



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 2019

New Zealand Fisheries Assessment Report 2020/11

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ISSN 1179-5352 (online)
ISBN 978-1-99-001773-5 (online)

April 2020



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EXECUTIVE SUMMARY

Michael, K.P.; Bilewitch, J.; Forman, J.; Hulston, D.; Moss, G. (2020). A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU 5) and the status of Bonamia (*Bonamia exitiosa*) in February 2019.

New Zealand Fisheries Assessment Report 2020/11. 74 p.

Stock assessments of Foveaux Strait oysters (OYU 5) are undertaken five-yearly. The last stock assessment survey was in 2017. Two-phase, random stratified stock assessment surveys have been undertaken since 1990. Since 1999, these surveys have sampled the same survey area (1054 km²) using the same methods and similar strata to ensure data from these surveys are comparable. An additional stratum (16 km²) was introduced by oyster skippers in 2007 and since then, the size of survey area has remained at 1070 km². 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 relatively high (commercial), moderate, and low oyster densities. Since 2012, 26 survey strata have been sampled for stock assessments. Annual, smaller surveys (Bonamia surveys) that focus sampling effort in the core commercial fishery area are undertaken in the years between stock assessments. The Bonamia survey area represents 14 of the 26 stock assessment survey strata (46% of the area), and 75% and 69% of the recruit-sized oyster population in 2012 and 2017 respectively. To allow Bonamia survey data to be incorporated into stock assessments, the remaining 12 stock assessment strata are combined into a single background, 15th stratum, thereby sampling the whole stock assessment area. These February surveys provide a “weather forecast” immediately before the oyster season begins in March. Bonamia surveys update information on:

- Oyster densities and population sizes of four size groups of oysters. Fishers high-grade their catches (return the smaller oysters that are above minimum legal size) to maximise the numbers of first grade oysters. “Commercial”-sized oysters were estimated for the first time in 2019 and represent the size group retained by fishers. “Commercial”-sized oysters are included in counts of recruit-sized oysters. Individual oysters were allocated to size groups based on their ability to pass through three standard rings with internal diameters of 65 mm, 58 mm, and 50 mm:
 - “Commercial”-sized oysters, ≥ 65 mm
 - Recruit-sized (minimum legal size) oysters, ≥ 58 mm
 - Pre-recruit oysters, ≥ 50 mm to 57 mm
 - Small oysters, 49 mm down to 10 mm in diameter.
- the status of *Bonamia exitiosa* infection (prevalence and intensity)
- estimates of disease mortality (from *B. exitiosa*) in the commercial fishery areas.

Together with estimates of recruitment from spat monitoring, catch sampling and survey estimates for small oysters, these data are important to better predict the future status of the fishery.

The February 2019 survey was undertaken in collaboration with the Bluff Oyster Management Company Ltd who provided a vessel, the survey dredge, and crews for the survey. Dredge sampling was consistent with previous surveys and survey data are comparable with others in the time series. Since 2013, testing for *B. exitiosa* infection used two methods to allow the time series of infection data from heart imprints recorded since 1986 to be adjusted for the higher levels of detection provided by polymerase chain reaction (PCR) methods. A quantitative PCR (qPCR) method was used between 2013 and 2017. An improved droplet digital PCR (ddPCR) method with a high level of precision and repeatability, superior levels of sensitivity, detection, and cost-effectiveness was used for the first time in 2018.

Population estimates are presented by stratum, and by three fishery areas: the Bonamia survey area (491.8 km²), the background stratum (578.4 km²), and the stock assessment survey area (1070.2 km²). Only five stations were sampled in the background stratum; estimates for the background stratum and the stock assessment survey area should therefore be viewed with caution. The commercial-sized oyster

population in the Bonamia survey area was 318.7 million oysters (95% CI 198.0–500.1). Commercial-sized oysters represented 58.9% of recruit-sized oysters in the Bonamia survey area and 61.9% in the stock assessment survey area. Recruit sized oysters in the Bonamia survey area increased 9.8% to 542.5 million, pre-recruit oysters increased 21.4% to 216.5 million, and small oysters increased 48.3% to 595.8 million in 2019.

Bonamia infection levels have been low since 2016. In 2019, estimates of the mean prevalence of *B. exitiosa* infection in recruit-sized oysters from the Bonamia survey area were 1.4% using heart imprints and 6.4% using ddPCR. Both estimates were similar to 2018 estimates. Bonamia mortality over the summer of 2018–19 in this area was low (2%), comprised of a pre-survey mortality of 0.7% and a post-survey mortality of 1.3%. Non-fatal infections (from heart imprints) represented 0.1% of the recruit-sized population in 2019.

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 likely to be driven by disease mortality from Bonamia and the levels of recruitment. Disease mortality was low in 2019 and is expected to remain low in 2020. Population sizes of the three size groups of oysters continued to increase between 2018 and 2019. Spat monitoring, catch sampling, and the survey data show increased recruitment to the oyster population, and increases in pre-recruit and small oysters will support future increases in recruit-sized oysters. In the medium-term, all the key indicators for the future rebuilding of the OYU 5 fishery are strongly positive.

1. INTRODUCTION

The Foveaux Strait oyster fishery (OYU 5) is a high value, nationally important 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 Fisheries New Zealand 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 done through accurate and frequent monitoring to support responsive management. Additionally, there is a collaborative fishery 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 Ltd (BOMC), customary and recreational fishers, and the then Ministry of Fisheries, now Fisheries New Zealand.

The haplosporidian parasite of flat oysters *Bonamia exitiosa* (Bonamia) was thought to be an endemic disease of Foveaux Strait oysters. A recent study found *B. exitiosa* has a broad geographic distribution and infects a number of oyster species (Hill-Spanik et al. 2015). Three Bonamia epizootics since 1985 (Doonan et al. 1994, Cranfield et al. 2005, and Michael et al. 2019b) have shown that mortality from Bonamia is oyster density dependent and a recurrent feature of the oyster population. This mortality is the principal driver of oyster population abundance during epizootics and recurrent mortality 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 (assuming near long-term average recruitment). Management of the fishery also recognises 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 by Michael et al. (2015a).

Since 2000, OYU 5 research has been directed by strategic research plans (Andrew et al. 2000, Michael & Dunn 2005, Michael 2010). In 2010, a strategic research plan (SRP) 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. An update of the SRP is overdue.

Two-phase, random stratified stock assessment surveys have been undertaken since 1990 (Cranfield et al. 1991, Fu et al. 2016). OYU 5 stock assessments, oyster surveys, and Bonamia surveys since 1999 are summarised by Michael et al. (2016). Since 1999, these surveys have sampled the same survey area (1054 km²) using the same methods and similar strata to ensure data from these surveys are comparable. An additional stratum (B1a, 16 km², Figure 1) was introduced by oyster skippers in 2007. Since then, the size of the Foveaux Strait oyster survey area has remained at 1070 km². Some of the original 1999 strata have been subdivided at various times to better define the areas with relatively high (commercial), moderate, and low oyster densities. Since 2012, 26 survey strata have been consistently sampled for stock assessments. Annual, smaller surveys (Bonamia surveys) that focus sampling effort in the core commercial fishery area are undertaken in the years between stock assessments (Michael et al. 2015b). The Bonamia survey area represents 14 of the 26 stock assessment survey strata (46% of the area) and 75% and 69% of the recruit-sized oyster population in 2012 and 2017 respectively. To allow Bonamia survey data to be incorporated into stock assessments, the remaining 12 stock assessment strata are combined into a single background, 15th stratum, thereby sampling the whole stock assessment area. The background stratum receives limited sampling effort.

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 oyster densities and population sizes of four size groups of live oysters and recruit-sized and pre-recruit new and old clocks (see Table 2 below and Michael et al. 2016 for definitions):

- “Commercial”-sized oysters ≥ 65 mm
- Recruit-sized (minimum legal size) oysters ≥ 58 mm
- Pre-recruit oysters ≥ 50 mm to 57 mm
- Small oysters 49 mm down to 10 mm in diameter.

Clocks are the articulated shells of recently dead oysters with the ligament attaching the two valves intact. In February surveys, new clocks are assumed to be oysters that have died since summer mortality from *Bonamia* began, and oysters that died up to three years before this are categorised as old clocks.

Survey data provide a useful forecast for the following season because oyster density and meat quality in localised high-density populations 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. 2015b). 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 thereby represents the core commercial fishery that has been consistent through the fluctuations in relative oyster abundance driven by *Bonamia* mortality. 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 stock assessment survey area from an additional 5 stations in the background stratum (Michael et al. 2015b). Surveys since have achieved CVs of 8%, 9%, 11%, and 11% for recruit-sized oysters in the *Bonamia* survey area in 2015, 2016, 2017, and 2018 respectively. In the stock assessment survey area, CVs of 9%, 7%, 9%, and 27% were achieved in 2015, 2016, 2017, and 2018 respectively (Michael et al. 2019a). These low coefficients of variation for population estimates are well below the 20% target set by Fisheries New Zealand for stock assessment surveys.

The current TACC for OYU 5 is 15 million oysters. At relatively low levels of catch (less than 30 million oysters per year), the trend in the abundance of recruit-sized oysters in the Foveaux Strait fishery is driven by disease mortality from *Bonamia* and the levels of recruitment to the population (spat settlement). Oyster spat settlement was low between the summers of 2009–10 and 2015–16 (see Figure 12) despite the population size of spawning-sized oyster densities increasing until 2012 (see Figure 29). Consequently, the numbers of small and pre-recruit oysters declined markedly and were unable to replace the large numbers of oysters killed by *Bonamia*. 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 the *Bonamia* mortality and low recruitment. The increased *Bonamia* mortality between 2013 and 2016 (200 million oysters between 2012 and 2014), and the continued low replenishment of spat to the oyster population, and pre-recruit sized oysters to the fishery, had resulted in a substantial decline in the recruit-sized oyster population. All sized groups of oysters declined between 2012 and 2017 and increased between 2017 and 2018 (Table 1).

Table 1: Mean population estimates for recruit-sized, pre-recruit, and small oysters in the Bonamia survey area and the stock assessment survey area in 2012, 2017, and 2018. Commercial-sized oyster populations were not estimated. The percentage decrease in mean population size between 2012 and 2017 (shaded tan) caused by Bonamia mortality, and the percentage increase in mean population size between 2017 and 2018 (shaded green) are also given. Estimates of mean population size from the stock assessment survey area in 2018 should be viewed with caution because of the limited sampling in the large background stratum.

	Mean population size (millions)			% decrease 2012-2017	% increase 2017-2018
	2012	2017	2018		
Oyster size: Bonamia survey area					
Commercial	NA	NA	NA	NA	NA
Recruit	688.1	363.6	494.1	47.2	35.9
Pre-recruit	297.4	123.1	178.4	58.6	44.9
Small	451.3	261.9	401.8	42.0	53.4
Oyster size: Stock assessment survey area					
Commercial	NA	NA	NA	NA	NA
Recruit	918.4	527.4	883.3	42.6	67.5
Pre-recruit	414.3	168.2	225.8	59.4	34.2
Small	612.2	361.6	552.5	40.9	52.8

The use of droplet digital polymerase chain reaction (ddPCR) since 2018 has improved the detection of low levels of Bonamia infection. Bonamia mortality has been low (less than 5%) since 2016, as has been the prevalence of fatal infections and non-fatal infections. The low oyster densities and low non-fatal infections suggest reduced transmission of Bonamia infection.

This report provides a summary of information from the fifth of the new series of Foveaux Strait oyster surveys in the Bonamia survey area undertaken in February 2019. 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 OYU 5 stock assessment. This survey was undertaken as part of the research for Ministry for Primary Industries programme OYS2017/01 (Objectives 1–4).

1.1 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.

1.1.1 Contracted Objectives (2018–2020)

1. Using a stratified random sampling design estimate the current recruited abundance and biomass of oysters within the area of the commercial Foveaux Strait oyster fishery with a target CV of $\leq 20\%$.
2. Using a stratified random sampling design estimate the annual mortality from Bonamia within the area of the commercial Foveaux Strait oyster fishery.
3. Using a stratified random sampling design estimate the prevalence and intensity of Bonamia within the area of the commercial Foveaux Strait oyster fishery.
4. Review all ddPCR procedures prior to undertaking any analysis of tissue samples at the beginning of each year's fishing survey.

1.1.2 Specific Objectives for the February 2019 survey

- Estimate oyster density and population size for four size groups (commercial, recruit, pre-recruit and small size) in the Bonamia survey area, the background stratum and the stock assessment survey area.
- Estimate the prevalence and intensity of *Bonamia exitiosa* (Bonamia) infection in recruit-sized oysters using droplet digital polymerase chain reaction (ddPCR) and heart imprints to maintain the long time series of infection data.
- Undertake pre-testing checks on all ddPCR procedures and reagents.
- Estimate summer mortality combining two different estimates of mortality:
 - Pre-survey mortality, the population size of recruit-sized new clocks and gapers
 - Projections of post-survey mortality from oysters with fatal infections (category 3–5 infections).

2. METHODS

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

2.1 Survey methods

Survey strata for the February 2019 survey were the same as for February Bonamia surveys since 2014 (Figure 1). The 2017 stock assessment survey sampled all the 26 strata (Michael et al. 2019b). The inclusion of a single large background stratum (see Figure 1) for Bonamia surveys ensures that the entire stock assessment survey area is sampled, and that data from these annual surveys can be included in future stock assessments for OYU 5.

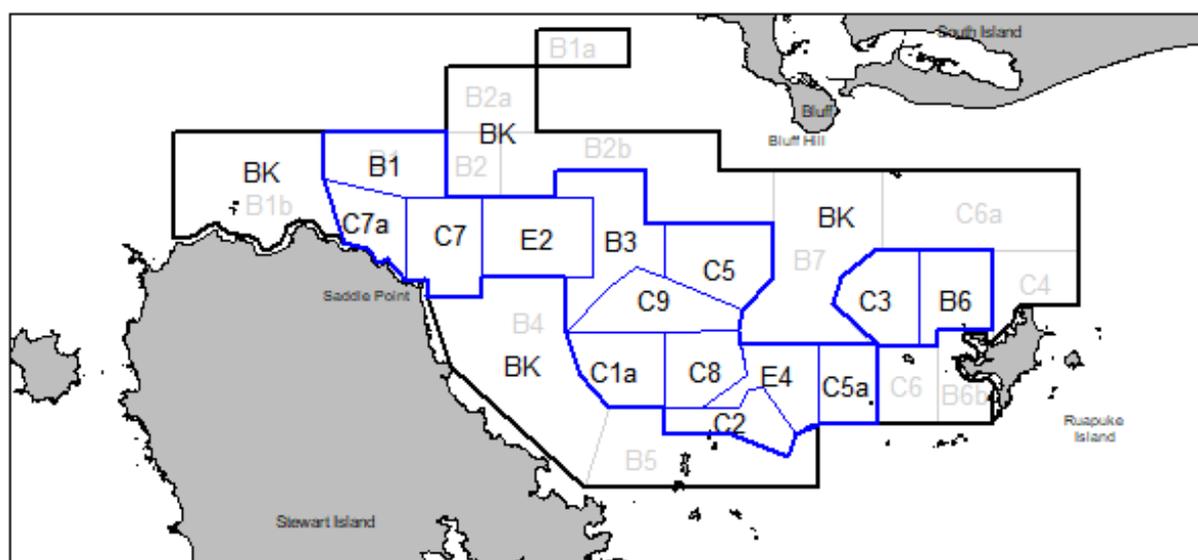


Figure 1: The 2019 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2019 survey strata representing the core commercial fishery area (Bonamia survey area) shown as blue lines. Strata are labelled with grey text. The remaining stock assessment survey strata which represent 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 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 2019

(Table 2). Rand_Stn (Doonan & Rasmussen 2012) was used to generate the location of 50 random first-phase stations 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. 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.

