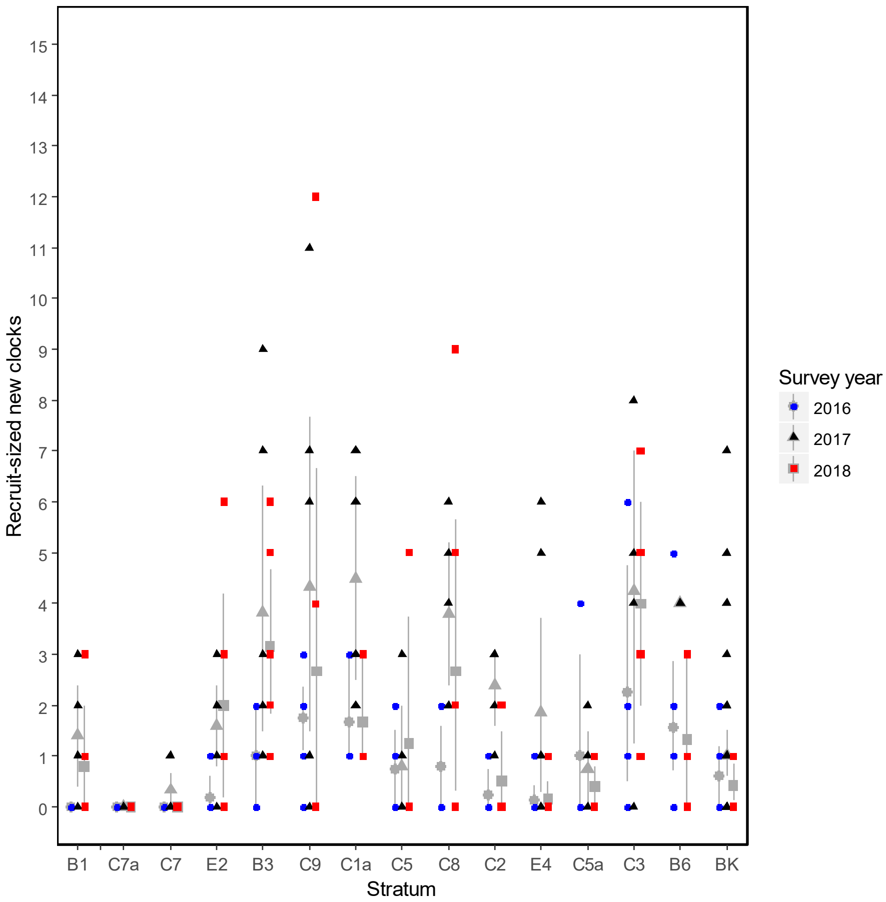

Figure 12: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized new clocks, their means and 95% confidence intervals (grey) by stratum sampled during the 2016 (blue), 2017 (black), and 2018 (red) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

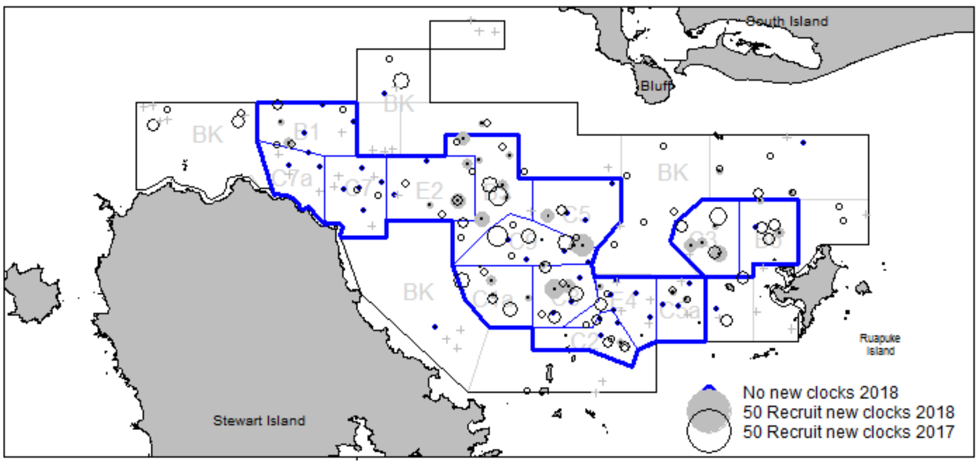

Figure 13: The distribution of recruit-sized new clocks and gaper densities combined in 2017 and 2018 (2017, open black circles; 2018, filled grey circles) showing the pre-survey mortality in February 2017 and 2018. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

Table 8: Absolute population estimates for recruit-sized new clocks in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2018 by stratum. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded light blue), upper and lower 95% confidence intervals (95% CI) in millions of oysters where a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres. Also given are the 2017 mean population estimates and percentage of the 2017 population size by stratum. The percentage of the 2017 estimate (% of 2017) is shaded green for increases in population size and tan for decreases.

Stratum	No. stns	Mean density	Density s.d.	CV	2018 Pop.n	B.lower 95%CI	B.upper 95%CI	Area km^2	2017 Pop.n	% of 2017
B1	4	0.00	0.00	1.00	0.1	0.0	0.3	78.2	0.4	23.9
B3	6	0.02	0.00	0.25	0.7	0.3	1.2	44.7	0.4	172.3
B6	3	0.01	0.00	0.66	0.2	0.0	0.5	30.0	0.6	32.6
C1a	3	0.01	0.00	0.41	0.3	0.0	0.5	31.3	0.7	36.4
C2	3	0.00	0.00	0.00	0.0	0.0	0.0	21.9	0.2	0.0
C3	3	0.01	0.01	0.39	0.5	0.1	0.9	32.7	0.9	53.1
C5	3	0.00	0.00	0.00	0.0	0.0	0.0	37.7	0.2	0.0
C5a	4	0.00	0.00	1.00	0.0	0.0	0.1	23.5	0.1	28.5
C7	5	0.00	0.00	0.00	0.0	0.0	0.0	36.1	0.1	0.0
C7a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.6	0.0	-
C8	5	0.01	0.01	0.65	0.4	0.0	0.9	26.8	0.5	73.2
C9	6	0.01	0.01	0.73	0.4	0.0	1.2	34.5	0.7	62.2
E2	3	0.01	0.00	0.66	0.3	0.0	0.7	42.8	0.3	93.4
E4	4	0.00	0.00	1.00	0.0	0.0	0.1	28.0	0.2	16.9
Core total	**55**	**0.01**	**0.00**	**0.19**	**2.9**	**1.6**	**4.6**	**491.8**	**5.3**	**53.9**
BK	5	0.00	0.00	1.00	0.6	0.0	1.8	578.4	2.7	20.9
Survey total	**60**	**0.00**	**0.00**	**0.23**	**3.4**	**1.7**	**5.8**	**1070.2**	**7.8**	**43.8**

Table 9: Absolute population estimates for pre-recruit new clocks in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2018 by stratum. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded light blue), upper and lower 95% confidence intervals (95% CI) in millions of oysters where a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres. Also given are the 2017 mean population estimates and percentage of the 2017 population size by stratum. The percentage of the 2017 estimate (% of 2017) is shaded green for increases in population size and tan for decreases.

Stratum	No. stns	Mean density	Density s.d.	CV	2018 Pop.n	B.lower 95%CI	B.upper 95%CI	Area km^2	2017 Pop.n	% of 2017
B1	4	0.00	0.00	0.00	0.0	0.0	0.0	78.2	0.0	-
B3	6	0.00	0.00	0.63	0.1	0.0	0.2	44.7	0.0	-
B6	3	0.00	0.00	1.00	0.1	0.0	0.3	30.0	0.1	97.4
C1a	3	0.00	0.00	1.00	0.1	0.0	0.3	31.3	0.2	50.8
C2	3	0.00	0.00	1.00	0.0	0.0	0.1	21.9	0.0	-
C3	3	0.00	0.00	1.00	0.1	0.0	0.2	32.7	0.1	52.6
C5	3	0.00	0.00	0.00	0.0	0.0	0.0	37.7	0.0	-
C5a	4	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	5	0.00	0.00	0.00	0.0	0.0	0.0	36.1	0.0	-
C7a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.6	0.0	-
C8	5	0.00	0.00	1.00	0.1	0.0	0.2	26.8	0.1	52.1
C9	6	0.00	0.00	0.00	0.0	0.0	0.0	34.5	0.3	-
E2	3	0.00	0.00	0.00	0.0	0.0	0.0	42.8	0.0	-
E4	4	0.00	0.00	0.00	0.0	0.0	0.0	28.0	0.0	-
Core total	**55**	**0.00**	**0.00**	**0.41**	**0.4**	**0.1**	**0.8**	**491.8**	**0.9**	**45.8**
BK	5	0.00	0.00	0.00	0.0	0.0	0.0	578.4	0.4	0.0
Survey total	**60**	**0.00**	**0.00**	**0.41**	**0.4**	**0.1**	**0.8**	**1070.2**	**1.3**	31.7

Table 10: Estimates of pre-survey mortality for core strata (Core strata) and the 2007 stock assessment survey area for recruit-sized and pre-recruit new clocks for the 2012, 2014–2018 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	0.9	0.7
2018	494.1	2.9	0.6	178.4	0.4	0.2

Stock assessment survey area

	Recruit-sized			Pre-recruit		
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
2018	883.3	3.4	0.4	225.8	0.4	0.2

4.4 The prevalence and intensity of Bonamia infection in core strata

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 eight stations in 2018 which included 72 stations of recruits and 6 stations of pre-recruits. In all, 1839 samples of heart imprint slides were sampled and archived. This sample comprised 1641 recruit-sized oysters, 176 pre-recruits, and 22 small oysters. Almost all of the samples (96.6%) were of recruit-sized oysters, which is similar to 2017 (96.3%), 2016 (96.6%), 2015 (97.2%), and to previous surveys. Only a subsample of these samples were screened (n= 379). Stations with fewer than 15 recruit and pre-recruit sized oysters (53, n= 11; 15, n= 7; 33, n= 6; 32, n= 6; 137, n= 4; and 16, n= 4) were not used in the analysis of infection.

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

ddPCR detection of Bonamia in oyster heart tissues

A summary of ddPCR samples tested and the corresponding heart imprint slides examined is shown in Table 11. Of the 1839 slides taken from random stations with more than 15 recruit and pre-recruit sized oysters in 2018, a subset of 379 heart imprint slides were examined for Bonamia infection. The remaining 1460 slides were from oysters screened using ddPCR and were not infected. In 2018, 92.2% of oysters had no detectable infection, which is higher than in 2017, (95.4%), 2016 (87.3%) 2015 (84.7%), 2014 (85.8%), and for 2010 to 2013 (90%, 88%, 89%, and 88% respectively). There were no ddPCR false negatives.

Table 11: The numbers of oyster heart tissue samples screened for Bonamia using ddPCR and heart imprints in 2018. Samples shown by recruit-sized and pre-recruit oysters. The total numbers of samples tested (Sample (N)), the numbers of samples that tested negative and positive (by size group) using ddPCR and from heart imprint slides are summarised. For each station, the sample of heart imprint slides screened included all ddPCR positives and three or more randomly selected ddPCR negative samples. There were no ddPCR false negatives.

ddPCR samples	Recruits	Pre-recruits	Recruits	Recruits	Pre-recruits	Pre-recruits
Bonamia infection	Sample (N)	Sample (N)	ddPCR-	ddPCR+	ddPCR-	ddPCR+
Heart	1689	150	1559	130	143	7
Histology samples						
Slides read (N)	379					
Heart imprints	**Recruits**	**Pre-recruits**				
Sample (N)	353	26				
Heart imprint -ve	320	25				
Heart imprint +ve	28	1				
Heart imprint NA*	5	0				

* No haemocytes visible on slide

Prevalence and intensity of infection in oysters by Bonamia

We assumed that all heart imprint slides corresponding to samples that were ddPCR negative, but not scored for Bonamia, were negative. Fixed stations and stations with too few oysters (stations 15, 16, 32, 33, 53, and 137) were excluded from the analysis of prevalence and intensity of infection (Table 12). 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 qPCR and ddPCR estimates (Table 12). The mean prevalence from heart imprints in 2018 (1.5%) was lower than in 2016 and 2017 Table 12), and lower than in older surveys (2009–2015, 7.5–15.3%). qPCR and ddPCR analysis of heart tissues was more sensitive than heart imprints (Table 12). Mean prevalence from ddPCR in 2018 was higher (7.8%) than from heart imprints (1.5%). In 2016 and 2017, mean prevalence from qPCR was 11.8% and 5.4% respectively, both higher than from heart imprints (Table 12).

Table 12: Comparisons of infection levels (prevalence and intensity) between 2016, 2017 and 2018. Number of samples for each method (N), mean and median prevalence (Prev %) and intensity estimated by heart imprints (Hist.), and prevalence from qPCR and ddPCR. Standard deviation (s.d.) and 5% and 95% percentiles (5% and 95%). Data from random stations sampled for Bonamia with more than 15 recruit and pre-recruit oysters in the sample.

			2016			2017			2018
	Hist. Prev (%)	Hist. Intensity	qPCR Prev (%)	Hist. Prev (%)	Hist. Intensity	qPCR Prev (%)	Hist. Prev (%)	Hist. Intensity	ddPCR Prev (%)
N	55	42	55	76	43	76	54	18	54
mean	7.5	2.8	11.8	4.3	3.4	5.4	1.5	3.1	7.8
median	4.0	2.5	8.0	4.0	3.5	4.0	0	3.0	6.0
s.d.	7.1	1.1	9.5	4.5	0.9	5.5	2.4	8.6	7.7
5%	5.7	2.5	9.3	0	2.0	4.0	0	1.9	0
95%	9.4	3.1	14.3	13.0	4.9	16.0	5.4	4.2	24.0

Details of recruit-sized oysters and densities by station, and their Bonamia infection status from histology and ddPCR are shown in Table A4.1, Appendix 4.

For the stations with infection, the median prevalence of infection in 2018 (6.0% from ddPCR, Table 12) was more than in 2017 (4.0%, qPCR) and less than in 2016 (8.0% qPCR). Intensity of infection was determined from heart imprints to maintain the time series of Bonamia survey data. Of the 7.8% of oysters with detectable infections in 2018, mean light (category 1 and 2 infections) was 2.1% (1–5% in 2010–2017), and 5.7% had category 3 and higher infections (4–11% in 2010–2017) which are normally fatal.

The prevalence of infection from heart imprints in 2018 is the lowest since 2007 (Figure 14). During periods of relatively high prevalence (2012–2015), qPCR showed higher prevalence than heart imprints, but levels were similar at relatively low prevalence (Figure 14). Prevalence from ddPCR in 2018 is higher than for heart imprints reflecting the increased sensitivity of PCR methods (Figure 14).

Most of the oysters screened with heart imprints that had detectable infections were fatally infected, i.e., the median intensity of infection was category three or above (Figure 15). The proportion of non-fatal infection is the lowest since 2007 (Figure 15).

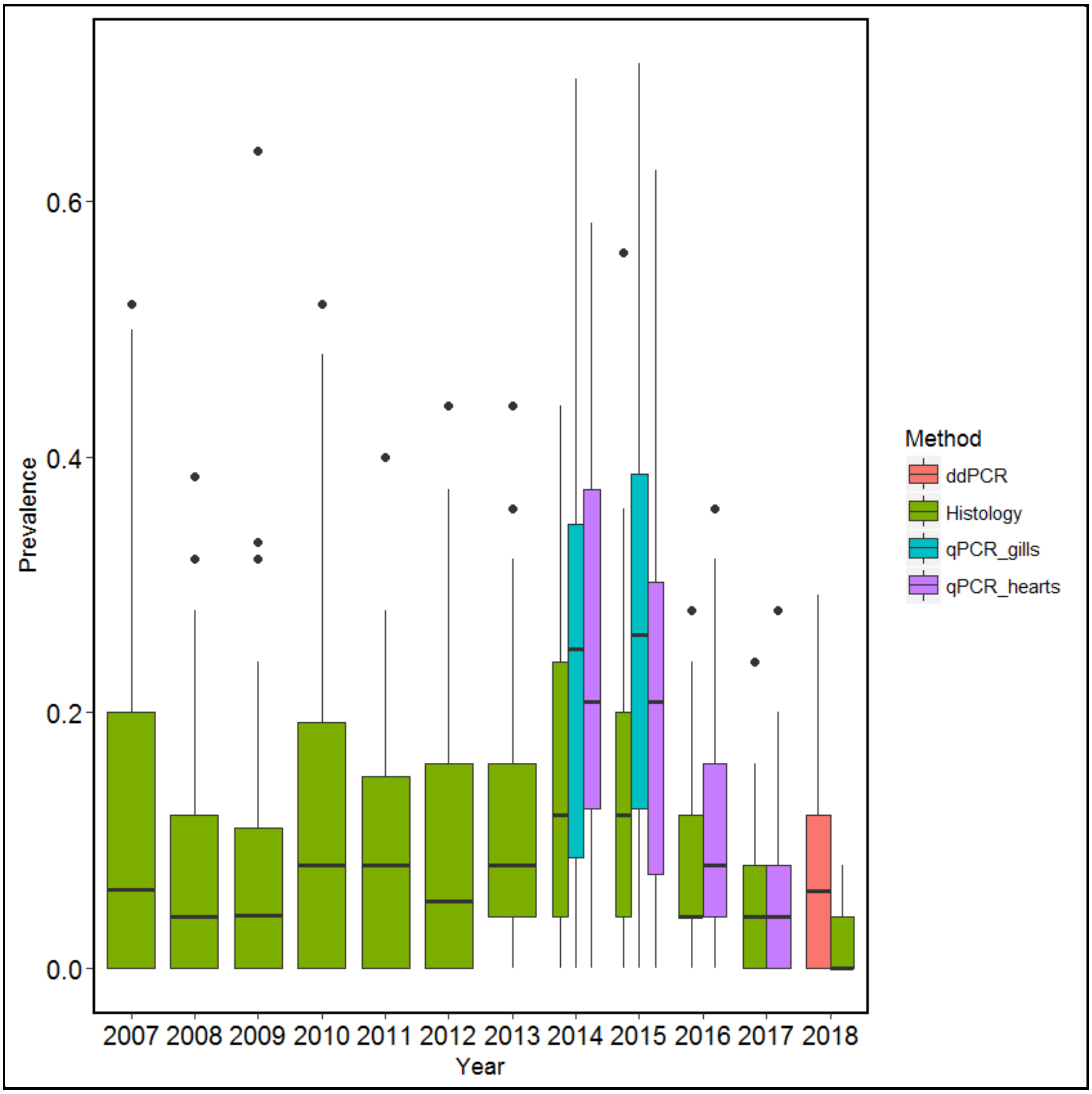

Figure 14: Boxplots of the median prevalence of Bonamia infection 2007–2018. The median prevalence of infection at all stations determined from histology (heart imprints) 2007–2013, and for qPCR heart tissues (qPCR_hearts) and gill tissues (qPCR_gills) in 2014 and 2015, but only heart tissues in 2016 and 2017. Heart tissues were only tested with ddPCR in 2018. Medians shown as solid lines, boxes represent 50th percentiles and whiskers 95th percentiles, and outliers as filled black circles.