Since 2007, increasing numbers of fixed stations have been sampled annually across the stock assessment survey area to provide a time series of data on changes in oyster density and Bonamia status in localised areas. In 2015, Fisheries New Zealand (then MPI) Shellfish Working Group agreed data from the 12 fixed stations add value to the information obtained from surveys. These 12 fixed stations were sampled in February 2019 (see Table 2 and Figure 2).

Catch sampling

Except for minor variations, dredge sampling has followed standard procedures for stock assessment and Bonamia surveys since October 2002: 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. Details of the standard procedures are given by Michael et al. (2015a). Two commercial oyster vessels have been used for surveys since 1999, F.V. *Golden Lea* 1999–2010 and F.V. *Golden Quest* 2011–2019, except in 2016 when the F.V. *Golden Lea* was used due to the unavailability of the F.V. *Golden Quest*. Stephen Hawke has skippered the survey vessel since 2011, to maintain consistency in the time series. 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. A traditional friction winch used to deploy the dredge on F.V. *Golden Quest* was replaced with a hydraulic winch system in 2014.

The catch from each tow (one per station) 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. Until 2019, live oysters from the catch were sorted in three size groups (Table 2). In 2019 a fourth commercial size was recorded (Table 2). Reference rings (65 mm, 58 mm, and 50 mm internal diameter) were used to ensure accurate allocation to each size group.

Table 2: In 2019, individual oysters were allocated to four size groups based on their ability to pass through three ring sizes with internal diameters of 65 mm, 58 mm, and 50 mm. Commercial-sized oysters were above the median size of oysters landed in the commercial catch. Small oysters were those that passed through a 50-mm ring, down to a size of about 10 mm in length estimated visually (*).

Oyster size	Upper ring limit (mm) (pass through)	Lower ring limit (mm) (unable to pass)
Commercial	NA	65
Recruit	65	58
Pre-recruit	58	50
Small	50	10*

Clocks and gapers were recorded in two size groups, recruits and pre-recruits. Clocks were further divided into two categories, new and old (see Michael et al. 2015a, figures 8–10). In February surveys, new clocks were defined as those with clean inner valves that had retained their lustre, but may have had some minor speckling from fouling organisms. The analysis assumes that new clocks were only those oysters that had died since summer mortality from Bonamia began. Oysters that died before this were categorised as old clocks and these oysters had shells that were fouled or in which the inner valves had lost their lustre. Old clocks can be covered in fouling organisms on both external and internal surfaces, and because the ligaments of oysters are thought to break down over about a three-year period, old clocks represent oysters that had died between 1 and 3 years previously (Cranfield et al. 1991). The

classification of old clocks may vary depending on habitat. Old clocks from sand habitats may be older because they may be filled with sand preventing the settlement of fouling organisms and they may be exposed to lower physical forces on the hinge that prolong the time that both valves remain attached to beyond three years. Gravel habitats are usually shallower with stronger tidal currents and higher swell energy and the valves of old clocks there may be disconnected much more quickly than three years, or the clocks (new and old) may be transported out of the fishery area by the strong tides.

The data recorded at each station included start and finish locations of the tow, depth, speed of tow; numbers of oysters, new clocks, and gapers caught by size group; percentage fullness of the dredge; wind force (Beaufort scale); stations where live bryozoans (*Cinctipora elegans*) were observed; and sediment type (see Appendix 1). The presence/absence of bycatch species was also recorded directly from the dredge contents. An example of the station data form is shown in Appendix 1 (see Michael et al. 2015a for details).

2.2 Estimates of oyster densities and population size

Oyster densities and population sizes for the four 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 stock assessment survey area. Estimates are presented by core strata where three or more randomly selected stations were sampled in February 2019 and these were compared with the estimates from strata sampled in 2016–2018 (Michael et al. 2016, 2019a, 2019b). Estimates for the four 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. 2015b) are given for comparison.

Estimates of absolute abundance and variance were calculated using standard stratified random sampling theory (Francis 1984, Jolly & Hampton 1990). The estimate of dredge efficiency of 0.17 (95% confidence intervals 0.13–0.22) from Dunn (2005) was used as a single scalar. The absolute population size of recruit, pre-recruit, and small oysters, and clocks, was calculated by 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 (m²), 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 bootstrapping, i.e., resampling with replacement of a normal distribution for which the variance is based on a CV of the population estimate and the error of the estimated dredge efficiency. The total error of the estimates of the population mean has two sources:

- the sampling error from the survey, where the survey estimate of population size follows a normal distribution and is based on standard survey sampling theory; and,

- the 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 summarised 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.

2.3 Methods to estimate the annual mortality from Bonamia

Although substantial winter mortality from Bonamia has occurred previously (Hine 1991), most mortality from *B. exitiosa* occurs in the summer. Summer mortality of recruit-sized oysters only is estimated by Bonamia surveys. 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 during the 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. Although estimates of 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 2.2). Post-survey mortality used the mean proportion of oysters with fatal infections (category 3–5 infections, see Diggles et al. 2003) in each stratum as a correction factor, i.e., $1 - \text{mean proportion of category 3–5 infections}$. 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. Estimates of fatally infected oysters by stratum and for the total population were made using scaled-up numbers of fatally infected oysters at each station and the same method used to estimate population size in section 2.2.

2.4 Methods to estimate the prevalence and intensity of Bonamia infection

Samples of up to 30 randomly selected recruit-sized oysters from each station were flown to the National Institute of Water and Atmospheric Research (NIWA) Wellington for *Bonamia exitiosa* testing. Oysters were generally processed the following day. A subsample of up to 25 recruit-sized oysters from each station was taken for heart imprints and droplet digital polymerase chain reaction (ddPCR) analysis to estimate the prevalence and intensity of Bonamia. For each sample, station and sample data were recorded on Bonamia sampling forms (Appendix 2 and Appendix 3 give an example and details). Data on size, general condition, and whether oysters were incubating larvae were recorded (see Appendix 3). Histological samples were taken from the first five oysters processed for heart imprints at each station.

Estimating prevalence and intensity of *Bonamia exitiosa* infection

Since 2013, testing for *B. exitiosa* infection used two methods to allow the time series of infection data from heart imprints recorded since 1986 to be adjusted for the higher levels of detection provided by polymerase chain reaction (PCR) methods. A quantitative PCR (qPCR) method was used between 2013 and 2017. An improved droplet digital PCR (ddPCR) method with a high level of precision and repeatability, superior levels of sensitivity, detection, and cost-effectiveness was used for the first time in 2018.

Prevalence of infection at each station is the proportion of the total sample number that tested positive for Bonamia infection using heart imprints and ddPCR (Bilewitch et al. 2018). The intensity of *B. exitiosa* infection was estimated using heart imprints and ddPCR. These estimates are not directly comparable because heart imprints score the numbers of *B. exitiosa* parasites in haemocytes using the methods of Diggles et al. (2003) and ddPCR estimates the numbers of *B. exitiosa* gene copies in the sample (see Appendix 3 for details). However, there is a good relationship between the increasing intensity of infection shown by heart imprints and an increase in the ratio of *B. exitiosa* DNA to *Ostrea chilensis* DNA (relative infection levels) in ddPCR samples (see Figure A3.3, Appendix 3).

Review of ddPCR procedures prior to testing and repeat testing

Before the samples from the 2019 survey were analysed, quality control of reagents and methods was undertaken (details in Appendix 3). Each 96-well plate tested included positive and negative controls. Reactions with less than 10^3 total droplets were repeated. 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. Each oyster sample determined: (1) whether Bonamia was present (within the limit of detection for ddPCR) and (2) the relative level of infection – this being directly comparable with heart imprint scores determined via histology. 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 (see Appendix 3 for details).

ddPCR testing

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 that tested positive by ddPCR were also examined to estimate prevalence. Oysters that tested negative for Bonamia using ddPCR analysis were assumed to also be negative for heart imprints. A randomly selected subsample of samples that tested negative by ddPCR were also 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 Bonamia and stock assessment survey areas.

A detailed account of the ddPCR method and testing is given by 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.

Heart imprints

The categorical score from heart imprints (see Table A3.1, Appendix 3) assumes that category 0 oysters are not infected. Previous studies (Diggles et al. 2003) suggested that stages 1 and 2 Bonamia infections are relatively light and do not appear to adversely affect the host, i.e., they are non-fatal. Stage 3 infections are much more elevated and systemic and are associated with minor tissue damage throughout the host. It is likely that stage 3 infections will almost always progress to stage 4 (Diggles et al. 2003). Stage 4 infections are systemic, and all tissues are congested with infected haemocytes; death appears inevitable. Stage 5 infections differ from those of stage 4 in that tissue damage is extreme throughout the animal, tissues have lost their integrity, and the oyster is near death. Stages 3–5 Bonamia infections represent fatal infections.

Relative infection levels from ddPCR increase slowly at heart imprint scores 1 and 2. At intensifying levels of infection (heart imprint score 3) and fatal infections (scores 4 & 5), the relative level of infection from ddPCR increases rapidly (see Figure A3.3, Appendix 3).

Mean intensity estimated from heart imprints is the mean frequency of stages 1–5 oysters (i.e., the mean stage of all oysters examined that had at least one Bonamia cell observed). Exact 95% confidence intervals are given for prevalence, determined from the *F*-distribution, i.e., for a proportion π , where

$\pi = r/n$ (where r is the number of oysters infected with *Bonamia* and n the number of oysters in the sample), the 95% confidence interval is determined by:

from heart imprint samples only

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

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

Population estimates of non-fatal and fatal *Bonamia* infection

Two methods were used to scale fatal and non-fatal infections to population estimates for recruit-sized oysters only, following the procedures in section 2.2. These estimates are presented by stratum, for the *Bonamia* survey area and stock assessment survey area. Method 1 used a correction factor from strata with three or more randomly selected stations only, i.e., target stations were not included. Method 2 used the total numbers of oysters in each *Bonamia* infection category (1–5) based on the estimated proportion of oysters in each infection category in the sample, and scaled to the total catch for each station. The overall intensity was calculated as the average *Bonamia* level in the population. Variance for prevalence and intensity were estimated using standard methods as for population estimates.

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

Under the new management plan for OYU 5, stock assessments are carried out five-yearly, with annual population and *Bonamia* surveys between assessments. The last assessment was completed in 2017 (Large et al. 2018a) 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% 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. The 2012 stock assessment based on a TACC of 15 million oysters predicted the population size to remain similar or decline by 23% with 10% and 20% mortality respectively. The recruit-sized population decreased by 44.5% in 2015 (509.9 million oysters), more than expected because recruitment had been very low since 2010.

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 previously used to select the most appropriate projection, the population estimates predicted were similar to the estimates of population size from subsequent surveys.

3. RESULTS

Sea conditions were good for sampling during the survey and tides were mostly swift spring tides. Observations from the survey suggest little pre-survey mortality (few new clocks), including in the eastern fishery areas where mortality has historically been 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 in 2019; the same percentage as in 2018. There was good growth in some areas, facilitating an increase in recruit-sized oysters. Many of these oysters were legal-sized, but not yet commercial-sized. Many more spat and 1–3 year-old oysters were observed on oysters in some areas in 2019.

Dredge efficiency is thought to be greatly reduced in areas densely populated with kāeo (*Pyura pachydermatina*) because the dredge skims above the seabed with little or no contact. Large numbers of kāeo and very few oysters were caught in stratum E4 (station 49), and stratum C5a (stations 24 and T1). Oyster density was most likely underestimated at these stations.

The efficiency of dredge sampling during the 2019 survey was consistent with previous surveys. Dredge tow lengths were almost all about 0.2 nautical miles (371 m) in length. Wind speeds were less than 10 knots, and sea conditions and dredge saturation were similar to previous surveys (Appendix 4, Figures A4.1–A4.3).

3.1 Survey operational detail

NIWA and the BOMC staff began the survey on the 6th of February 2019 and finished on the 12th of February, sampling on six days during this period. The oyster vessel F.V. *Golden Quest* successfully sampled all 72 stations (Table 3). The locations of survey tows are shown in Figure 2, and the numbers of stations sampled in each stratum are given in Table 3. A few allocated stations couldn't be sampled because of rough ground; first-phase stations 17, 25, 28, 51, and 55 were replaced by stations 84, 104, 98, 140, and 144 respectively. A number of second-phase stations also couldn't be sampled: 141, 142, and 143.

Twenty-five 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 67 of the 72 stations. For samples with fewer than 25 recruit-sized oysters, samples included pre-recruit and small oysters: stations 24 ($n=25$, small only), 48 ($n=25$, small only), 53 ($n=21$), 12 ($n=18$), and 2 ($n=13$). Oyster samples were couriered to NIWA 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 3: The numbers of first-phase, second-phase, and fixed stations sampled in each stratum during the February 2019 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	4	3		44.7
B6	3			30.1
B6a	5		2	*
BK	3			578.3
C1a	3			31.3
C2	3		1	21.9
C3	3		1	32.7
C5	3	2	1	37.7
C5a	4		1	23.5
C7	3			36.1
C7a	4			23.6
C8	6		1	26.8
C9	4			34.5
E2	3		2	42.8
E4	4		2	28.0
Totals	55	5	12	1 070.2

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

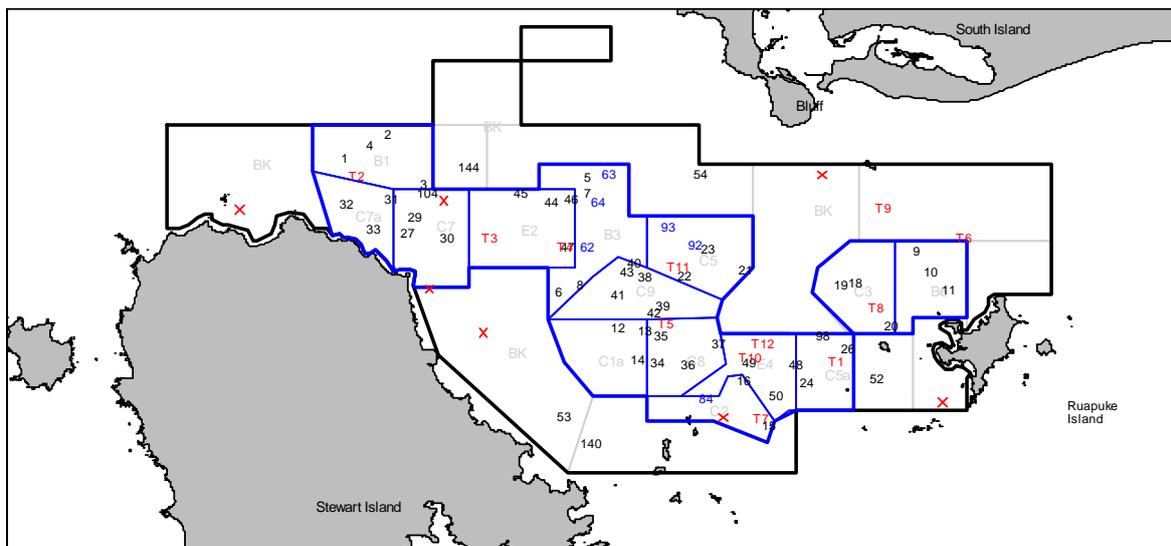


Figure 2: The 2019 survey area with the 2007 survey boundary shown as a heavy, black outer line, the Bonamia survey area as a heavy blue line, and the 2019 Bonamia survey strata shown as blue lines. The remaining stock assessment survey strata (light grey lines) in the large background stratum were merged into a single stratum (BK). First-phase station numbers are in black text, second-phase in blue, and fixed stations in red text. Red crosses denote stations that couldn't be towed because of foul ground.

3.2 Oyster abundance

Changes in oyster densities between 2016 and 2019

Plots of catches adjusted to the standard tow length (0.2 nautical miles) from the 2016, 2017, 2018, and 2019 surveys for recruit-sized, pre-recruit, and small oysters, and 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 were spatially patchy. Low catches of all three size groups of oysters in strata C5a and E4 may be due to reduced dredge efficiency caused by dense stands of kāeo. Catches of these size groups in the background stratum (BK) were similar or lower.

At stratum level, catches of recruit-sized oysters generally varied by region across the fishery area (Figure 3). Strata north of a line between Saddle Point (Stewart Island) and North Head (Ruapuke Island) (B1, C7, C7a, E2, B3, C5, C3, and B6) had similar or higher catches in 2019 than in 2016–2018 and high catches from individual sample tows in 2019. Some strata south of this line (C9, C1a, C2, and E4) had lower catches in 2019, whereas another (C8) had highly variable catches (Figure 3).

Catches of pre-recruit-sized oysters in 2019 were generally more variable and higher than in 2016–2018 (Figure 4), especially in strata C7, C7a, E2, B3, C2, C3, C5, and B6. The catches of pre-recruits in strata C1a, C9, E4, C5a and BK remained low between 2016 and 2019 (Figure 4). Relatively high catches of small oysters were patchy in 2019 (Figure 5). At the stratum level, catches were generally similar or higher across the fishery between 2018 and 2019 compared with between 2016 and 2017 (Figure 5). The number of small oysters reflects the trend of increasing recruitment to the population indicated by spat monitoring.

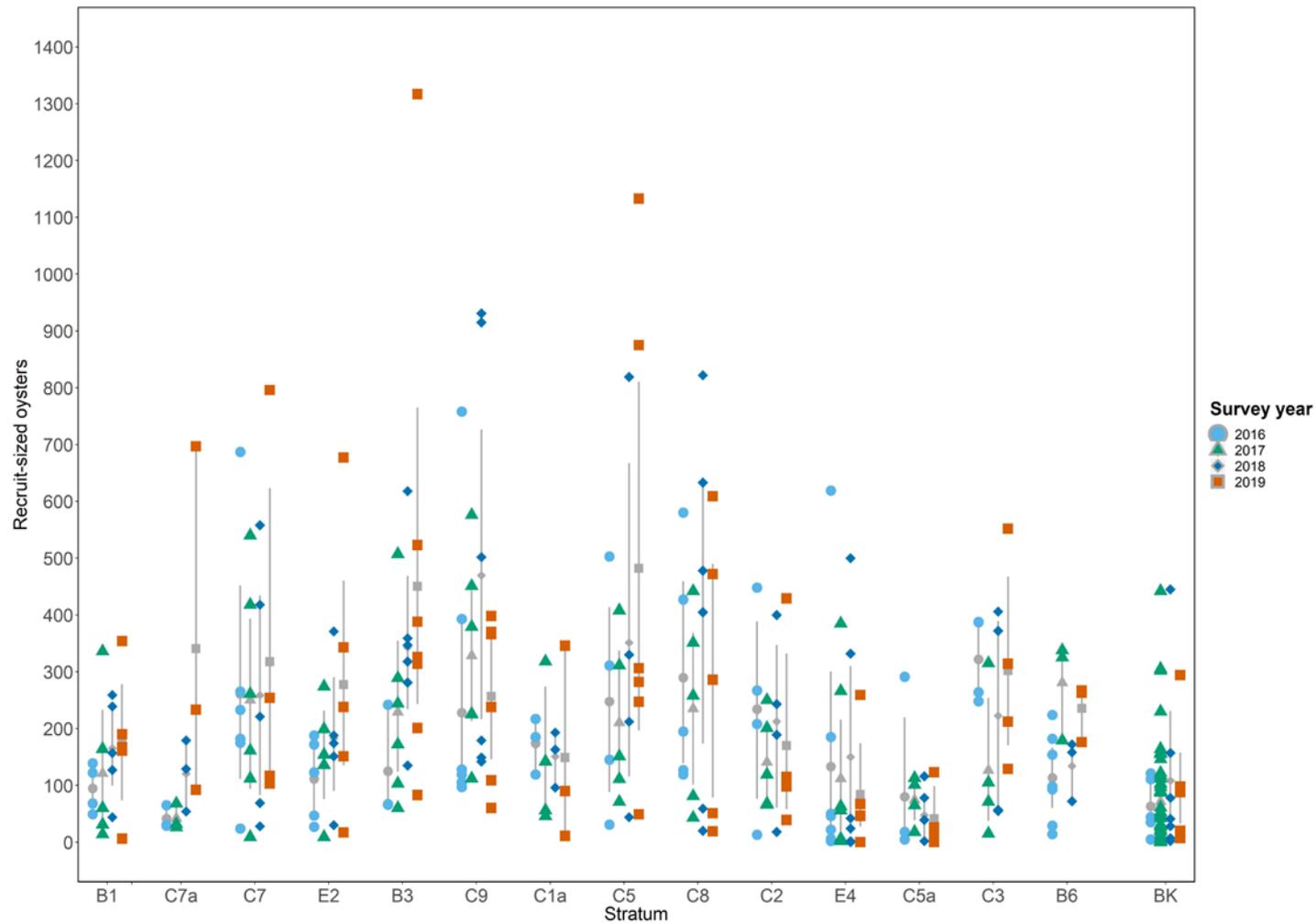


Figure 3: The numbers of recruit-sized oysters (≥ 58 mm in diameter) per tow, means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines) by stratum, for surveys during 2016–2019. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2016 survey are shown as light blue filled circles, 2017 as green filled triangles, 2018 as dark blue filled diamonds, and 2019 as brown filled squares. *Bonamia* survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthest right.

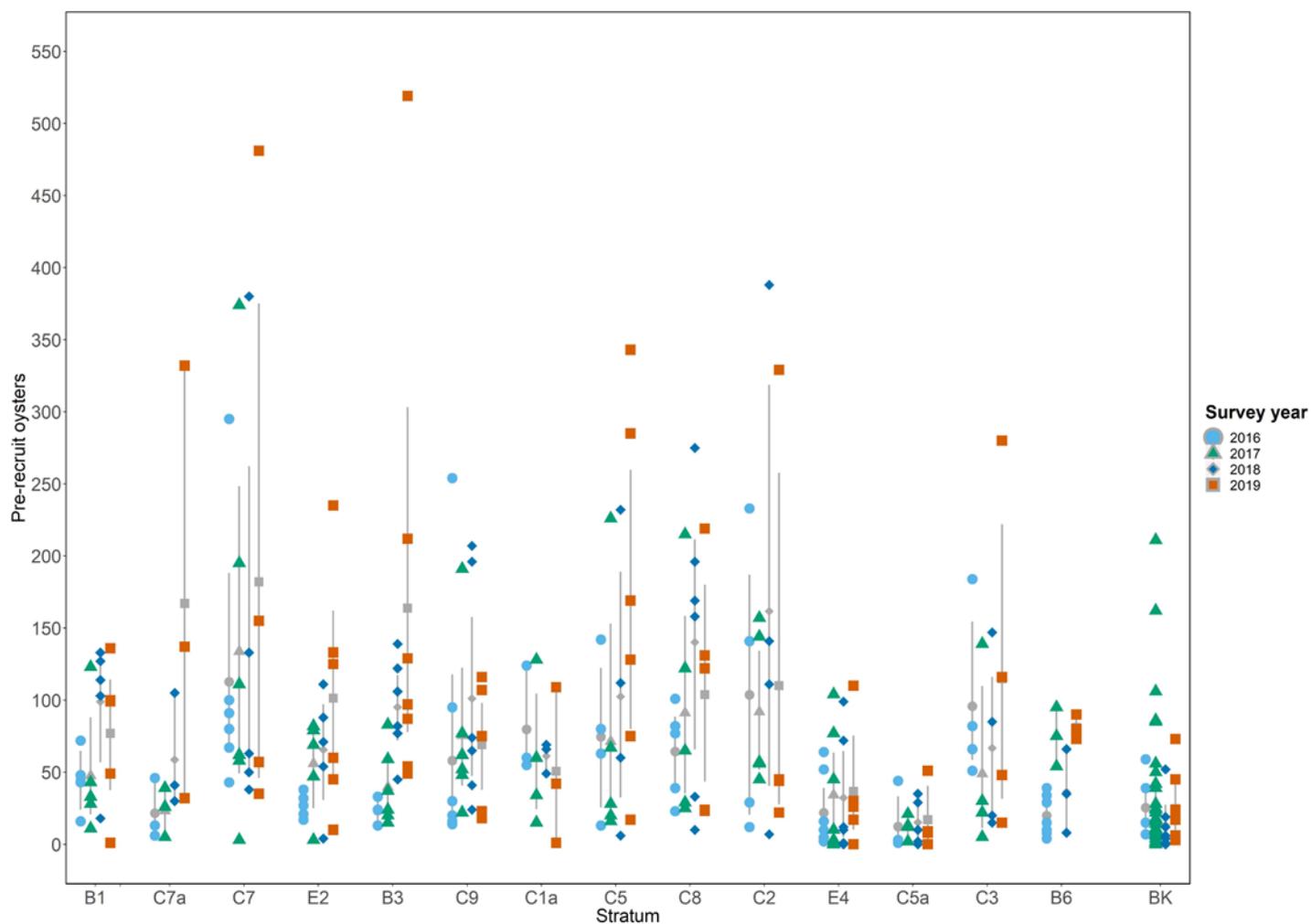


Figure 4: The numbers of pre-recruit- oysters ($\geq 50\text{--}57$ mm in diameter) per tow, means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines) by stratum, for surveys during 2016–2019. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2016 survey are shown as light blue filled circles, 2017 as green filled triangles, 2018 as dark blue filled diamonds, and as 2019 brown filled squares. *Bonamia* survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthest right.

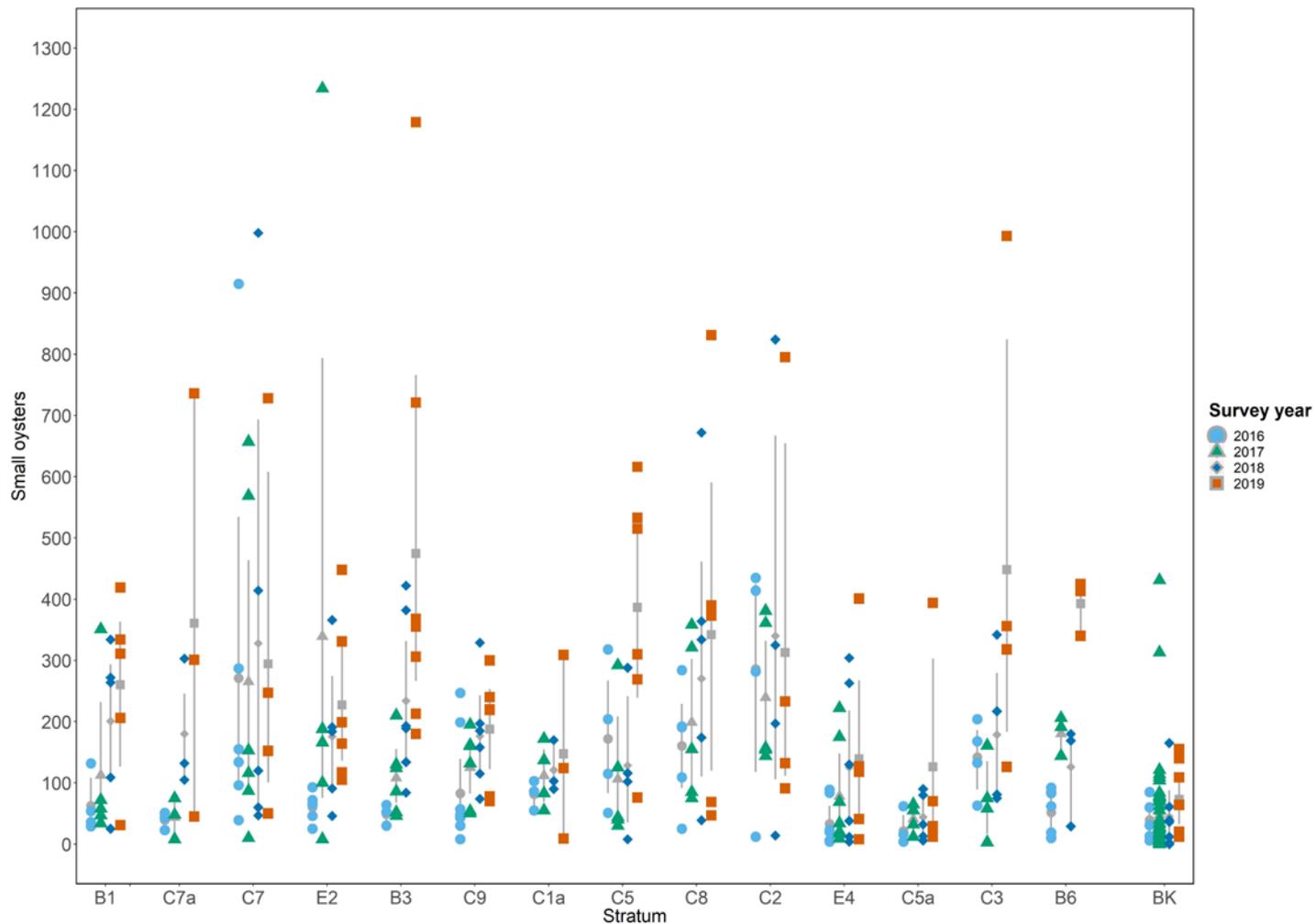


Figure 5: The numbers of small oysters (10–49 mm in diameter) per tow, means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines) by stratum, for surveys during 2016–2019. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2016 survey are shown as light blue filled circles, 2017 as green filled triangles, 2018 as dark blue filled diamonds, and 2019 as brown filled squares. Bonamia survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthest right.

Survey estimates of population size

Estimates of absolute population size for commercial-sized, recruit, pre-recruit, and small oysters from the February 2019 survey are given by stratum in Tables 4–7: 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). The population estimates in the Bonamia survey area for recruit-sized, pre-recruit, and small oysters from the 2012, and 2016–2019 surveys are shown in Table 8, and for 2012, and 2014–2019 in Figure 6. Fisheries Assessment Reports for Foveaux Strait oyster and Bonamia surveys 2010–2018 that provide estimates of oyster density, population size, and CVs for all size groups are referenced in Table A6.1 (Appendix 6).