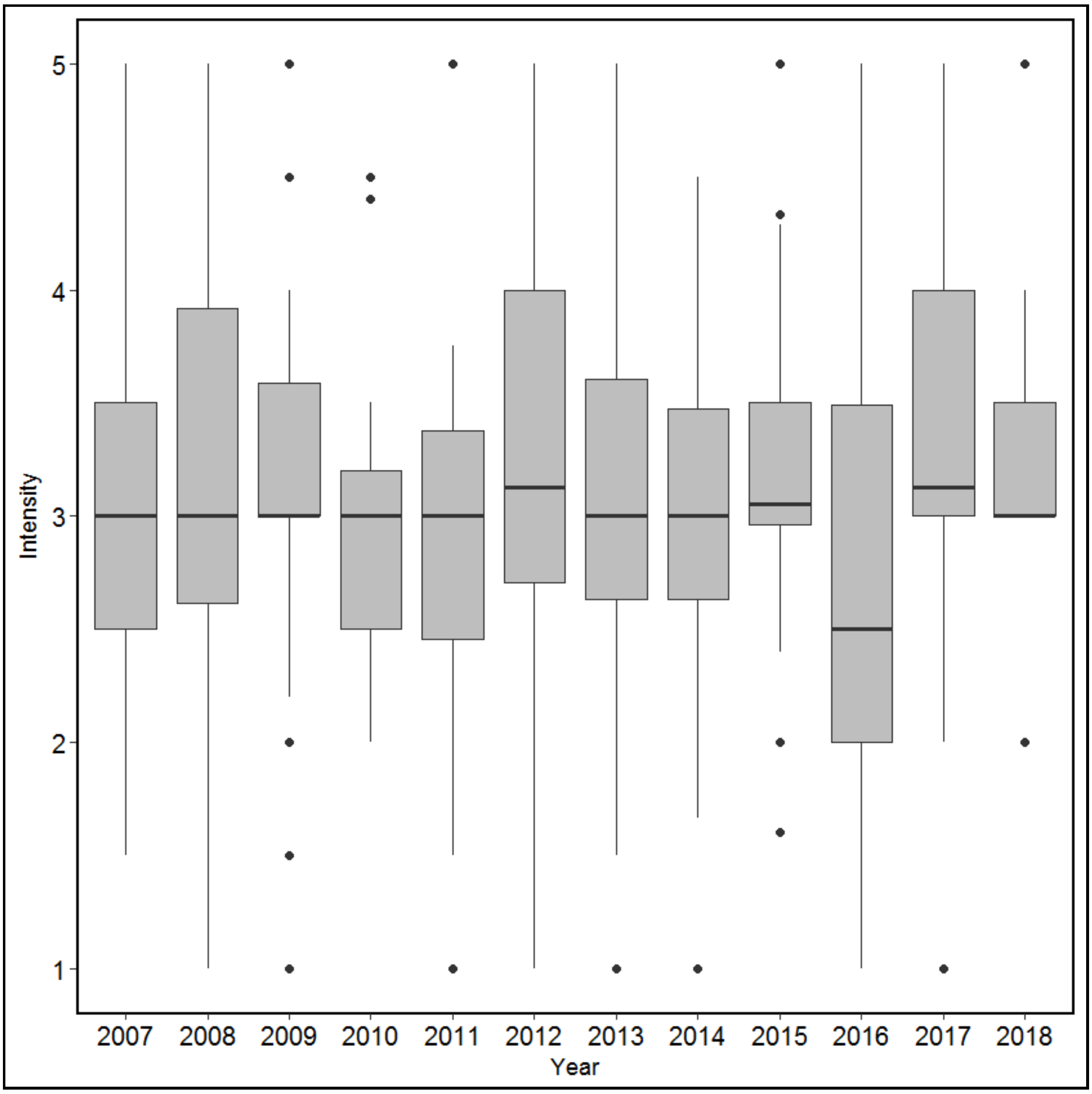

Figure 15: Boxplots of the mean intensity of Bonamia infection 2007–2018. The mean intensity of infection at all stations determined from histology. Medians shown as solid lines, boxes represent 50 percentiles and whiskers 95 percentiles, and outliers are shown as filled black circles.

Changes in the distribution of prevalence and intensity of Bonamia infection

The distribution of the prevalence of Bonamia infection estimated from heart imprints, qPCR and ddPCR analyses between 2016 and 2018 is widespread, but spatially patchy over multiple scales (Figures 16–18). In in all years, qPCR and ddPCR detected higher numbers of infected oysters than were detected by heart imprints (Figures 16–18).

Generally, relatively high prevalence has persisted in southern and eastern fishery areas between 2016 and 2018, and relatively low and patchy infection in the western and central areas. Infected stations were interspersed with stations with no detectable infection (shown as filled blue circles) In 2018, prevalence of infection appeared to increase in southern areas (Strata C2, C8, and E4) and remain similar in eastern areas (Strata C3 and B6, Figure 18), however this may be due to the higher sensitivity of ddPCR.

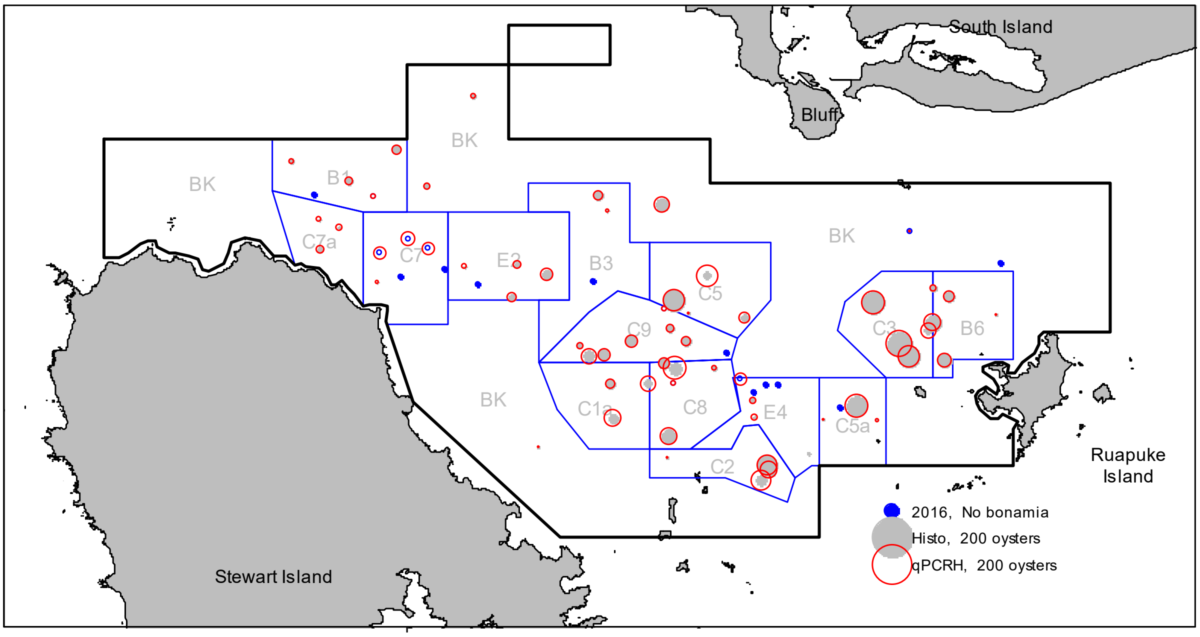

Figure 16: The distributions of Bonamia infection in February 2016 estimated from heart imprints, and qPCR analysis of heart tissues only. Numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and qPCR heart tissues (qPCRH, open red circles). Stations with no Bonamia (filled blue circles). The 2007 survey area (black outer line), the core strata (blue lines), and the stratum labels in grey.

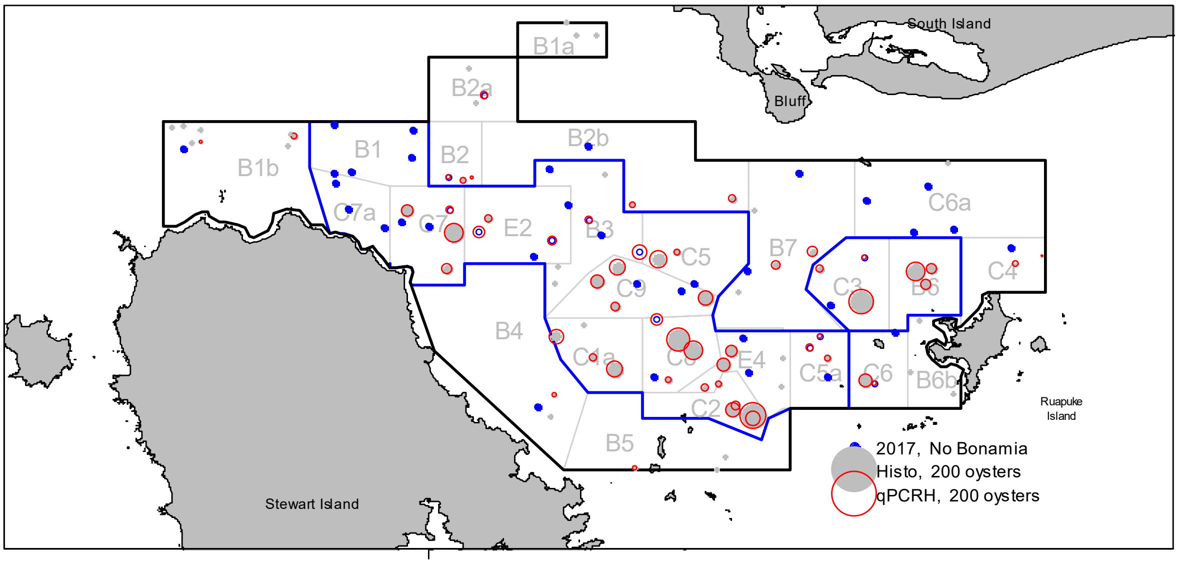

Figure 17: The distributions of Bonamia infection in February 2017 estimated from heart imprints, and qPCR analysis of heart tissues only. Numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and qPCR heart tissues (qPCRH, open red circles). Stations with no Bonamia (filled blue circles). The 2007 survey area (black outer line), the core strata (blue lines), and the stratum labels in grey.

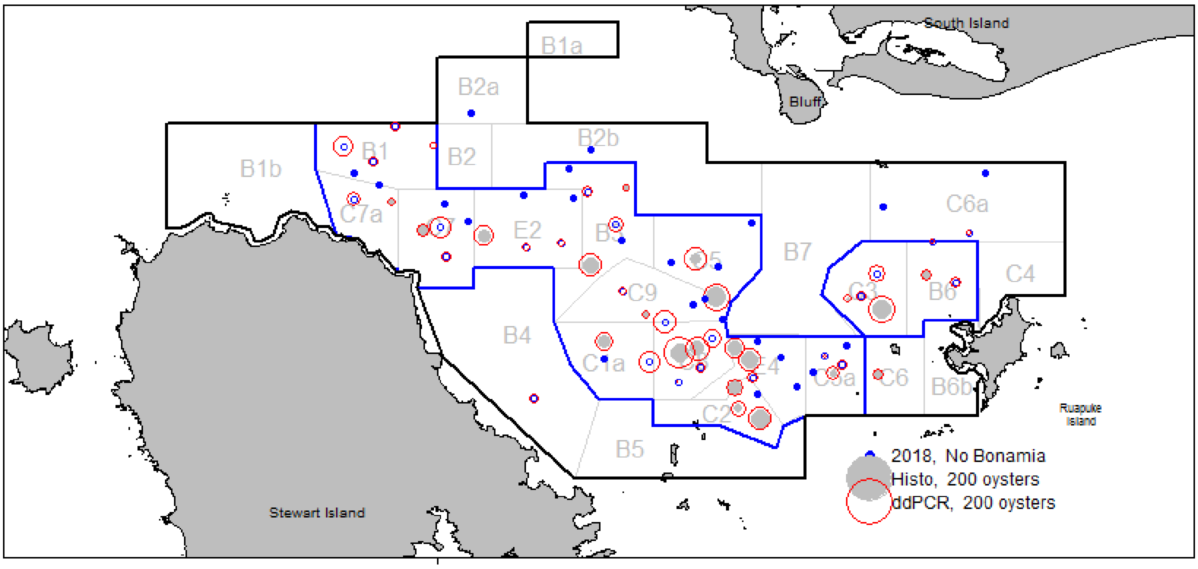

Figure 18: The distributions of Bonamia infection in February 2018 estimated from heart imprints, and ddPCR analysis of heart tissues only. Numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and ddPCR heart tissues (qPCRH, open red circles). Stations with no Bonamia (filled blue circles). The 2007 survey area (black outer line), the core strata (blue lines), and the stratum labels in grey.

Between 2012 and 2015, widespread fatal infections caused substantial oyster mortality over the fishery area (Michael et al. 2016). By 2016 Bonamia mortality had markedly reduced oyster density, but fatal infection levels were also reduced markedly and confined to the fishery areas east of a line between Saddle Point and Bluff Hill (Figure 19). Recruit-sized oyster densities were similarly low in 2017, infection was low and patchy, and fatal infections more widespread, extending in to western fishery areas (Figure 20). In 2018, recruit-sized oyster densities increased markedly, and the levels of infection (almost all fatal) were similar to 2017. Fatal infections were more patchily distributed than in 2017, interspersed with sites with no detectable infection (Figure 21).

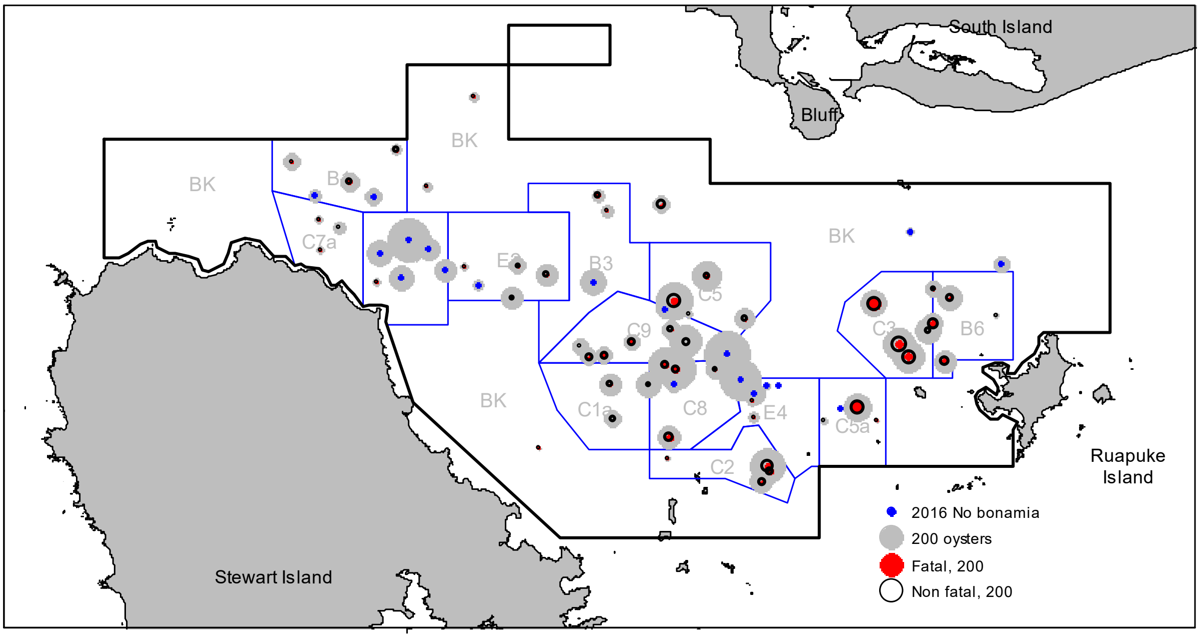

Figure 19: The distributions of oysters and Bonamia infection in February 2016. Numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no Bonamia (filled blue circles). The 2007 survey area (black outer line), the core strata (blue lines), and the stratum labels in grey.

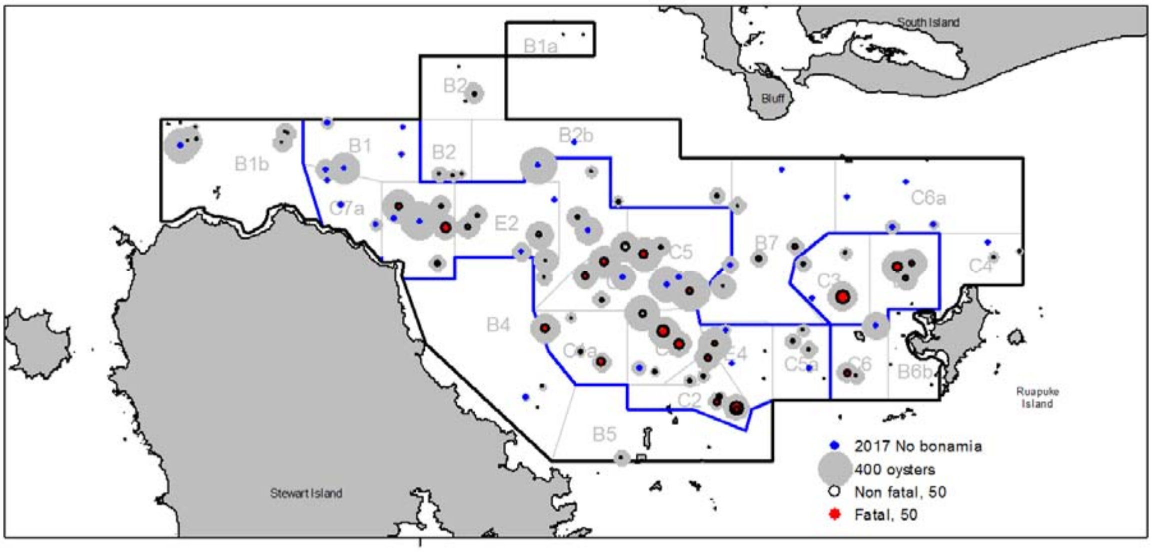

Figure 20: The distributions of oysters and Bonamia infection in February 2017. Numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no Bonamia (filled blue circles). The 2007 survey area (black outer line), the core strata (blue lines), and the stratum labels in grey.

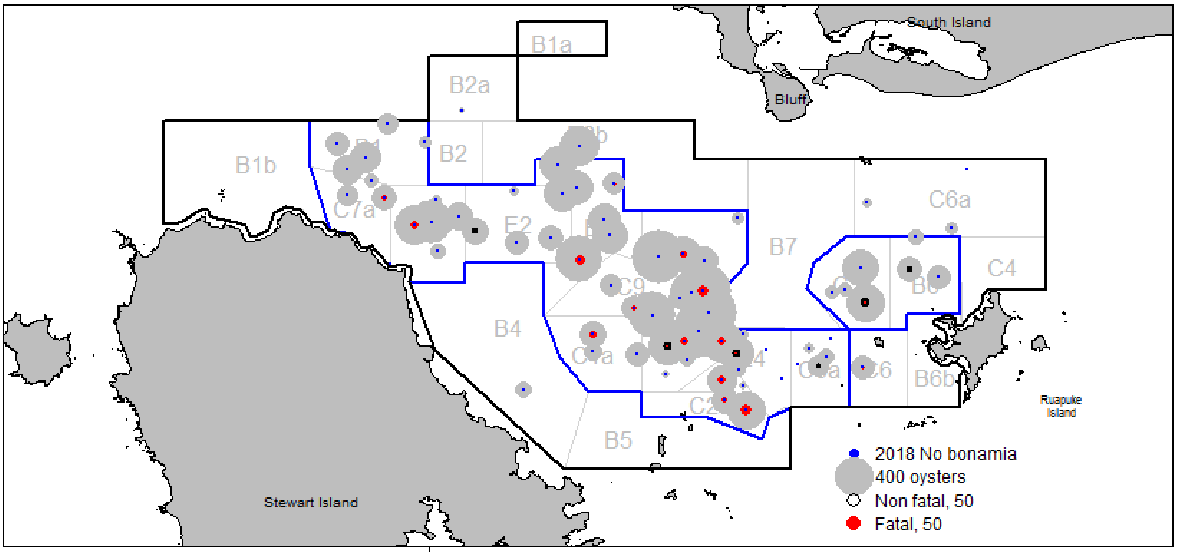

Figure 21: The distributions of oysters and Bonamia infection in February 2018. Numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no Bonamia (filled blue circles). The 2007 survey area (black outer line), the core strata (blue lines), and the stratum labels in grey.

Patterns in the distribution of prevalence and intensity of infection between 2012 and 2018 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 little fishing since 2008 because of the low meat quality there.

The total numbers of recruit-sized oysters infected with Bonamia

The prevalence of Bonamia infection in recruit-sized oysters in core strata, estimated from heart imprints (categories 1–5) declined 90.1%, from 89.5 million (95% CI 50.8–146.1) in 2014 to 8.2 million (95% CI 3.5–14.3) in 2018 (Table 13). Prevalence over the whole of the 2007 survey area declined 93.4%, from 176.1 million (95% CI 63.9–325.3) in 2014 to 11.6 million (95% CI 3.3–22.6) in 2018 (Table 13). Most of the infected oysters in 2018 had fatal infections; only 11.0%, 0.9 million (95% CI 0.0–2.2) were non-fatal infections in the core strata and very few had non-fatal infections in the background stratum (see Table 15).

Population prevalence estimated from ddPCR was 436.6% higher in core strata, 35.8 million (95% CI 20.2–58.6), and 417.2% higher in the 2007 stock assessment survey area, 48.4 million (95% CI 24.9–80.6) (Table 14).