Comparisons between the population estimates for the background stratum should be made with caution because 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 where the 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.

Fishers high-grade their catches (return the smaller oysters that are above minimum legal size) to maximise the numbers of first grade oysters. The density and population size of commercial-sized oysters were estimated for the first time in 2019 (Table 4) and represent the size group retained by fishers. Of the recruit-sized population, 59% was of commercial size in the core strata and 61.9% in the stock assessment survey area (Tables 4 & 5). Mean density in the core strata was 0.65 oysters m^{-2} and ranged from 0.06 to 1.33 oysters m^{-2} (Table 4). Oyster densities for strata C5a and E4 are likely to be underestimated.

The density and population size of recruit-sized oysters in core strata increased by 9.8% between 2018 and 2019 (Table 5). 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} and increasing further in 2019 to 1.10 oysters m^{-2} (see Tables 5 & 8). Usually an increase in recruit-sized oyster density results in an increased catch rate; however, the large numbers of fast growing and thin legal-sized oysters were 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 51% (0.56 oysters m^{-2}) of that in the Bonamia survey area in 2019 (Table 5), lower than in 2018 (67%, 0.67 oysters m^{-2}), and higher than in 2017 (0.28 oysters m^{-2}).

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, increased to 494.1 million oysters in 2018, and further increased to 542.5 million oysters in 2019. Between 2012 and 2019, data from 55 stations produced CVs for all core strata combined between 8.0% and 13.0%. 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, increased to 389.2 million oysters in 2018, and decreased to 325.5 million oysters in 2019. The CVs have increased from 19.7% in 2012 (stations sampled, $n=62$), to 59.0% in 2018 ($n=5$), but decreased to 17% in 2019. It is not likely that the five stations are a good representation of the size of the oyster population in the background stratum despite the size of the 2019 CV, and caution is advised when population estimates from the stock assessment survey area are compared. Recruit-sized oyster population sizes (and mean densities) increased in half of the strata in 2019 (Table 5). Some estimates may have been affected by the abundance of k̄āeo (which reduced the efficiency of sampling). Increases in population size ranged from about 23% to 183% and decreases ranged from 1% to 72% (Table 5).

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 and 0.44 oysters m^{-2}

in 2019 (Table 6). The population size declined from 297.4 million oysters in 2012 to 89.2 million oysters in 2015 and increased to 216.5 million oysters in 2019. Pre-recruit population size increased by 21.4% between 2018 and 2019 (Table 6). The population size in the background stratum was similar between 2017 and 2018, 45.0 million oysters and 47.4 million oysters respectively, and doubled to 93.4 million oysters in 2019 (Table 6). The population size in the 2007 stock assessment survey area increased by 37.2% from 225.8 million oysters in 2018 to 309.8 million oysters in 2019. Pre-recruit oyster population sizes (and mean densities) increased in half of the strata between 2018 and 2019 (Table 6), the same strata with increases for recruits. Increases in population size ranged from about 21% to 186% and decreases ranged from 17% to 58% (Table 6).

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, 401.8 million oysters in 2018, and a further 48.3 % to 595.8 million oysters in 2019 (Table 7). 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 by 51.2% in 2018 to 150.8 million oysters and further increased by 80.4% to 272.0 million oysters (Table 7). Overall, the population size of small oysters in the stock assessment 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 and further increased to 867.8 million oysters in 2019 (Table 7). Small oyster population sizes (and mean densities) increased in all strata except C2, C7, E2, and E4 (Table 7). Increases in population size ranged from about 7% to 376% and decreases from about 9.5% to 45% (Table 7).

In 1995 and 1997, Cranfield et al. (1999) estimated Current Annual Yield (CAY) from a “commercial population” that reflected the patchy distribution of oyster density. Cranfield et al. (1999) defined the commercial population as the recruited population in the stock assessment survey area above a density of 400 oysters per tow (equivalent to about 6–8 sacks per hour during commercial dredging). This threshold was based on a 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 and the Shellfish Working Group requested that these estimates be included in this report. Table 9 shows estimates of commercial population size, using the catch of recruit-sized oysters at each station minus 400 oysters, for the 2019 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, two in 2015, six in 2016, three in 2017, five in 2018, and six in 2019 (B3, C5, C7, C7a, C8, and E2). 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 and to 0.41 oysters m^{-2} in 2019. In the background stratum, oyster density was 0.02 oysters m^{-2} in 2017, increasing to 0.43 oysters m^{-2} in 2018 and further increasing to 0.56 oysters m^{-2} in 2019.

Table 4: Absolute population estimates for commercial-sized (≥ 65 mm in diameter) oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2019 by stratum. Columns give the mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n), upper and lower 95% confidence intervals (95% CI) in millions of oysters where a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area (km²)) in square kilometres.

Stratum	Mean density	Density s.d.	CV	2019 Pop.n	B.lower 95% CI	B.upper 95% CI	Area (km ²)
B1	0.33	0.11	0.33	25.8	8.7	48.3	78.2
B3	1.33	0.43	0.33	59.5	20.9	110.6	44.7
B6	0.71	0.06	0.08	21.4	14.1	31.9	30.0
C1a	0.46	0.32	0.70	14.5	0.0	37.8	31.3
C2	0.18	0.05	0.28	3.9	1.6	7.1	21.9
C3	0.61	0.12	0.20	19.9	10.5	32.6	32.7
C5	1.09	0.52	0.47	41.1	2.5	89.0	37.7
C5a	0.06	0.03	0.54	1.3	0.0	2.9	23.5
C7	0.76	0.34	0.45	27.3	3.2	57.5	36.1
C7a	0.82	0.45	0.55	19.3	0.0	44.1	23.6
C8	0.52	0.26	0.49	13.8	0.4	30.3	26.8
C9	0.87	0.19	0.22	30.1	15.3	50.9	34.5
E2	0.89	0.45	0.51	38.0	0.0	85.4	42.8
E4	0.10	0.06	0.59	2.7	0.0	6.6	28.0
Core total	0.65	0.08	0.13	318.7	198.0	500.1	491.8
BK	0.38	0.15	0.41	218.2	41.9	442.8	578.4
Survey total	0.50	0.09	0.18	536.9	296.9	869.8	1 070.2

Table 5: Absolute population estimates for recruit-sized oysters (≥ 58 mm in diameter), including commercial sized oysters, in the core strata (Stratum), background stratum (BK), and for the stock assessment survey area (Survey total) sampled in 2019 by stratum. Columns give the mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the 2019 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 2018 mean population estimates (2018 Pop.n) and the 2019 mean population size represented as a percentage of the 2018 mean population size by stratum. The percentage change from the 2018 estimate (% of 2018) is shaded green for increases in population size in 2019 and tan for decreases.

Stratum	Mean density	Density s.d.	CV	2019 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km ²)	2018 Pop.n	% of 2018
B1	0.64	0.20	0.32	49.9	17.2	92.7	78.2	56.1	-11.0
B3	2.19	0.75	0.34	97.8	32.0	184.1	44.7	74.3	+31.7
B6	1.15	0.15	0.13	34.5	21.2	53.4	30.0	19.7	+75.1
C1a	0.73	0.49	0.68	22.7	0.0	58.4	31.3	22.9	-0.8
C2	0.41	0.11	0.27	8.9	3.9	15.8	21.9	16.1	-44.8
C3	1.06	0.26	0.25	34.7	16.2	59.4	32.7	25.8	+34.4
C5	1.73	0.69	0.40	65.1	12.9	132.1	37.7	33.9	+92.0
C5a	0.07	0.04	0.55	1.6	0.0	3.6	23.5	4.5	-65.3
C7	1.54	0.78	0.51	55.7	0.6	123.4	36.1	45.2	+23.3
C7a	1.65	0.90	0.54	39.1	0.0	88.6	23.6	13.8	+183.0
C8	1.01	0.52	0.51	27.1	0.0	60.5	26.8	46.7	-41.9
C9	1.26	0.29	0.23	43.5	21.1	74.5	34.5	78.0	-44.2
E2	1.33	0.70	0.53	56.7	0.0	129.9	42.8	38.6	+47.0
E4	0.19	0.10	0.52	5.2	0.0	11.8	28.0	18.4	-71.8
Core total	1.10	0.14	0.13	542.5	337.0	851.0	491.8	494.1	+9.8
BK	0.56	0.23	0.41	325.5	59.2	664.4	578.4	389.2	-16.4
Survey total	0.81	0.14	0.17	868.0	489.4	1394.7	1070.2	883.3	-1.7

Table 6: Absolute population estimates for pre-recruit (50–57 mm in diameter) oysters in the core strata (Stratum), background stratum (BK), and for the stock assessment survey area (Survey total) sampled in 2019 by stratum. Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (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 2018 mean population estimates (2018 Pop.n) and the 2019 mean population size represented as a percentage of the 2018 mean population size by stratum. The percentage change from the 2018 estimate (% of 2018) is shaded green for increases in population size in 2019 and tan for decreases.

Stratum	Mean density	Density s.d.	CV	2019 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km ²)	2018 Pop.n	% of 2018
B1	0.35	0.14	0.41	27.1	4.9	55.1	78.2	36.5	-25.7
B3	0.80	0.30	0.38	35.6	9.0	69.7	44.7	20.6	+72.9
B6	0.40	0.02	0.06	11.9	7.9	17.5	30.0	5.3	+123.6
C1a	0.25	0.15	0.62	7.7	0.0	19.0	31.3	9.3	-16.9
C2	0.18	0.04	0.20	3.9	2.1	6.5	21.9	9.3	-57.8
C3	0.29	0.14	0.50	9.5	0.1	20.8	32.7	6.4	+48.2
C5	0.72	0.27	0.38	27.1	6.2	54.1	37.7	10.3	+162.8
C5a	0.03	0.01	0.50	0.6	0.0	1.4	23.5	1.3	-50.3
C7	0.88	0.50	0.56	31.9	0.0	74.0	36.1	23.1	+38.3
C7a	0.81	0.43	0.53	19.2	0.0	43.1	23.6	6.7	+186.3
C8	0.37	0.15	0.40	9.8	2.1	19.7	26.8	14.8	-33.5
C9	0.34	0.08	0.24	11.7	5.5	20.2	34.5	16.8	-30.6
E2	0.43	0.25	0.58	18.3	0.0	43.6	42.8	14.2	+29.1
E4	0.08	0.04	0.56	2.1	0.0	5.0	28.0	3.8	-43.7
Core total	0.44	0.07	0.15	216.5	129.6	346.1	491.8	178.4	+21.4
BK	0.16	0.06	0.36	93.2	26.3	180.7	578.4	47.4	+96.6
Survey total	0.29	0.04	0.15	309.8	183.7	484.7	1070.2	225.8	+37.2

Table 7: Absolute population estimates for small (10–57 mm in diameter) oysters in the core strata (Stratum), background stratum (BK), and for the stock assessment survey area (Survey total) sampled in 2019 by stratum. Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (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 2018 mean population estimates (2018 Pop.n) and the 2019 mean population size represented as a percentage of the 2018 mean population size by stratum. The percentage change from the 2018 estimate (% of 2018) is shaded green for increases in population size in 2019 and tan for decreases.

Stratum	Mean density	Density s.d.	CV	2019 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km ²)	2018 Pop.n	% of 2018
B1	1.08	0.33	0.31	84.2	30.4	154.8	78.2	70.1	+20.0
B3	2.31	0.65	0.28	103.0	43.5	183.4	44.7	50.7	+103.2
B6	1.92	0.14	0.07	57.5	38.0	85.3	30.0	18.5	+210.9
C1a	0.72	0.42	0.59	22.5	0.0	54.0	31.3	18.5	+21.4
C2	0.74	0.21	0.28	16.2	6.8	29.1	21.9	19.2	-15.7
C3	1.29	0.35	0.27	42.3	18.5	74.0	32.7	19.9	+112.8
C5	1.67	0.42	0.25	62.8	28.5	110.0	37.7	13.2	+375.7
C5a	0.18	0.08	0.47	4.2	0.4	9.0	23.5	3.7	+14.3
C7	1.43	0.72	0.50	51.7	0.9	114.0	36.1	57.1	-9.5
C7a	1.76	0.99	0.56	41.5	0.0	95.7	23.6	20.6	+101.4
C8	1.08	0.46	0.42	28.8	4.6	59.2	26.8	24.9	+15.7
C9	0.92	0.19	0.20	31.8	16.7	53.1	34.5	29.5	+7.7
E2	0.98	0.24	0.24	41.8	19.7	72.1	42.8	42.2	-1.1
E4	0.27	0.16	0.59	7.6	0.0	18.2	28.0	13.9	-45.3
Core total	1.21	0.12	0.10	595.8	385.4	912.5	491.8	401.8	+48.3
BK	0.47	0.13	0.27	272.0	116.4	481.8	578.4	150.8	+80.4
Survey total	0.81	0.09	0.11	867.8	544.0	1315.4	1070.2	552.5	+57.1

Table 8: Percentage changes in the population size of recruit-sized, pre-recruit, and small oysters in the Bonamia survey area in 2012, 2016–2019. Columns give the mean oyster density per square metre (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, 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
2019	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2018-2019
Recruit	1.10	0.13	542.5	337.0	851.0	+9.8
Pre-recruit	0.44	0.15	216.5	129.6	346.1	+21.4
Small	1.21	0.10	595.8	385.4	912.5	+48.3

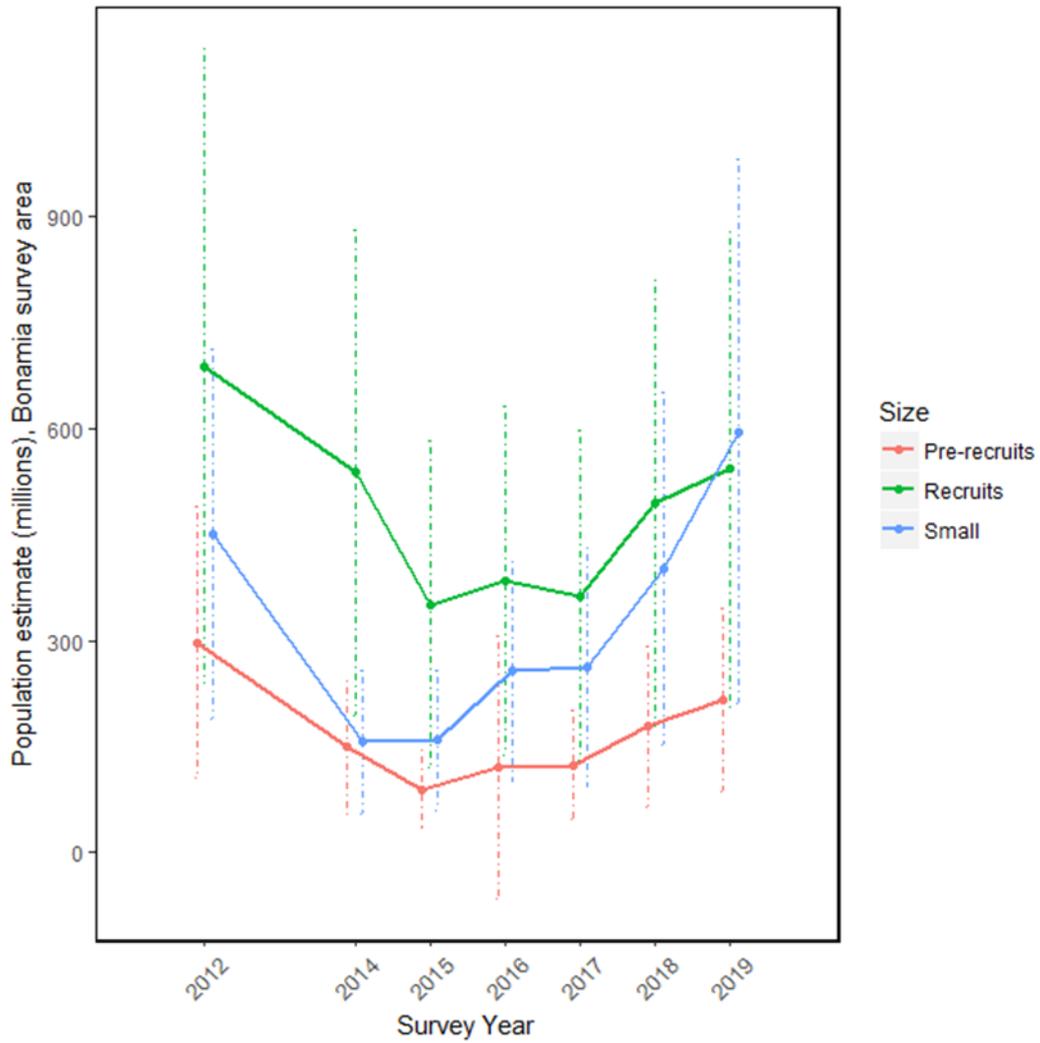


Figure 6: Mean population sizes and bootstrapped 95% confidence intervals for recruit-sized, pre-recruit, and small oysters in the Bonamia survey area between 2012 and 2019.

Table 9: Absolute population estimates for 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 stock assessment survey area (Survey total) sampled in 2019 by stratum. Columns give the mean oyster density per square metre (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 2018 mean population estimates (2018 Pop.n) and the 2019 mean population size represented as a percentage of the 2018 mean population size by stratum. The percentage change from the 2018 estimate (% of 2018) is shaded green for increases in population size in 2019 and tan for decreases.

Stratum	Mean density	Density s.d.	CV	2019 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km ²)	2018 Pop.n	% of 2018
B1	0.00	0.00	0.00	0.0	0.0	0.0	78.2	0.0	-
B3	1.28	0.92	0.72	57.0	0.0	151.1	44.7	22.5	+53.4
B6	0.00	0.00	0.00	0.0	0.0	0.0	30.0	0.0	-
C1a	0.00	0.00	0.00	0.0	0.0	0.0	31.3	0.0	-
C2	0.00	0.00	0.00	0.0	0.0	0.0	21.9	0.0	-
C3	0.00	0.00	0.00	0.0	0.0	0.0	32.7	0.0	-
C5	0.87	0.87	1.00	32.7	0.0	103.0	37.7	0.0	-
C5a	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	0.96	0.96	1.00	34.7	0.0	110.6	36.1	34.0	+2.0
C7a	1.13	1.13	1.00	26.8	0.0	85.9	23.6	0.0	-
C8	0.58	0.58	1.00	15.4	0.0	48.9	26.8	44.6	-65.4
C9	0.00	0.00	0.00	0.0	0.0	0.0	34.5	64.8	0.0
E2	0.83	0.83	1.00	35.5	0.0	113.6	42.8	0.0	-
E4	0.00	0.00	0.00	0.0	0.0	0.0	28.0	16.9	0.0
Core total	0.41	0.16	0.39	202.1	46.7	399.1	491.8	182.9	+10.5
BK	0.56	0.23	0.41	325.5	59.2	664.4	578.4	250.6	+29.9
Survey total	0.81	0.14	0.17	868.0	489.4	1394.7	1070.2	433.4	+100.3

Changes in the distribution of live oysters

The February 2019 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 2019, and hence the survey is not likely to have estimated the distributions of oyster density well for live commercial-sized, recruit, pre-recruit, and small oysters outside the core strata (delimited by a blue line in Figures 7–10). These distributions of oysters are compared with the last Bonamia survey in 2018 which also sampled 72 stations in total, mostly in the commercial fishery area.

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 7–10). At most sites across the core fishery area, the highest densities of recruit-sized oysters comprised substantial proportions of commercial-sized oysters (Figure 7). The numbers of localised areas of relatively high recruit-sized oyster densities continued the increasing trend seen between the 2017 and 2018 surveys, with further increases between 2018 and 2019 (Figure 8), probably the result of relatively low (about 5%) Bonamia mortality over the last two summers.

The densities of pre-recruit oysters are patchily distributed and relatively low (Figure 9 and Table 8). Although densities generally increased throughout the fishery area between 2018 and 2019, densities are still low in some central fishery areas. The highest pre-recruit oyster densities were in strata C7, C7a, B3, C5, and C2 (see Figure 1 and Figure 9). 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 fast-growing small oysters reaching recruit-size in a single summer.

The distribution of small oyster densities (Figure 10) shows similar patterns to recruit-sized and pre-recruit oyster densities. Densities increased markedly across the entire fishery between 2018 and 2019 and small oysters are widespread throughout the fishery, but their distribution is patchy. The few sites sampled in 2018 and again in 2019 showed marked increases in densities. Small oysters are less vulnerable to Bonamia mortality. The increasing densities reflect increased recruitment to the oyster population, consistent with increased spat settlement since 2015.

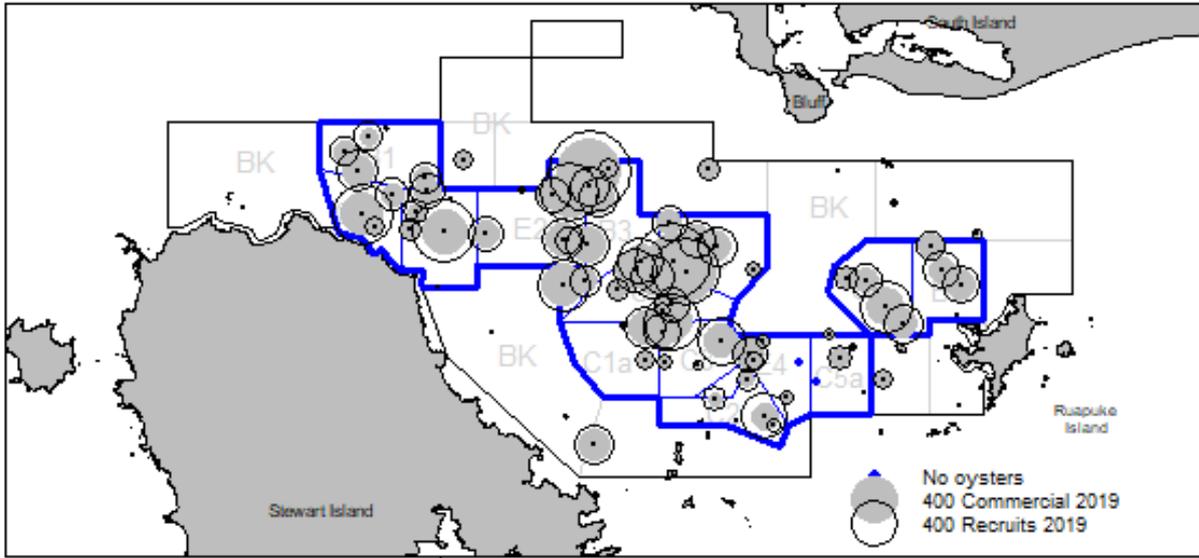


Figure 7: Density (numbers of oysters per standard tow representing an area swept of 1221 m²) of commercial-sized (filled grey circles) and recruit-sized (open black circles) oysters sampled during the February 2019 survey. Blue filled circles denote no oysters caught. The *Bonamia* survey area is shown by the blue lines.

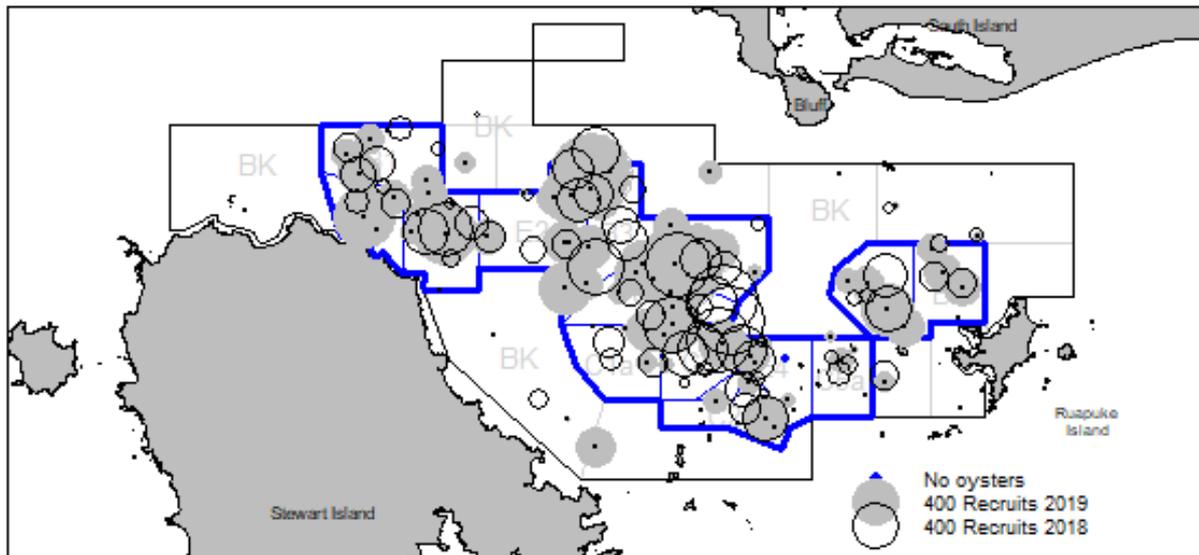


Figure 8: Density (numbers of oysters per standard tow representing an area swept of 1221 m²) of recruit-sized oysters sampled during the February surveys in 2019 (filled grey circles) and in 2018 (open black circles). Blue filled circles denote no oysters caught. The *Bonamia* survey area is shown by the blue lines.

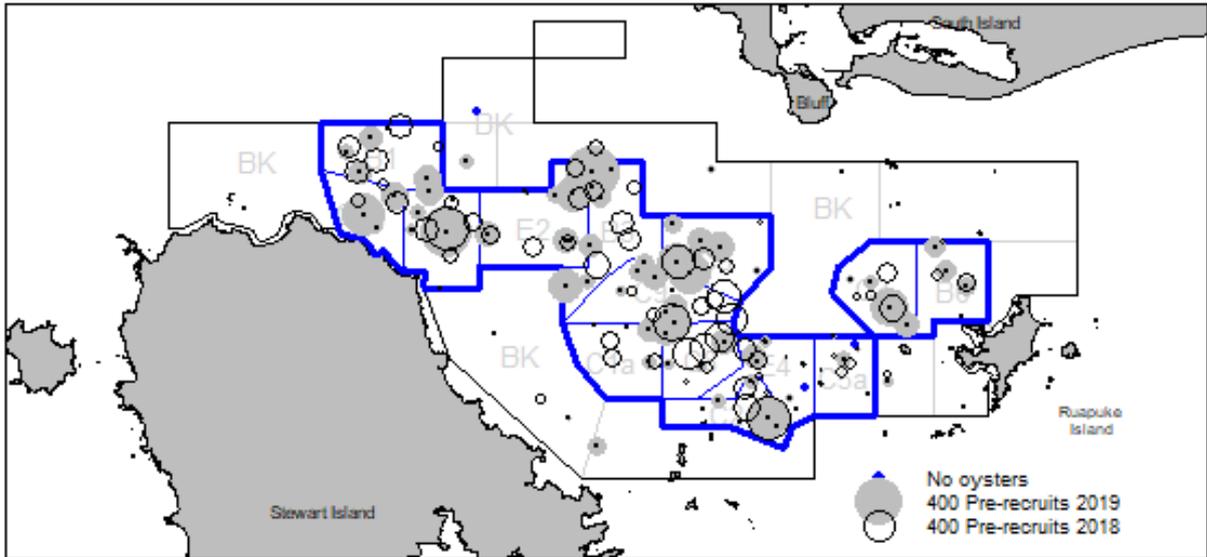


Figure 9: Density (numbers of oysters per standard tow representing an area swept of 1221 m²) of pre-recruit sized oysters sampled during the February surveys in 2019 (filled grey circles) and in 2018 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

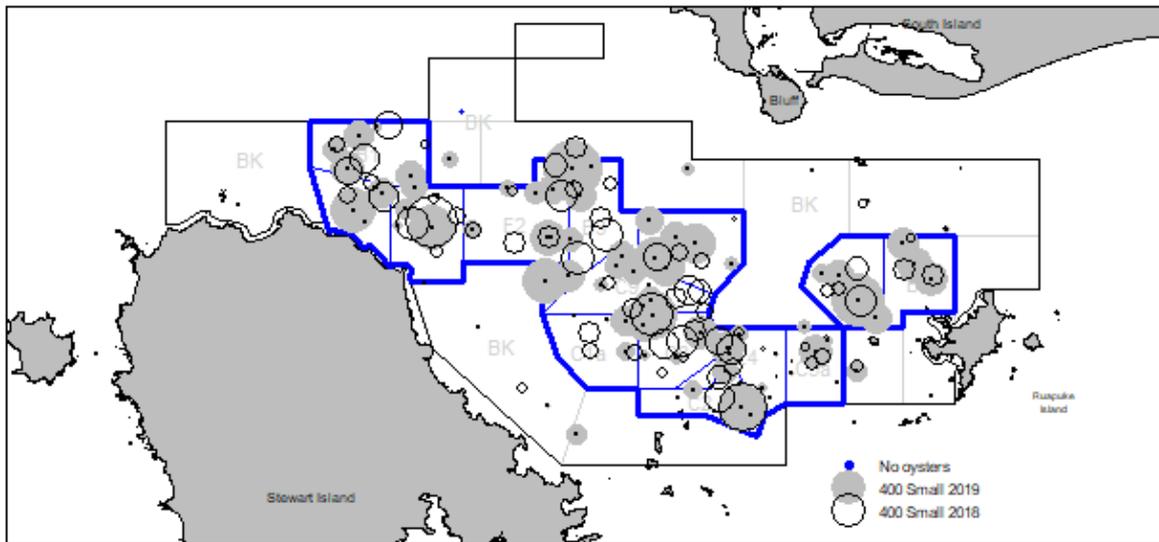


Figure 10: Density (numbers of oysters per standard tow representing an area swept of 1221 m²) of small oysters sampled during the February surveys in 2019 (filled grey circles) and in 2018 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue 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 2019 (Figure 11). Small oysters per recruit were generally low in number between 1995 and 2001, suggesting reduced recruitment to the population at a time when the numbers of recruit-sized oysters were increasing and relatively high compared with 1993 data (Figure 11). 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 remained low until 2016, whereas 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. 2018b), and the numbers of settlers recorded on spat collectors (Figure 12). 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).