Table 13: 2018 estimates of recruit-sized oysters with Bonamia infection (prevalence), estimated by heart imprints, scaled to population size in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total). The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95% CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	4	0.00	0.00	0.00	0.0	0.0	0.0	78.2
B3	6	0.02	0.02	0.82	1.1	0.0	3.1	44.7
B6	3	0.01	0.01	1.00	0.3	0.0	1.1	30.0
C1a	3	0.02	0.02	1.00	0.8	0.0	2.5	31.3
C2	3	0.04	0.03	0.61	1.0	0.0	2.3	21.9
C3	3	0.00	0.00	1.00	0.1	0.0	0.3	32.7
C5	3	0.02	0.02	1.00	0.7	0.0	2.3	37.7
C5a	4	0.01	0.01	1.00	0.1	0.0	0.5	23.5
C7	5	0.02	0.02	1.00	0.6	0.0	1.9	36.1
C7a	3	0.01	0.01	1.00	0.3	0.0	0.9	23.6
C8	5	0.05	0.03	0.64	1.3	0.0	3.3	26.8
C9	6	0.03	0.03	0.82	1.2	0.0	3.3	34.5
E2	3	0.00	0.00	0.00	0.0	0.0	0.0	42.8
E4	4	0.02	0.02	1.00	0.7	0.0	2.2	28.0
Core total	55	0.02	0.00	0.27	8.2	3.5	14.3	491.8
BK	5	0.01	0.01	1.00	3.4	0.0	10.9	578.4
Survey total	60	0.01	0.00	0.35	11.6	3.3	22.6	1070.2

Table 14: 2018 estimates of recruit-sized oysters with Bonamia infection (prevalence), <u>estimated from ddPCR</u>, scaled to population size in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total). The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95% CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	4	0.08	0.04	0.48	6.0	0.4	12.9	78.2
B3	6	0.07	0.04	0.52	3.2	0.0	7.1	44.7
B6	3	0.05	0.02	0.35	1.5	0.4	2.8	30.0
C1a	3	0.12	0.07	0.52	3.9	0.0	8.8	31.3
C2	3	0.08	0.04	0.51	1.9	0.0	4.1	21.9
C3	3	0.08	0.03	0.45	2.6	0.3	5.4	32.7
C5	3	0.08	0.08	1.00	3.0	0.0	9.4	37.7
C5a	4	0.03	0.02	0.88	0.6	0.0	1.8	23.5
C7	5	0.07	0.04	0.57	2.5	0.0	5.9	36.1
C7a	3	0.05	0.03	0.66	1.1	0.0	2.7	23.6
C8	5	0.19	0.08	0.44	5.1	0.7	10.8	26.8
C9	6	0.07	0.06	0.82	2.3	0.0	6.7	34.5
E2	3	0.01	0.01	1.00	0.4	0.0	1.4	42.8
E4	4	0.06	0.05	0.72	1.8	0.0	4.7	28.0
Core total	55	0.07	0.01	0.18	35.8	20.2	58.6	491.8
BK	5	0.02	0.01	0.63	12.5	0.0	31.0	578.4
Survey total	60	0.05	0.01	0.21	48.4	24.9	80.6	1070.2

Table 15: 2018 estimates of recruit-sized oysters with non-fatal infections (category 1 and 2), estimated by heart imprints, scaled to population size in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total). The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	4	0.00	0.00	0.00	0.0	0.0	0.0	78.2
B3	6	0.00	0.00	0.00	0.0	0.0	0.0	44.7
B6	3	0.01	0.01	1.00	0.3	0.0	1.1	30.0
C1a	3	0.00	0.00	0.00	0.0	0.0	0.0	31.3
C2	3	0.00	0.00	0.00	0.0	0.0	0.0	21.9
C3	3	0.00	0.00	0.00	0.0	0.0	0.0	32.7
C5	3	0.00	0.00	0.00	0.0	0.0	0.0	37.7
C5a	4	0.01	0.01	1.00	0.1	0.0	0.4	23.5
C7	5	0.00	0.00	0.00	0.0	0.0	0.0	36.1
C7a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.6
C8	5	0.02	0.02	1.00	0.4	0.0	1.3	26.8
C9	6	0.00	0.00	0.00	0.0	0.0	0.0	34.5
E2	3	0.00	0.00	0.00	0.0	0.0	0.0	42.8
E4	4	0.00	0.00	0.00	0.0	0.0	0.0	28.0
Core total	55	0.00	0.00	0.62	0.9	0.0	2.2	491.8
BK	5	0.00	0.00	0.00	0.0	0.0	0.0	578.4
Survey total	60	0.00	0.00	0.62	0.9	0.0	2.2	1070.2

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

Before 2016, the distribution of non-fatal (category 1 and 2 infections) infections was widespread and variable across the fishery. The prevalence of non-fatal infection varied at small spatial scales; stations with relatively high prevalence were often close to stations with low prevalence or no infection. Stations with high numbers of non-fatal infection are likely to be subjected to heightened Bonamia mortality in the future. Stations with non-fatal infections in 2016 were considerably fewer than in previous surveys, and mainly in central (C5 and C9) and eastern (C3 and B6) fishery areas, east of a line between Bluff Hill and Saddle Point (Figure 22). The numbers of stations with non-fatal infections were greatly reduced in 2017 (Figure 23) with the occasional station in central, southern and eastern stations. In 2018, non-fatal infections were detected at relatively few, isolated stations in western, southern and eastern areas, however, prevalence was higher than in 2017 (Figures 23 and 24) – probably due to the increased sensitivity of ddPCR to detect low-level infections.

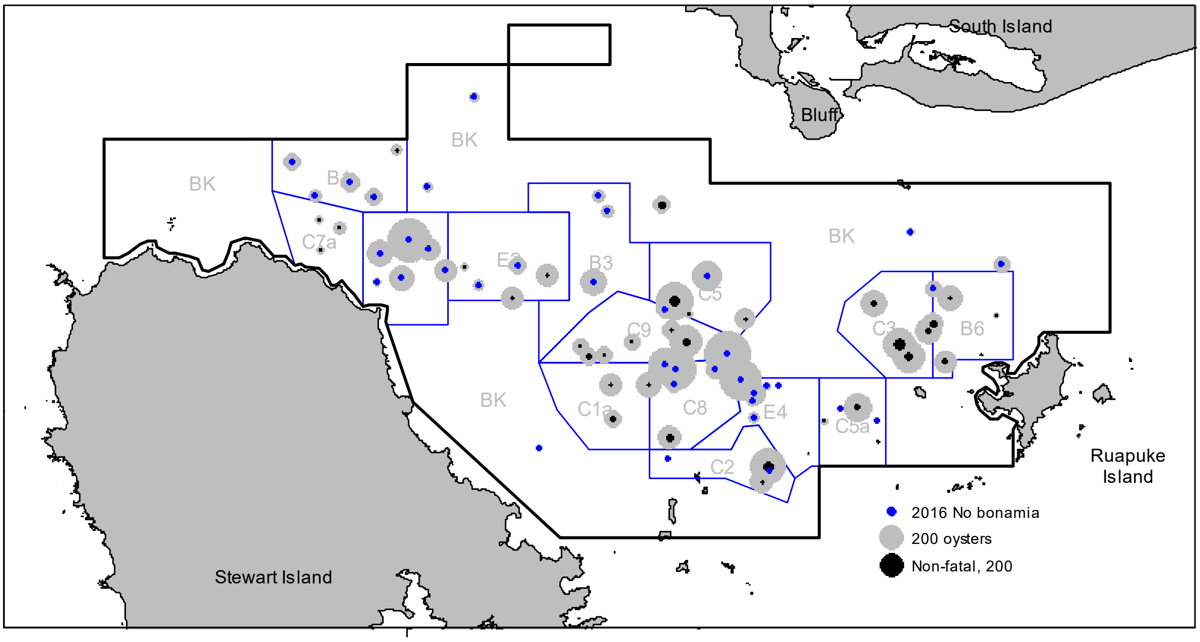

Figure 22: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (open black circles, the numbers of oysters scaled to the size of the catch) in February 2016. Stations with no Bonamia infection are shown by blue circles.

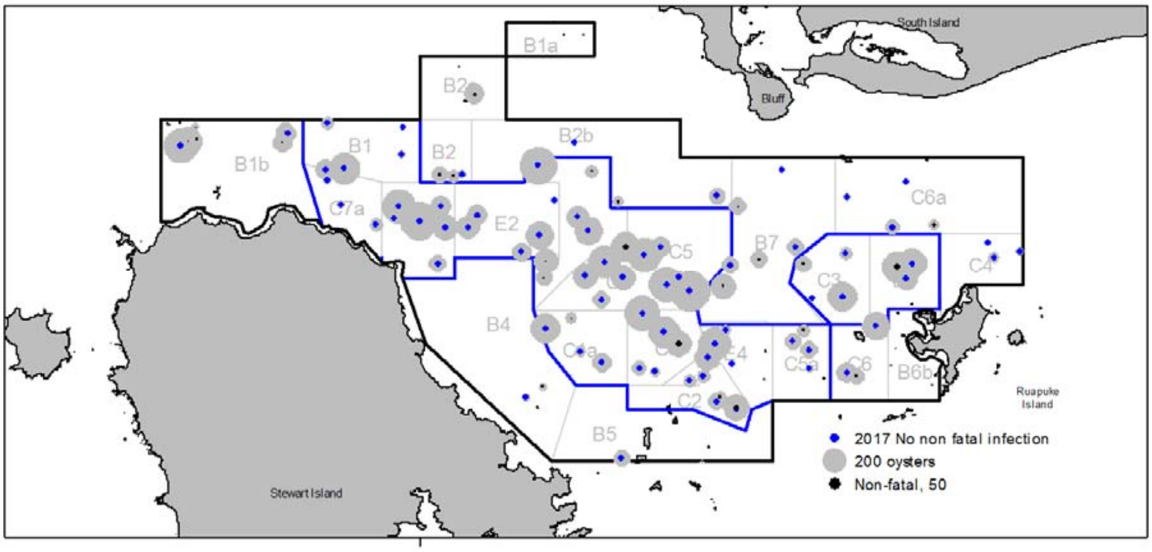

Figure 23: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (open black circles, the numbers of oysters scaled to the size of the catch) in February 2017. Stations with no Bonamia infection are shown by blue circles.