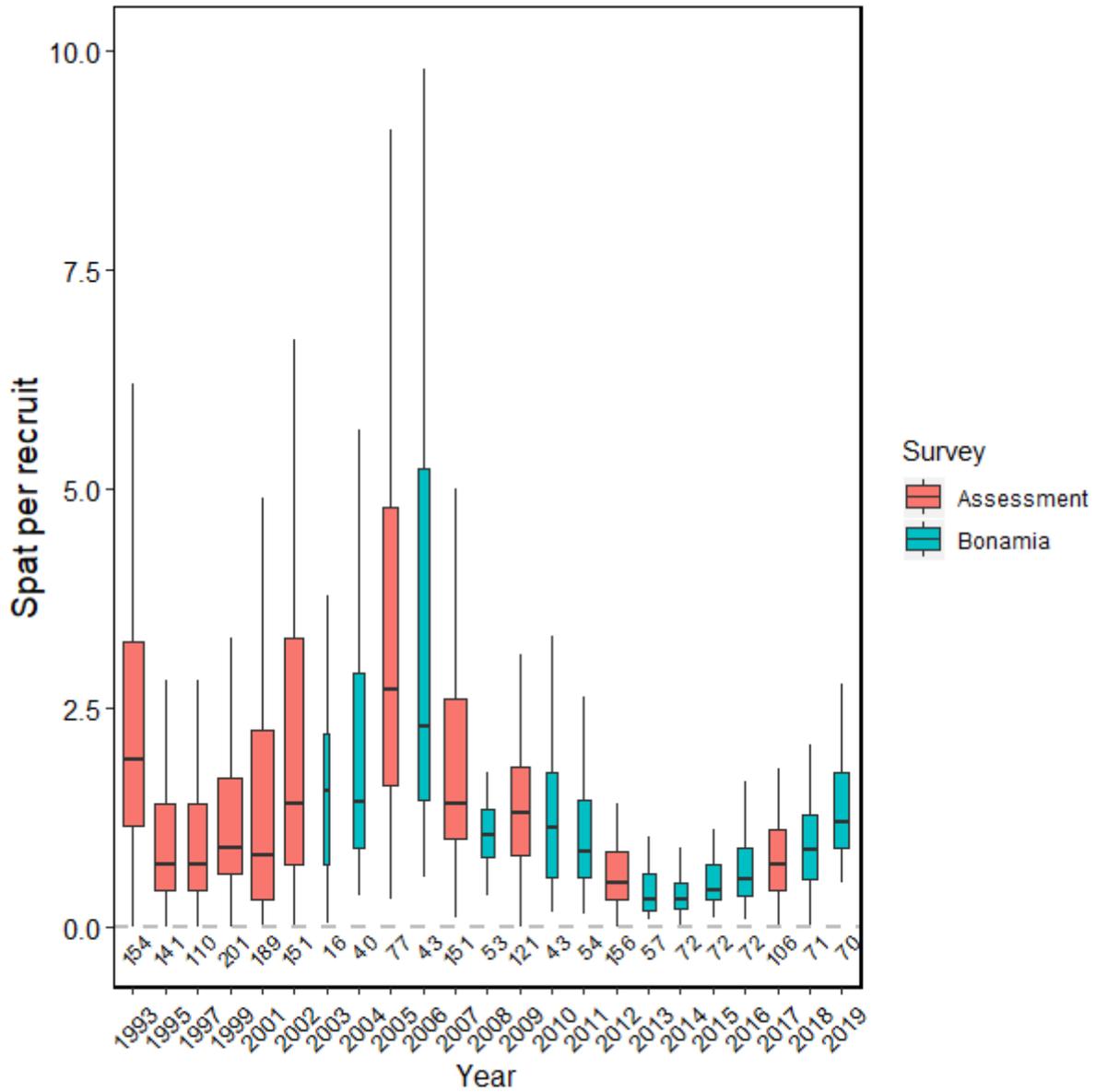


Figure 11: The numbers of small oysters per recruited oyster sampled between 1993 and 2019 stock assessment surveys (Assessment), and Bonamia surveys (Bonamia). Medians are shown as solid lines, boxes represent 50th percentiles (25–75%), and whiskers 90th percentiles (5–95%). Outliers smaller than 5% and greater than 95% have not been plotted for ease of visualisation. The number of stations sampled each year varied between 16 and 201 (shown below boxes as black text).

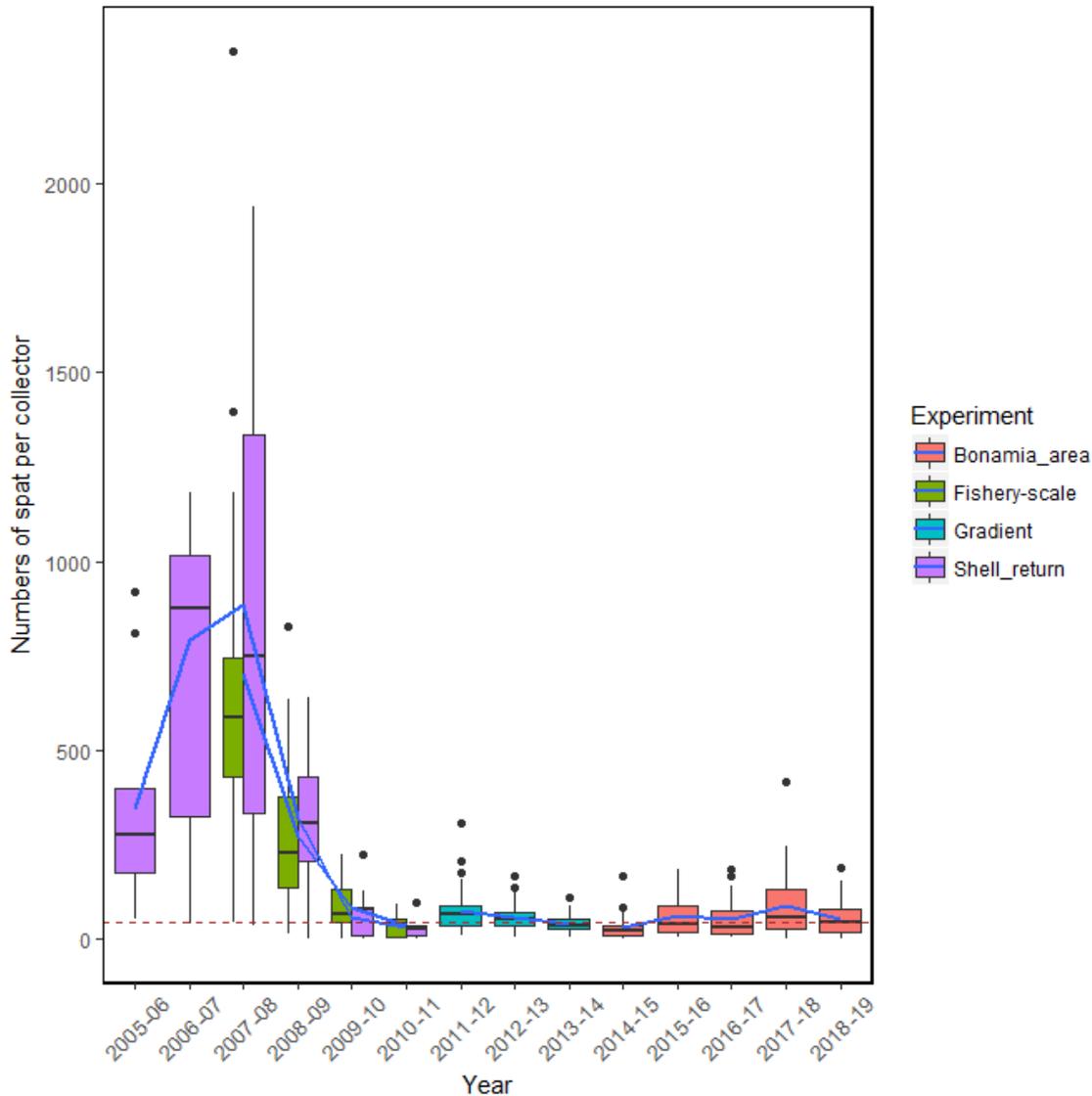


Figure 12: The total numbers of spat per collector sampled over the summers of 2005–06 to 2018–19. Spat settlement shows the success of spawning and indicates the levels of replenishment to the oyster population. Data represent four different experiments and different areas: the shell return site and fishery scale experiments, the gradient experiment in the central fishery area and fishery scale monitoring that began over the summer of 2014–15. Brown dashed horizontal line denotes mean recruitment in 2018–19.

3.3 Estimates of oyster mortality before and during the February 2019 survey

Descriptive statistics for the percentages of recruit-sized and pre-recruit new clocks and gapers sampled between 2016 and 2019 are given in Table 10. Low percentages of recruit-sized new clocks and gapers suggest that pre-survey mortality has remained low (see Michael et al. 2016 for 2012, 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.

There were very few gapers observed during the February 2019 survey, six stations (10.9%) had one or two recruit-sized gapers. One station (2.5%) had a single pre-recruit gaper in 2019. A larger proportion of stations recorded gapers in 2012 (26%), 2014 (14%), and in 2018 (28.1%); a smaller proportion in 2015 (6.9%) and 2016 (4.2%); and the same numbers of stations in 2018 (10.9 %) (see Appendix 6 for a list of survey reports).

Table 10: The number of stations and descriptive statistics for the percentages of new clocks and gapers for recruit and pre-recruit size groups. Percentages are new clocks and gapers to new clocks, gapers, and oysters combined, sampled from survey tows with more than 50 live oysters for surveys 2016–2019.

Year	Percentage new clocks and gapers							
	Recruit-sized				Pre-recruits			
	2016	2017	2018	2019	2016	2017	2018	2019
No. stations sampled	52	74	55	57	26	40	40	40
Median	0.4	1.2	0.3	0.8	0.0	0.0	0.0	0.0
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maximum	3.0	12.7	9.1	3.6	1.2	5.8	3.0	5.5
5th percentile	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
95th percentile	0.6	5.0	2.8	2.5	0.1	3.27	2.2	1.9
No. zero stations	22	13	23	11	22	21	33	21
% zero stations	42.3	17.6	41.8	19.3	84.6	52.5	82.5	52.5

The distributions of recruit-sized new clocks between 2016 and 2019 are compared in Figure 13. 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 in 2016 and spread across the fishery area (Figure 13). In 2018 recruit-sized new clock densities were lower and generally confined to the east of a line between Bluff Hill and Saddle Point (see Figure 1 & Figure 14) with many sites in the west showing no pre-survey mortality. The distribution of pre-survey mortality was widespread and locally variable (Figure 14), and new clock densities were higher at stations where recruit-sized oyster densities were higher in 2019, mostly in strata designated as commercial (C7a, B3, C5, and C3).

The population size of recruit-sized new clocks in core strata was 41.3% higher in 2019 (4.1 million) than in 2018 (2.9 million) (Table 11). No stratum had recruit-sized new clock densities above 0.02 m⁻² in 2019 (Table 11). Changes in the numbers of recruit-sized new clocks by strata ranged from 67.3% less to 33.1% more (Table 11).

The population size of pre-recruit new clocks in core strata was also higher in 2019 (1.0 million) than in 2018 (0.4 million) (Table 12). Only five of the fifteen strata had pre-recruit new clocks in 2019, and the densities in these five strata (B3, B6, C1a, C3, and C8) were very low. Between 2018 and 2019, changes in the numbers of pre-recruit-sized new clocks by strata ranged from 67.3% less to 117.9% more.

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 2019, ranging between 0.4% and 1.4% (Table 13). It was higher in 2012, 2014, and 2015, 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 2019, and between 3.2% and 7.6% in 2012, 2014, and 2015 (Table 13).

Pre-survey mortality of pre-recruit oysters in core strata was also low between 2016 and 2019, ranging between 0.2% and 0.7% (Table 13). It was higher in 2012, 2014, and 2015 ranging between 2.4% and 2.9%. Pre-survey mortality of pre-recruit oysters in the stock assessment survey area was similar to core strata between 2016 and 2018, ranging between 0% and 0.8%, and between 2.3% and 3.6% in 2012, 2014, and 2015.

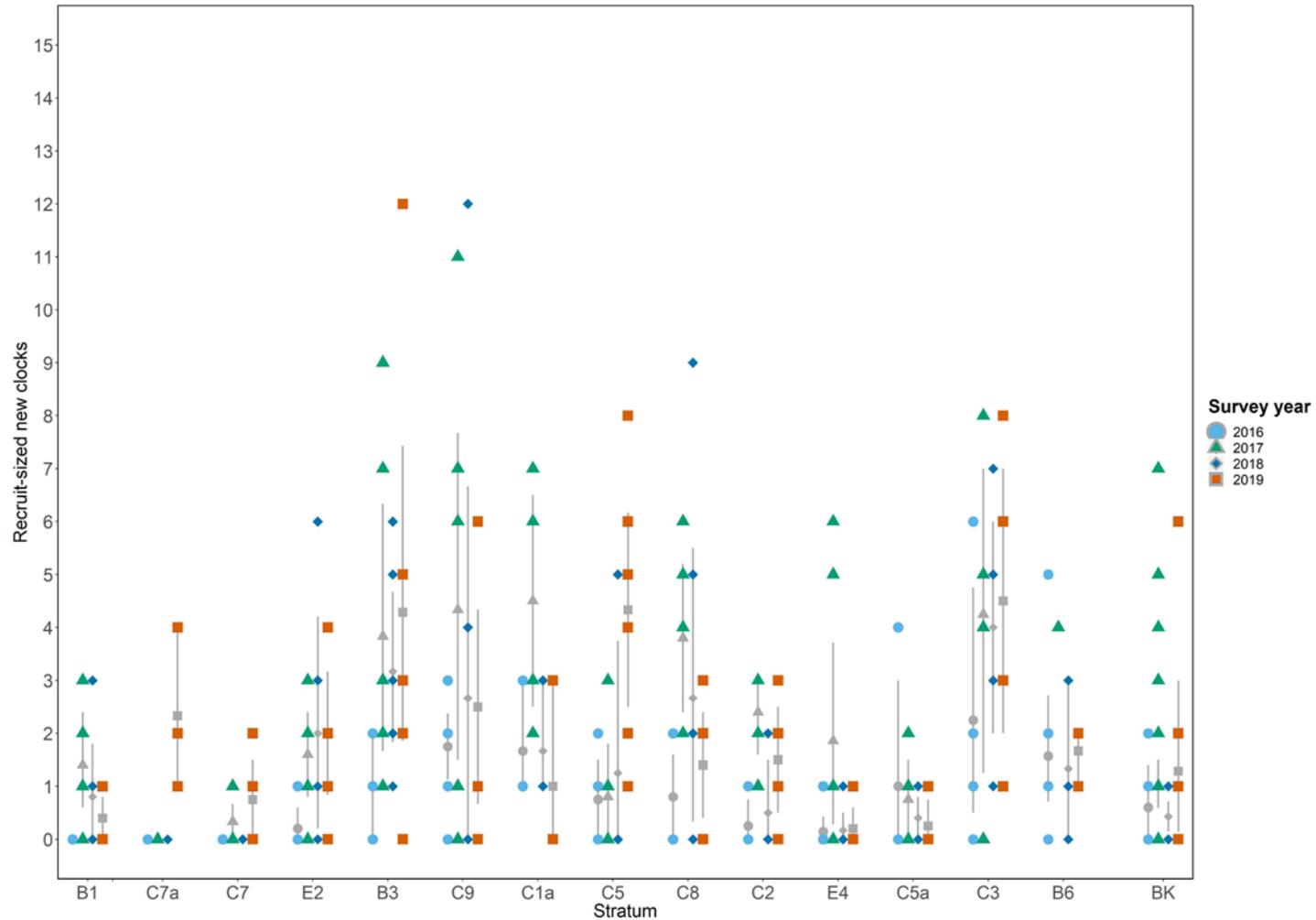


Figure 13: The numbers of recruit-sized new clocks (≥ 58 mm in diameter) per tow, means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines) by stratum, for surveys 2016–19. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2016 survey are shown as light blue filled circles, 2017 as green filled triangles, 2018 as dark blue filled diamonds, and 2019 as brown filled squares. Bonamia survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthest right.

Table 11: Absolute population estimates for recruit-sized (≥ 58 mm in diameter) new clocks in the core strata (Stratum), background stratum (BK), and for the stock assessment survey area (Survey total) sampled in 2019 by stratum. Columns give 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 2018 mean population estimates (2018 Pop.n) and the 2019 mean population size represented as a percentage of the 2018 mean population size by stratum. The percentage of the 2018 estimate (% of 2018) is shaded green highlights for increases in population size in 2019, tan highlights for decreases, and – for no change.

Stratum	Mean density	Density s.d.	CV	2019 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2018 Pop.n	% of 2018
B1	< 0.01	< 0.01	1.00	0.1	0.0	0.3	78.2	0.1	-4.9
B3	0.02	0.01	0.34	0.9	0.3	1.7	44.7	0.7	+33.1
B6	0.01	< 0.01	0.20	0.2	0.1	0.4	30.0	0.2	+22.3
C1a	< 0.01	< 0.01	1.00	0.2	0.0	0.5	31.3	0.3	-49.3
C2	< 0.01	< 0.01	0.58	0.1	0.0	0.3	21.9	0.0	-
C3	0.02	0.01	0.43	0.5	0.1	1.1	32.7	0.5	+5.7
C5	0.02	0.01	0.31	0.8	0.3	1.4	37.7	0.0	-
C5a	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	< 0.01	< 0.01	0.64	0.1	0.0	0.3	36.1	0.0	-
C7a	0.01	< 0.01	0.39	0.3	0.1	0.5	23.6	0.0	-
C8	< 0.01	< 0.01	0.58	0.1	0.0	0.3	26.8	0.4	-67.3
C9	0.01	0.01	0.45	0.4	0.0	0.9	34.5	0.4	+6.0
E2	0.01	< 0.01	0.58	0.3	0.0	0.7	42.8	0.3	+4.6
E4	0.00	0.00	0.00	0.0	0.0	0.0	28.0	0.0	-
Core total	0.01	0.00	0.14	4.1	2.5	6.4	491.8	2.9	+41.3
BK	0.01	0.01	0.62	5.1	0.0	12.4	578.4	0.6	+745.6
Survey total	0.01	0.00	0.35	9.2	2.7	17.8	1070.2	3.4	+169.8

Table 12: Absolute population estimates for pre-recruit (50–57 mm in diameter) new clocks in the core strata (Stratum), background stratum (BK), and for the stock assessment survey area (Survey total) sampled in 2019 by stratum. columns give 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 2018 mean population estimates (2018 Pop.n) and the 2019 mean population size represented as a percentage of the 2018 mean population size by stratum. The percentage of the 2018 estimate (% of 2018) is shaded green highlights for increases in population size in 2019, tan highlights for decreases, and – for no change.

Stratum	Mean density	Density s.d.	CV	2019 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km ²)	2018 Pop.n	% of 2018
B1	0.00	0.00	0.00	0.0	0.0	0.0	78.2	0.0	-
B3	< 0.01	< 0.01	0.31	0.2	0.1	0.4	44.7	0.1	+117.9
B6	< 0.01	< 0.01	0.58	0.1	0.0	0.3	30.0	0.1	+47.5
C1a	< 0.01	< 0.01	1.00	0.1	0.0	0.3	31.3	0.1	+1.5
C2	0.00	0.00	0.00	0.0	0.0	0.0	21.9	0.0	-
C3	< 0.01	< 0.01	1.00	0.1	0.0	0.2	32.7	0.1	-46.9
C5	< 0.01	< 0.01	0.61	0.1	0.0	0.4	37.7	0.0	-
C5a	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	0.00	0.00	0.00	0.0	0.0	0.0	36.1	0.0	-
C7a	< 0.01	< 0.01	0.76	0.2	0.0	0.6	23.6	0.0	-
C8	0.00	0.00	1.00	0.0	0.0	0.1	26.8	0.1	-67.3
C9	0.00	0.00	0.00	0.0	0.0	0.0	34.5	0.0	-
E2	< 0.01	< 0.01	1.00	0.1	0.0	0.2	42.8	0.0	-
E4	0.00	0.00	1.00	0.0	0.0	0.1	28.0	0.0	-
Core total	< 0.01	< 0.01	0.26	1.0	0.5	1.8	491.8	0.4	+157.2
BK	0.00	0.00	0.00	0.0	0.0	0.0	578.4	0.0	-
Survey total	< 0.01	< 0.01	0.26	1.0	0.5	1.8	1070.2	0.4	+157.2

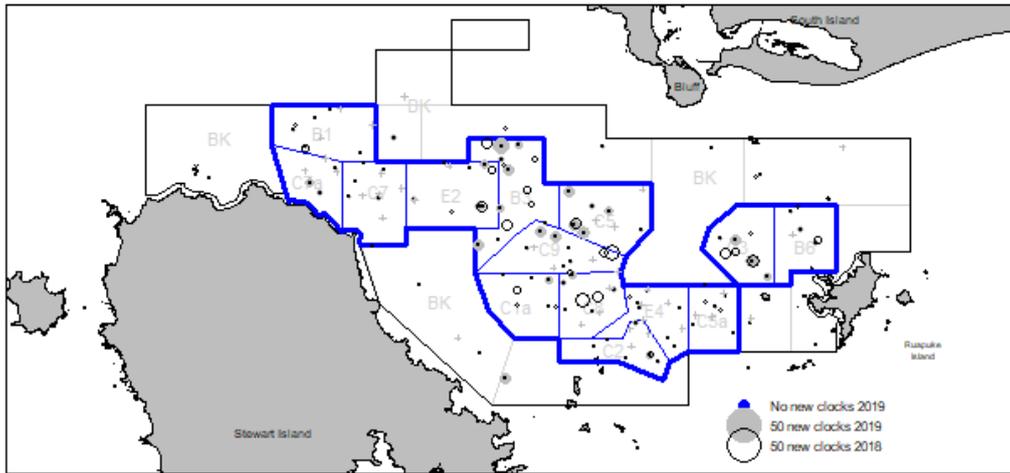


Figure 14: The distribution of recruit-sized new clocks and gaper densities combined in 2018 and 2019 (2018, open black circles and 2019, filled grey circles) showing the pre-survey mortality in February 2018 and 2019. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

Table 13: Estimates of pre-survey mortality for core strata (Bonamia survey area) and the stock assessment survey area for recruit-sized and pre-recruit new clocks (millions) for the 2012, 2014–2019 surveys. Estimates are from randomly selected stations only. Pre-survey mortality (% mort) is calculated as the percentage of new clocks over new clocks and oysters combined.

Bonamia survey area

Year	Recruit-sized			Pre-recruit		
	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
2019	542.5	4.1	0.8	216.5	1.0	0.5

Stock assessment survey area

Year	Recruit-sized			Pre-recruit		
	Oysters	New clocks	% mort	Oysters	New clocks	% mort
2012	918.4	30	3.2	414.3	12.0	2.8
2014	1 020.9	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
2019	868.0	9.2	1.1	309.8	0.0	0.0

3.4 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 seven stations in 2019; this included 61 stations of recruits only and 4 stations of recruits and pre-recruits. In all, 1726 samples of heart imprint slides were sampled and archived. This sample comprised 1659 recruit-sized oysters, 38 pre-recruits, and 29 small oysters. Almost all of the samples (96.1%) were of recruit-sized oysters, which is similar to 2018 (96.6%), 2017 (96.3%), 2016 (96.6%), and 2015 (97.2%). Only a subsample of these samples were screened ($n=140$). Stations with fewer than 15 recruit and pre-recruit sized oysters (station 2, $n=13$; station 24, $n=0$; and station 48, $n=0$) 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 14. Of the 1659 slides taken from random stations with more than 15 recruit and pre-recruit sized oysters in 2019, a subset of 140 heart imprint slides were examined for *Bonamia* infection. The remaining 1519 slides were from oysters screened using ddPCR and were not infected. In 2019, 97.0% of oysters had no detectable infection, a higher percentage than previous years: in 2018 (92.2%), 2017 (95.4%), 2016 (87.3%), 2015 (84.7%), 2014 (85.8%), and for 2010 to 2013 (90%, 88%, 89%, and 88% respectively), see Table A6.1 (Appendix 6) for references.

Table 14: The numbers of oyster heart tissue samples screened for *Bonamia* using ddPCR and heart imprints in 2019. Samples were recruit-sized oysters. The total numbers of samples tested (Sample (N)), the numbers of samples that tested negative and positive 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. NA* means no haemocytes were visible on the slide,

ddPCR samples	Recruits	Recruits	Recruits
Bonamia infection	Sample (N)	ddPCR-	ddPCR+
Heart	1659	1614	45
Histology samples			
Slides read (N)	140		
Heart imprints	Recruits		
Sample (N)	140		
Heart imprint -ve	104		
Heart imprint +ve	36		
Heart imprint NA*	0		

Prevalence and intensity of infection in oysters by *Bonamia*

Estimates of the prevalence and intensity of *Bonamia* infection assume that all heart imprint slides corresponding to samples that were ddPCR negative, but not scored for *Bonamia*, were negative. Fixed stations and stations with less than 25 recruit-sized oysters (stations 24, 48, 2, 26, 12, 98, 53, 45, 36, 54, 42, 21, 41, 34, and 15) were excluded from the analysis of prevalence and intensity of infection (Table 15). Infection intensity was estimated from heart imprint slides using the categorical score of Diggles et al. (2003) to maintain the established time series of data.

Heart imprints underestimate the true prevalence of Bonamia infection and are lower than qPCR and ddPCR estimates (Table 15). The mean prevalence from heart imprints in 2019 (1.9%) was higher than in 2018 but lower than in 2017, 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. Mean prevalence from ddPCR in 2019 was higher (7.4%) than from heart imprints. In 2017 and 2018, mean prevalence from ddPCR was 5.4% and 7.8% respectively, both higher than from heart imprints. Differences between ddPCR and heart imprints are in part determined by the intensity of infection.

Table 15: Comparisons of infection levels (prevalence (Prev %) and intensity (Inten)) between 2017, 2018, and 2019. Number of samples for each method (N), mean and median prevalence and intensity estimated by heart imprints (Hist.), and prevalence from qPCR and ddPCR, and standard deviation (s.d.) and 5% and 95% percentiles (5% and 95%) are reported. Data are from random stations sampled for Bonamia with more than 15 recruit and pre-recruit oysters in the sample.

	2017			2018			2019		
	Hist. Prev (%)	Hist. Inten	qPCR Prev (%)	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)
N	76	43	76	54	18	54	40	22	55
Mean	4.3	3.4	5.4	1.5	3.1	7.8	1.9	3.4	7.4
Median	4	3.5	4	0	3	6	0	3.3	0
s.d.	4.5	0.9	5.5	2.4	8.6	7.7	3.0	1.0	9.7
5%	0	2	4	0	1.9	0	0	2.0	0
95%	13	4.9	16	5.4	4.2	24	8	5.0	20

Details of recruit-sized oysters and densities by station, and their Bonamia infection status from histology and ddPCR, are shown in Table A5.1, Appendix 5.

Intensity of infection was determined from heart imprints to maintain the time series of Bonamia survey data. Of the 7.4% of oysters with detectable infections in 2019, mean light (category 1 and 2) infections was 0.3% (1–5% in 2010–2018), and 1.2% had category 3 and higher infections (4–11% in 2010–2018) which are normally fatal. A comparison between the categorical intensity of infection from heart imprints using the methods of Diggles et al. (2003) and intensity from ddPCR calculated as the ratio of the concentration of Bonamia targets to the concentration of β -actin targets in each sample is given in Appendix 3.

The prevalence of infection from heart imprints in 2019 is similar to 2018, which was the lowest since 2007 (Figure 15). During periods of relatively high prevalence (2012–2015), qPCR showed higher prevalence than heart imprints, but levels were similar at relatively low prevalence (Figure 15). Prevalence from ddPCR in 2019 is higher than for heart imprints reflecting the increased sensitivity of PCR methods (Figure 15).

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 16). The proportion of non-fatal infection is the lowest since 2007.

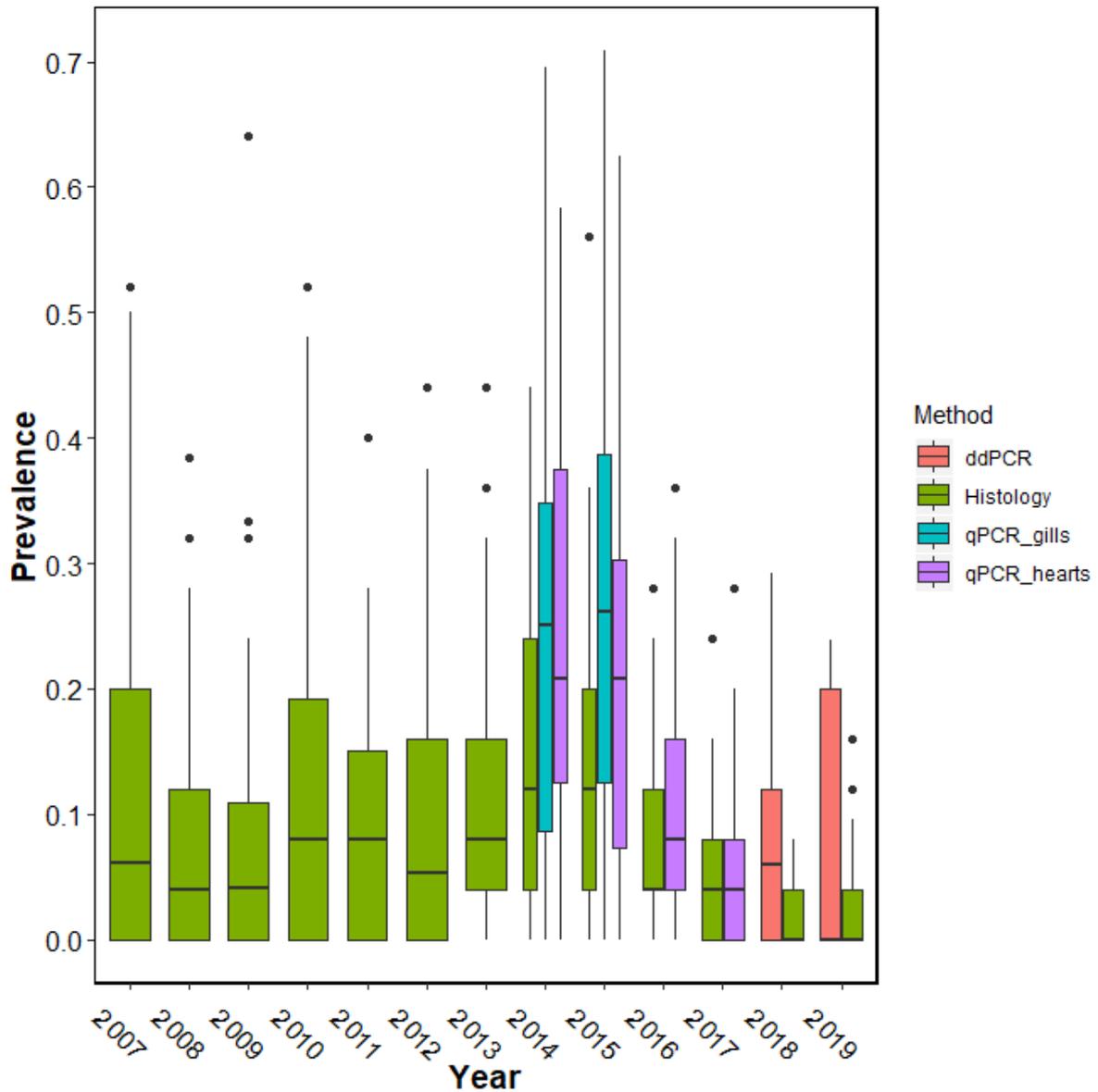


Figure 15: Boxplots of the median prevalence of Bonamia infection 2007–2019. The median prevalence of infection at all stations determined from histology (heart imprints) 2007–2013, and for qPCR heart tissues (qPCR_hearts), and gill tissues (qPCR_gills) in 2014 and 2015, but only heart tissues in 2016 and 2017. Heart tissues were only tested with ddPCR in 2018 and 2019. Medians shown as solid lines, boxes represent 50th percentiles and whiskers 95th percentiles, and outliers as filled black circles.