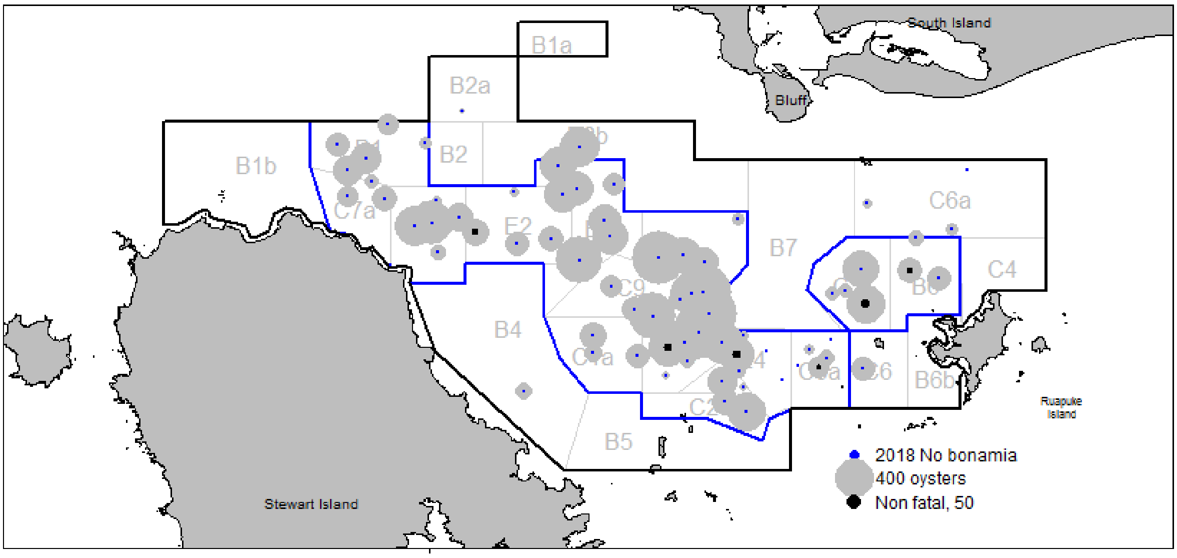

Figure 24: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (open black circles, the numbers of oysters scaled to the size of the catch) in February 2018. Stations with no Bonamia infection are shown by blue circles.

4.5 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 (see Table 8). In 2018, pre-survey mortality in all core strata combined was estimated to be 2.9 million recruit-sized oysters (95% CI 1.6–4.6), 0.6% of the recruit-sized population. Projections of post-survey mortality (within about two months of sampling) from the proportion of oysters with categories three and higher (fatal) infections scaled-up to the size of the total recruit-sized oyster population are given below. Two methods are used 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 fatally infected oysters in the stratum; and 2, post-survey mortality was estimated from the numbers of infected oysters at each sample station scaled to the catch, then to stratum, and to the survey area level. Estimates may differ a little when the numbers of fatal infections and oyster densities are more variable in any given strata.

Projected short-term mortality from Bonamia infections

Post-survey mortality of recruit-sized oysters was estimated for core strata with three or more randomly selected stations. Projected short-term mortality using the mean proportion of oysters infected with category 3 and higher infections in the catch was used to calculate a correction factor for each stratum (1 (the total catch) less the mean proportion of oysters infected with Bonamia, Table 16)) and this correction factor was applied to the mean oyster density estimated from all random tows. Using this method, post-survey mortality of oysters was projected to reduce the recruit-sized oyster population in core strata by 15.7 million oysters (3.2%) from 494.1 million oysters at the time of the survey (February 2018) to 478.4 million oysters (Table 16) by early in the new oyster season (March 2018). Estimates of the numbers of recruit sized oysters in core strata differ from Table 3 because some stations were omitted from the estimate because too few oysters were caught for a Bonamia sample. Post-survey mortality of recruit-sized oysters by stratum (Table 16) ranged from no mortality in strata C2 and C3 to 7.1% in E4.

Post survey mortality in 2018 was similar to 2017 in core strata, 4.9 million oysters (3.0%), slightly lower overall than in 2016 (14.8 million, 3.8%), and markedly lower than in 2015, 34.4 million oysters (10.0%).

The estimates of post-survey mortality in core strata from fatally infected oysters scaled to the size of the catch were lower than those estimated using averaged correction factors, 7.3 million oysters (1.5%) (Table 17). Five of the fourteen strata in 2018 (C9, B3, C2, C8, and C1a) accounted for 67% of the projected mortalities.

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

Summer mortality was estimated as the percentage of all recruit-sized oyster deaths in the population, from the time mortality began at the beginning of summer to the end of the seasonal mortality (about mid-March). This summer mortality was between 1.6% and 3.0% of the recruit-sized population in core strata (Table 18). Summer mortality was similar between 2016 and 2018, and much lower than between 2012 and 2015 (Table 18).

Table 16: Absolute population estimates for recruit-sized oysters after projected mortality from Bonamia based on category 3 and higher infections in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in February 2018. The number of randomly selected stations sampled (No. stns), the correction factor applied to each stratum (Correction factor), the mean oyster density per m^2 (Mean density), standard deviation (s.d.) of the density estimate, coefficient of variation (CV) of the oyster density, mean population size at the time of survey (Pop.n1, filled grey), mean post mortality population size (Pop.n2, filled blue) in millions of oysters, upper and lower 95% confidence intervals (CI) for the post-mortality estimate, the area of each stratum (Area.km^2), and percentage mortality by stratum.

Core Strata	No. stns	Correction factor	Mean density	Density s.d.	CV	Pop.n1	Pop.n2	Lower 95%CI	Upper 95%CI	Area.km^2	Mortality
B1	4	0.94	0.72	0.22	0.30	56.1	52.6	19.7	96.0	78.2	6.2
B3	6	0.94	1.66	0.31	0.19	74.3	69.6	39.0	112.4	44.7	6.4
B6	3	0.96	0.66	0.15	0.23	19.7	18.9	9.2	32.0	30.0	4.0
C1a	3	0.93	0.73	0.14	0.19	22.9	21.4	11.7	35.0	31.3	6.5
C2	3	1.00	0.73	0.33	0.45	16.1	16.1	1.7	33.7	21.9	0.2
C3	3	1.00	0.79	0.52	0.66	25.8	25.8	0.0	65.0	32.7	0.0
C5	3	0.96	0.90	0.37	0.41	33.9	32.5	6.4	65.4	37.7	4.3
C5a	4	0.97	0.19	0.13	0.68	4.5	4.4	0.0	11.1	23.5	3.1
C7	5	0.99	1.25	0.49	0.39	45.2	44.9	9.8	88.8	36.1	0.8
C7a	3	0.96	0.58	0.18	0.30	13.8	13.2	5.0	24.1	23.6	4.3
C8	5	0.98	1.74	0.72	0.41	46.7	45.6	8.4	93.0	26.8	2.3
C9	6	1.00	2.26	0.73	0.32	78.0	77.8	26.4	145.8	34.5	0.2
E2	3	1.00	0.90	0.49	0.54	38.6	38.5	0.0	89.0	42.8	0.2
E4	4	0.93	0.66	0.59	0.89	18.4	17.1	0.0	51.7	28.0	7.3
Core total	**55**	**-**	**-**	**-**	**-**	**494.1**	**478.4**	**-**	**-**	**-**	**3.2**
BK	5	0.98	0.67	0.40	0.59	389.2	381.9	0.0	917.4	578.4	1.9
Survey total	**60**	**-**	**0.83**	**0.22**	**0.27**	**883.3**	**860.2**	**367.5**	**1529.2**	**1070.2**	**2.6**

Table 17: Scaled up estimates of the population size of recruit-sized oysters with fatal infections (category 3–5) estimated by heart imprints in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area sampled in February 2018. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded blue), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	4	0.00	0.00	0.00	0.0	0.0	0.0	78.2
B3	6	0.02	0.02	0.82	1.1	0.0	3.1	44.7
B6	3	0.00	0.00	0.00	0.0	0.0	0.0	30.0
C1a	3	0.02	0.02	1.00	0.8	0.0	2.5	31.3
C2	3	0.04	0.03	0.61	1.0	0.0	2.3	21.9
C3	3	0.00	0.00	1.00	0.1	0.0	0.3	32.7
C5	3	0.02	0.02	1.00	0.7	0.0	2.3	37.7
C5a	4	0.00	0.00	0.00	0.0	0.0	0.0	23.5
C7	5	0.02	0.02	1.00	0.6	0.0	1.9	36.1
C7a	3	0.01	0.01	1.00	0.3	0.0	0.8	23.6
C8	5	0.03	0.02	0.62	0.9	0.0	2.2	26.8
C9	6	0.03	0.03	0.82	1.2	0.0	3.4	34.5
E2	3	0.00	0.00	0.00	0.0	0.0	0.0	42.8
E4	4	0.02	0.02	1.00	0.7	0.0	2.2	28.0
Core total	**55**	**0.01**	**0.00**	**0.29**	**7.3**	**2.9**	**13.0**	**491.8**
BK	5	0.01	0.01	1.00	3.4	0.0	10.8	578.4
Survey total	**60**	**0.01**	**0.00**	**0.37**	**10.7**	**2.9**	**20.9**	**1070.2**

Table 18: 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**	**2018**	**2015**	**2016**	**2017**	**2018**
Recruit-sized new clocks (NC)	30.0	23.7	3.6	7.8	3.4	13.5	1.4	5.3	2.9
Post-survey mortality									
Correction factor	81.1	49.0	24.3	18.3	23.1	34.4	14.8	13.4	15.7
Scaled catch	56.9	50.9	24.8	*12.9	10.7	31.6	14.8	10.9	7.3
Combined summer mortality									
Correction factor +NC	111.1	72.7	27.9	26.1	26.5	47.9	16.2	18.7	18.6
Scaled catch +NC	86.9	74.6	28.4	*20.7	14.1	45.1	16.2	16.2	10.2
Population before summer mortality									
Recruit-sized oysters +NC	948.4	533.6	564.7	535.2	886.7	364.9	386.6	368.9	497.0
Percent summer mortality									
Correction factor +NC	11.7	13.6	4.9	4.9	3.0	13.1	4.2	5.1	3.7
Scaled catch +NC	9.2	14.0	5.1	*3.9	1.6	12.4	4.2	4.4	2.1

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

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

Disease mortality and recruitment to the fishery are the main drivers of future stock size in the OYU 5 fishery. The 2017 stock assessment for OYU 5 suggests that an annual commercial harvest of up to 30 million oysters is not likely to have a significant effect on the future (1–5 years) status of the stock (Figure 25).

Between 1993 and 1999, the fishery rebuilt rapidly from a historically low size, driven by low or non-detectable Bonamia mortality and high recruitment to the fishery. After the second low point in the fishery in 2005, the fishery was again rebuilding rapidly driven by good spat-fall and juvenile survival, and a Bonamia mortality of about 10% of the recruit-sized population. The population of recruit-sized oysters continued to increase until 2012, and this high number of recruits should have led to an increase in recruitment, however, recruitment declined to low levels (regime shift to low recruitment) and remained low until 2015. The low recruitment to the fishery combined with a continuing Bonamia mortality of about 10% flattened the stock trajectory between 2010 and 2013 (Figure 25).

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 between 2012 and 2017. Recruit-sized oysters 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) (Michael et al. 2017). Bonamia mortality declined over the stock area to about 5% of the recruit-sized population in 2016 and 2017. These low levels of mortality have not been recorded since 1998.

The current status of the fishery suggests an increase in future recruit-sized stock abundance. The recruit-sized population increased around 36% between 2017 and 2018, as did pre-recruit sized oysters (around 45%) and small oysters (around 53%). Significant recruitment to the oyster population was recorded by spat monitoring, catch sampling, and during the February 2018 survey. Bonamia mortality was low (2–3%) over the summer of 2017–18. The future status of the fishery is best represented by series “a” in Figure 25 which assumes no Bonamia mortality. Moreover, non-fatal infections have declined to about 0.1% of the recruit-sized population, suggesting low Bonamia mortality in 2019. The low oyster densities and low non-fatal infections suggest reduced transmission of disease.

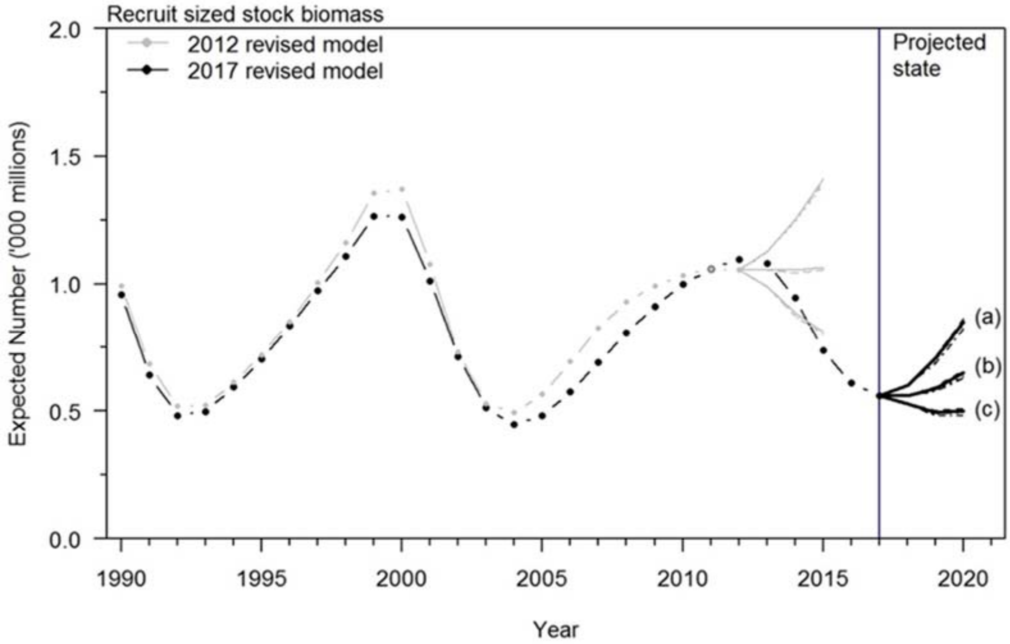

Figure 25: Model estimates of recent recruit-sized stock abundance and projected recruit-sized stock abundance with catches of 7.5 (solid line), 15 (dash dot), and 30 million oysters (dash line) under assumptions of (a) no disease mortality, (b) disease mortality of 0.10 y^{-1}, and (c) disease mortality of 0.20 y^{-1}, for the 2012 (grey dot dash line) and 2017 (black dot dash line) revised models (Figure reproduced from Large et al. 2017a).

5. DISCUSSION

The current programme of five-yearly stock assessments has placed greater onus on the annual Bonamia surveys to monitor changes in the oyster population in commercial fishery areas as well as the status of Bonamia. February Bonamia surveys provide a "weather forecast" immediately before the oyster season begins. The Bonamia survey area is 46% of the stock assessment survey area, and represented 75% and 69% of the recruit-sized oyster population in 2012 and 2017 respectively, thereby providing updated information on oyster densities in the important commercial fishery areas. This forecast also updates the status of infection and estimates of disease mortality, together with estimates of recruitment from spat monitoring, catch sampling and survey estimates, which are important in determining the trajectory of the stock. The limited sampling in the background stratum also allows these data to be incorporated into stock assessments. These surveys achieved low coefficients of variation (CVs) for population estimates, well below the 20% set by MPI for stock assessment surveys. A CV of 11% was obtained for estimates of recruit-sized oysters in the Bonamia survey area in 2018.

The objectives of Bonamia surveys have changed over time, see Michael et al. (2016) for details. A new time-series of Bonamia and oyster surveys which have incorporated a fully randomised, two-phase sampling design and a standard Bonamia survey area to make these surveys comparable from year to year was established in 2014. The February 2018 survey is the fourth in this new time-series. Because both estimates of new clocks and fatal infections are scaled to the size of the oyster population, better estimates of oyster density from randomised, two-phase sampling are likely to give more precise estimates of total summer mortality.

5.1 Survey results

This survey used the same vessel, skipper and crew, and standard sampling methods for Foveaux Strait surveys. Sampling conditions during the 2018 survey were not expected to have affected dredge efficiency. Additionally, the CVs obtained for population estimates from the survey are the same as those predicted for recruit sized oysters (CV of 11%). The timing of Bonamia surveys coincides with a period of peak seasonal mortality from Bonamia and the shedding of infective particles. In 2018, some Bonamia mortality had occurred before the survey (estimated as new clocks), but most of the mortality was expected shortly after the survey (category three and greater infections), suggesting that the survey effectively sampled summer mortality.

NIWA has transferred the previous qPCR-based methodology for the detection of Bonamia infection to an improved ddPCR method. ddPCR will also provide for quantification of infection for future Bonamia surveys. Overall, the new method provides correspondence of normalised quantification to histological scorings, a high level of precision and repeatability, superior levels of sensitivity and detection and cost-effectiveness. The use of ddPCR in 2018 most likely improved the detection of low level infection, increasing our estimates of prevalence in the Foveaux Strait population, however prevalence was still relatively low at about 7.0% of recruit-sized oysters in the Bonamia survey area.

Stock assessment and Bonamia surveys estimate oyster densities, and mean population sizes by stratum and for the Bonamia survey and stock areas. The design of the surveys does not describe the spatial structure of the stock well, especially the distribution of high density patches of large oysters important to fishers. Oyster density and meat quality in the highest-density patches determine commercial catch rates. Five of the 14 strata had recruit-sized oyster densities greater than 400 oysters per survey tow, however, a lot of these oysters were only just recruit-sized and do not contribute to the harvestable population. Consideration should be given to including a fourth “commercial” size category for surveys. The size of this category should be informed by oyster skippers and processors.

5.2 Outlook for the 2018 oyster season

At relatively low levels of catch (less than 30 million oysters per year), the future trend in the abundance of oysters in the Foveaux Strait fishery is driven by disease mortality from Bonamia and the levels of recruitment (spat settlement). Levels of oyster spat settlement had been low between the summers of 2009–10 and 2015–16 despite the population size of spawning sized-oysters increasing until 2012. Consequently, the numbers of small and pre-recruit oysters have been declining. Until 2012, Bonamia killed 8–12% of recruit-sized oysters, and fishing removed 1–2% of the recruited population. The recruit-sized oyster population was increasing, albeit slowly, despite this Bonamia mortality. The increased numbers of oysters killed by Bonamia since 2013 (200 million oysters in 2013), and the continued low replenishment of spat to the oyster population and medium-sized oysters to the fishery, resulted in a significant decline in the recruit-sized oyster population size in 2017.

Fishers target high density patches of “commercial-sized” oysters. In 2017, 66% of the catch was 70 mm in length or larger (recruit size is about 58 mm in length or larger). Between 2012 and 2017, Bonamia mortality has greatly reduced the numbers and extent of high-density patches with commercial-sized oysters, and oysters were generally distributed at low densities across the fishery area. Catch rates had fallen from 5.6 sacks per hour (S/H) in 2010 to 2.9 S/H in 2017. Oysters growing to recruit size in 2018 may not grow to “commercial size” for another 1–2 years. Therefore, catch rates in 2018 are expected to remain similar or to fall further.

In the medium-term, all the key indicators for the future rebuilding of the fishery are strongly positive. The three size groups of oysters increased between 35% and 53% between 2017 and 2018. Spat monitoring, catch sampling and the survey data show significant recruitment to the oyster population, and increases in pre-recruit and small oyster will support future increases in recruit-sized oysters. Bonamia mortality was low in 2018, and is it is expected to be low in 2019. At this low mortality, the

2017 stock assessment predicts that by 2020, recruit-sized oysters will increase by about 41%. The medium-term outlook for the OYU 5 fishery is for increasing commercial-sized oyster densities and catch rates.

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8. APPENDIXICES

8.1 Appendix 1: Survey station form

FOVEAUX STRAIT OYSTER SURVEY, STATION DATA RECORD

Vessel name

Recorder ....

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

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

Finish position: Latitude ° . S | Longitude ° . E

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

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

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

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

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

Sediment type

Circle the main type (one only)

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

Comments:

1 Nautical mile = 1.853 km

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

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

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

8.2 Appendix 2: Survey Bonamia form

Bonamia Testing: Laboratory Sampling Record Page___of____

Project code:

Site:

Station number/ID	Sampling Date	Sampling Time		
		:	Date processed	
Sampled primarily for:	B.e	B. o	Other	Heart/Gill plate#	Extra Gillx2 plate #

Oyster no	Length (mm)	Height (mm)	Size category	Gaper	Colour of larvae	Gonad section	Heart Imprint Score		
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									

Site description/comments:	Recorder	Checker	Heart/Gill plate 25th	Extra Gillx2 plate25th

Size catagory: **R**= recruits - do not pass through 58mm ring.
P= pre-recruits - pass through 58mm ring but not 50mm ring.
S = small pre-recruits- pass through 50mm ring.

Gaper : **G** = Gaper,N= No Gaper.

Colour of Larvae: **N**= None, **W**= white, , **G**= grey, **B** = black, **Y**=gold

Gonad section: **Y** =yes ,**N**= no.

Sampled primarily for: **B.e** = Bonamia exitiosa (**Y**= yes **N**= No) If all oysters are large, randomly select 25 from all the sample including gapers, then Ps and finally Ss i.e., do not target or avoid gapers as these biases the sample.
B.o= Bonamia ostreae (**Y**=yes **N**= No) Target gapers first, then all large oysters the make up with Ps and Ss if required.
Other: Specify eg. **APX** or **N** =No. **NOTE ALL MISSING VALUES PUT IN N**

8.3 Appendix 3: Survey conditions and comparability

Dredge tow lengths were almost all 0.2 nautical miles (371 m) in length (Figure A3.1). All oyster and clock densities were standardised to a 0.2 nautical mile standard tow length for analysis. Most of the survey stations were sampled in wind conditions less than 10 knots (Figure A3.2). The median wind force was 2 on the Beaufort scale (4−6 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 2018 survey was undertaken in similar conditions to the February 2017 survey.

Oyster dredges are considered saturated and cease fishing before the end of tow when they are more than 80% full on landing (Cranfield pers. comm.). Dredge saturation may lead to an underestimate of oyster density. No dredge was landed more than 80% full. Dredge fullness ranged from 1% to 80% with a median fullness of 40%, the same as in 2017 – 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 which increases the quantities of dead shell. Dredge saturation is not likely to have had a large effect on sampling effectiveness in the 2018 survey (Figure A3.3). 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 2018 survey data; future surveys will identify stations with this pattern in the distribution of catch. Five stations were landed over 70% full in 2018 with catches of 28, 135, 189, 318, 330, 371, 400, 445, and 822 recruit-sized oysters. Oyster densities may have been underestimated at these stations.

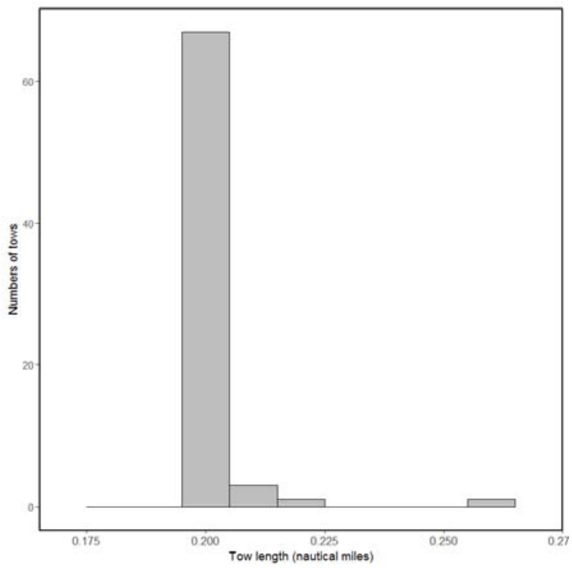

Figure A3.1: Distribution of dredge tow lengths from the February 2018 survey. The standard tow length was 0.2 nautical mile (371 m).

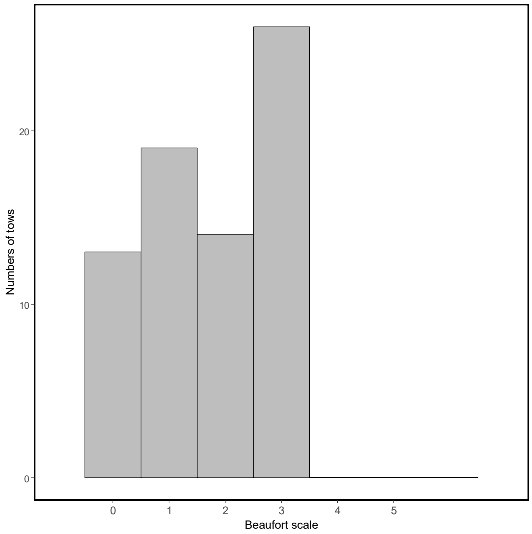

Figure A3.2: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2018. Beaufort scale: 0, < 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

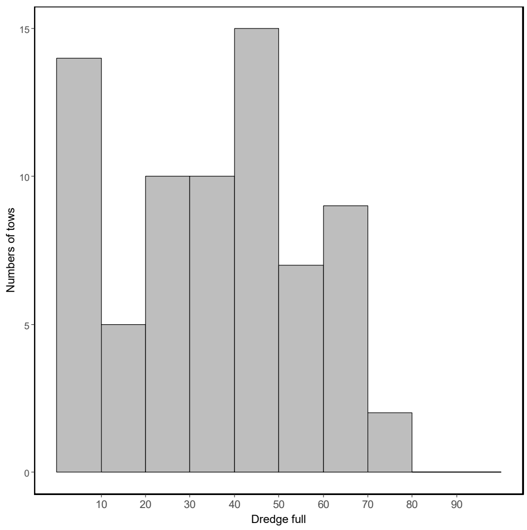

Figure A3.3: Distribution of dredge fullness recorded for survey tows in February 2018. No tows were landed with a dredge fullness of greater than 80%. Unpublished video data suggests that dredge saturation may occur below 80% full.

8.4 Appendix 4: 2018 survey catch and infection details.

Table A4.1: Details of recruit-sized oysters (Recruits) and densities m^{-2} (Density) by stratum (Str) and station (Stn); the numbers of oysters tested (Total) and numbers of uninfected (Un.inf) samples, samples with non-fatal infections (NF.inf) and fatal infections (Fat.inf) based on category 3 higher infections, and mean intensity of infection (Int) from heart imprints. The percentage prevalence of Bonamia infection detected by heart imprints (%Prev) and by ddPCR (%ddP) from the February 2018 survey.

Str	Stn	Recruits	Density	Total	Un.inf	NF.inf	Fat.inf	%Prev	Int	% ddP
B1	1	157	0.75	25	25	0	0	0.0	NA	24
B1	2	127	0.61	25	25	0	0	0.0	NA	8
B1	3	259	1.24	25	25	0	0	0.0	NA	4
B1	4	44	0.21	25	25	0	0	0.0	NA	12
B3	5	135	0.65	25	24	0	1	4.0	3	4
B3	6	318	1.46	25	25	0	0	0.0	NA	4
B3	7	359	1.71	25	25	0	0	0.0	NA	0
B3	8	281	1.35	25	25	0	0	0.0	NA	8
B3	9	618	2.96	25	24	0	1	4.0	4	8
B3	10	347	1.64	25	25	0	0	0.0	NA	0
B6	11	172	0.82	25	24	1	0	4.0	2	8
B6	12	158	0.75	25	25	0	0	0.0	NA	8
B6	13	72	0.35	25	25	0	0	0.0	NA	4
BK	14	445	2.12	25	25	0	0	0.0	NA	0
BK	15	7	0.03	7	7	0	0	0.0	NA	0
BK	16	2	0.01	4	4	0	0	0.0	NA	0
BK	17	157	0.76	25	24	0	1	4.0	3	8
BK	18	78	0.38	25	25	0	0	0.0	NA	12
C1a	19	96	0.46	25	25	0	0	0.0	NA	0
C1a	20	193	0.93	25	23	0	2	8.0	4	16
C2	23	243	1.16	25	23	0	2	8.0	3.5	12
C2	24	18	0.09	25	25	0	0	0.0	NA	0
C3	25	55	0.26	25	24	0	1	4.0	3	12
C3	26	372	1.79	25	25	0	0	0.0	NA	8
C3	27	57	0.27	25	25	0	0	0.0	NA	20
C5	28	212	1.00	25	25	0	0	0.0	NA	0
C5	29	44	0.21	25	25	0	0	0.0	NA	0
C5	30	330	1.43	25	24	0	1	4.0	3	16
C5a	31	39	0.19	25	25	0	0	0.0	NA	4
C5a	32	2	0.01	6	6	0	0	0.0	NA	17
C5a	33	2	0.01	6	6	0	0	0.0	NA	0
C5a	34	116	0.55	25	24	1	0	4.0	2	16
C7	35	558	2.63	25	25	0	0	0.0	NA	8
C7	36	28	0.13	25	25	0	0	0.0	NA	0
C7	37	418	1.99	25	24	0	1	4.0	4	4
C7	38	221	1.06	25	25	0	0	0.0	NA	0
C7	39	69	0.32	25	25	0	0	0.0	NA	16
C7a	40	54	0.25	25	25	0	0	0.0	NA	0
C7a	41	129	0.61	25	25	0	0	0.0	NA	16
C7a	42	179	0.85	25	24	0	1	4.0	3	4
C8	43	822	3.93	25	25	0	0	0.0	NA	4
C8	44	59	0.28	25	25	0	0	0.0	NA	16

Table A5.1: Continued

Str	Stn	Recruits	Density	Total	Un.inf	NF.inf	Fatal.inf	%Prev	Int	% ddP
C8	45	20	0.10	25	25	0	0	0.0	NA	4
C9	46	179	0.85	25	24	0	1	4	3	4
C9	47	149	0.71	25	25	0	0	0	NA	0
C9	48	931	4.43	25	25	0	0	0	NA	0
C9	49	142	0.68	25	25	0	0	0	NA	4
E2	50	371	1.78	25	25	0	0	0	NA	0
E2	51	30	0.14	25	25	0	0	0	NA	0
E2	52	151	0.72	25	25	0	0	0	NA	4
E4	53	0	0	11	11	0	0	0	NA	0
E4	55	500	2.37	25	24	0	1	4	3	8
C1a	77	163	0.76	25	25	0	0	0	NA	28
C2	89	189	0.91	25	24	0	1	4	5	12
C8	120	478	2.3	25	24	0	1	4	4	12
C8	121	405	1.93	25	23	1	1	8	3	24
C9	126	915	4.17	25	24	0	1	4	3	8
C9	128	502	2.43	25	25	0	0	0	NA	0
E4	137	2	0.01	4	4	0	0	0	NA	0
E4	138	42	0.2	24	24	0	0	0	NA	29
C5a	T1	78	0.38	25	25	0	0	0	NA	12
BK	T10	28	0.13	25	25	0	0	0	NA	0
E4	T11	24	0.11	25	25	0	0	0	NA	0
E4	T12	332	1.6	25	23	1	1	8	3	16
B1	T2	239	1.14	25	25	0	0	0	NA	0
E2	T3	188	0.88	25	23	1	1	8	3.5	20
E2	T4	174	0.83	25	25	0	0	0	NA	4
C8	T5	633	3.03	25	25	0	0	0	NA	8
BK	T6	41	0.2	25	25	0	0	0	NA	8
C2	T7	400	1.9	25	23	0	2	8	3	12
C3	T8	406	1.93	24	22	1	1	8.3	2	17
C5	T9	819	3.9	25	25	0	0	0	NA	0