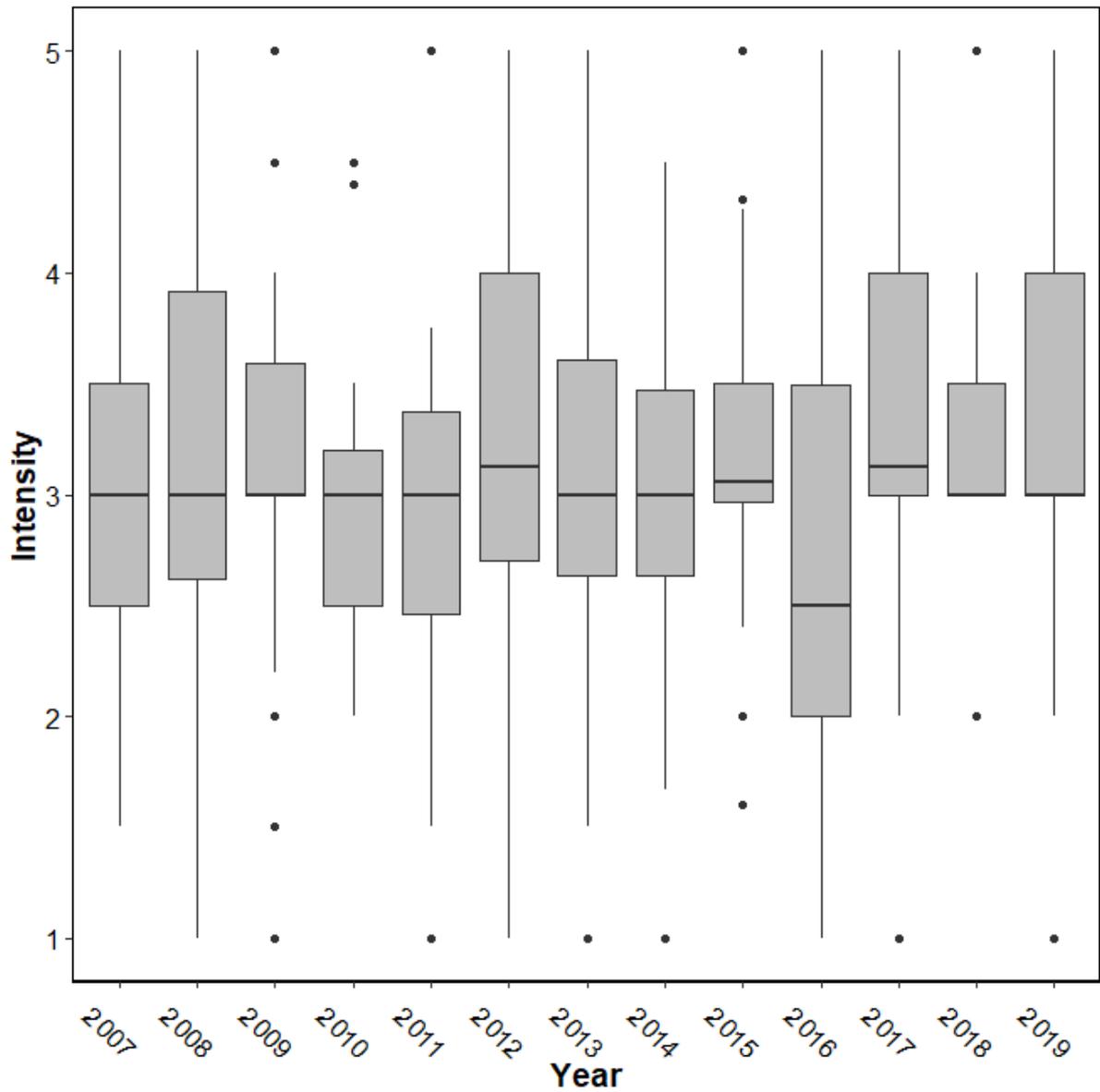


Figure 16: Boxplots of the mean intensity of Bonamia infection 2007–2019. The mean intensity of infection at all stations was determined from histology. Medians are shown as solid lines, boxes represent 50th percentiles, and whiskers 95th percentiles, and outliers are shown as filled black circles.

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 2019 is widespread, but spatially patchy over multiple scales (Figures 17–20). In all years, qPCR and ddPCR detected higher numbers of infected oysters than were detected by heart imprints (Figures 17–20).

Generally, relatively high prevalence persisted in southern and eastern fishery areas between 2016 and 2018, and relatively low and patchy infection occurred in the western and central areas (Figures 17–19). Infected stations were interspersed with stations with no detectable infection (shown as filled blue circles, Figures 17–19). In 2018, the prevalence of infection appeared to increase in southern areas (strata C2, C8, and E4) and remained similar in eastern areas (strata C3 and B6, Figure 19), however this may be due to the higher sensitivity of ddPCR. In 2019, the distribution became patchier and there was greater variation in the prevalence estimated by heart imprints and ddPCR, possibly because of an increase in low level infections not easily detected by heart imprints (Figure 20).

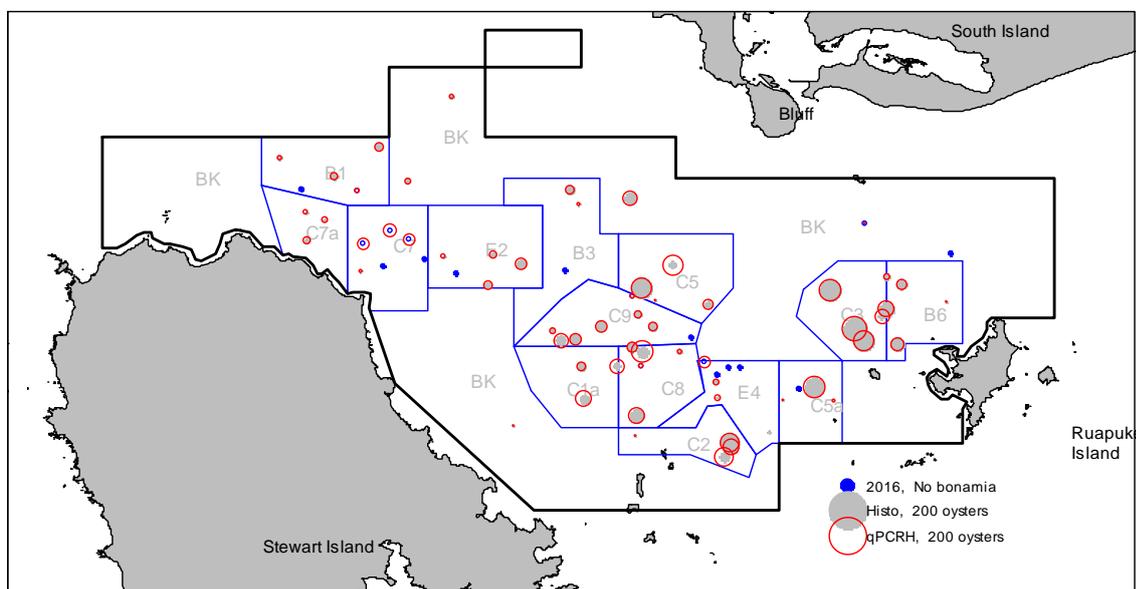


Figure 17: The distributions of Bonamia infection in February 2016 estimated from heart imprints and qPCR analysis of heart tissues only: numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and qPCR heart tissues (qPCRH, open red circles) and stations with no Bonamia (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

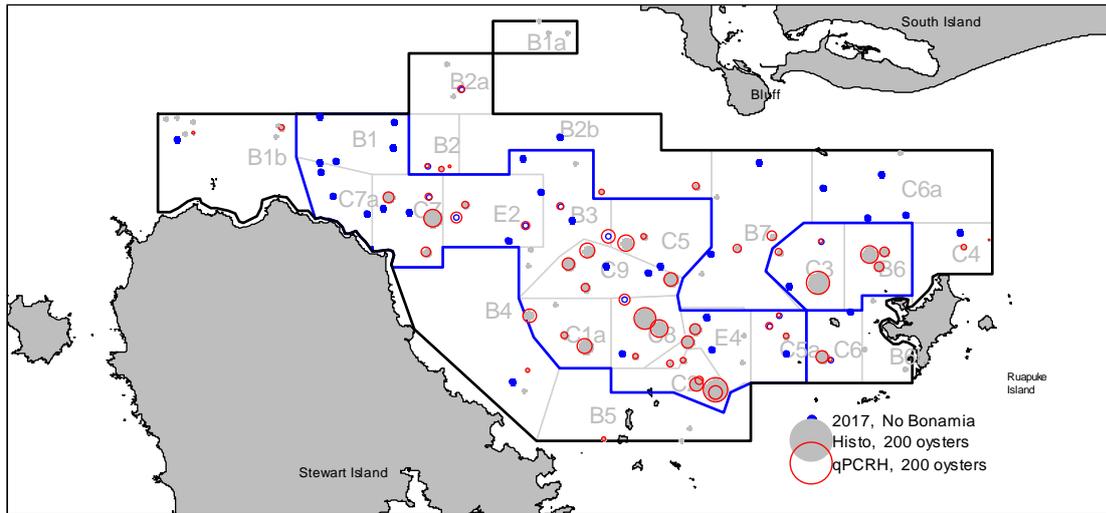


Figure 18: The distributions of *Bonamia* infection in February 2017 estimated from heart imprints and qPCR analysis of heart tissues only: numbers of oysters with *Bonamia* infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and qPCR heart tissues (qPCRH, open red circles) and stations with no *Bonamia* (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

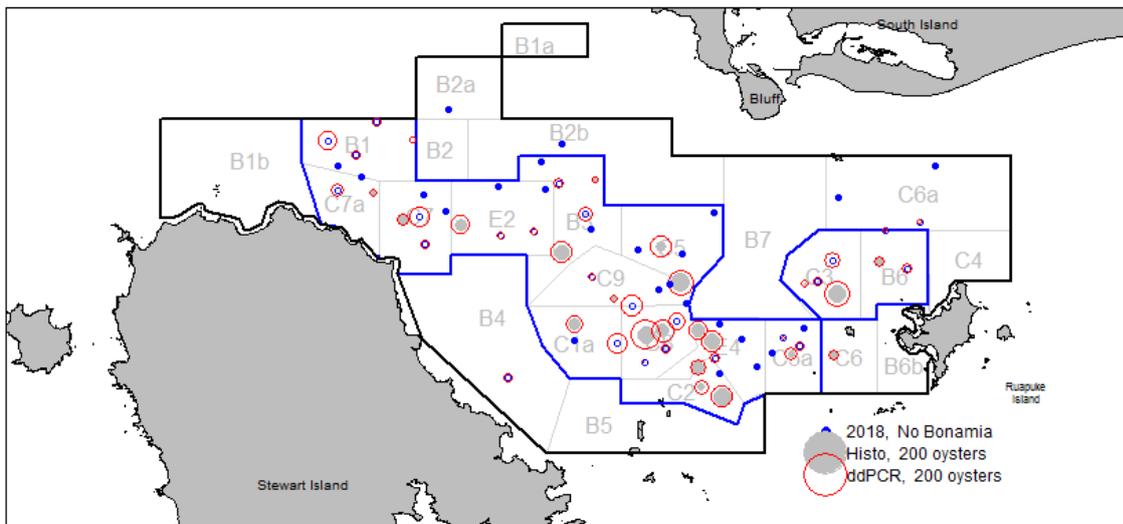


Figure 19: The distributions of *Bonamia* infection in February 2018 estimated from heart imprints and ddPCR analysis of heart tissues only: numbers of oysters with *Bonamia* infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and ddPCR heart tissues (ddPCR, open red circles) and stations with no *Bonamia* (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

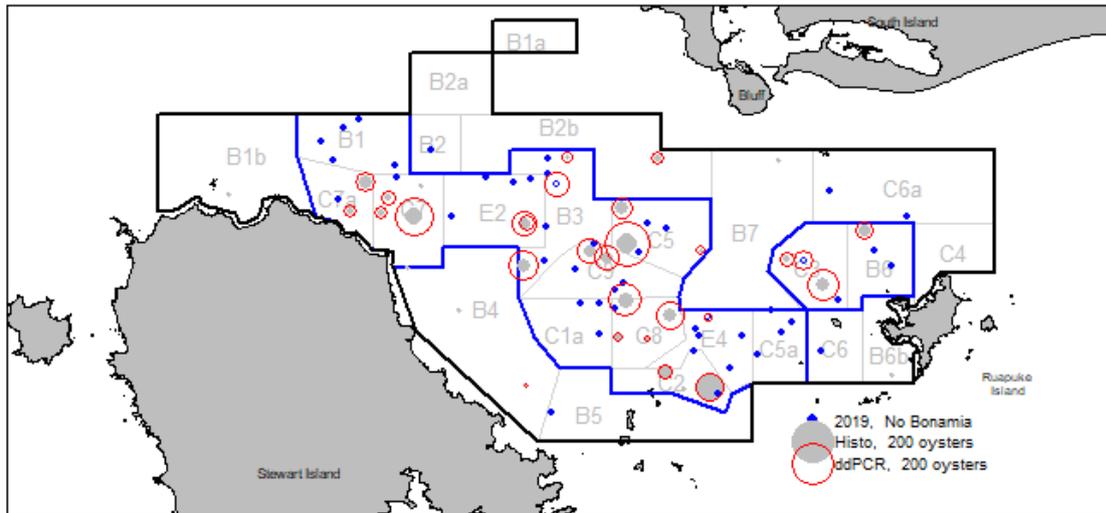


Figure 20: The distributions of *Bonamia* infection in February 2019 estimated from heart imprints and ddPCR analysis of heart tissues only: numbers of oysters with *Bonamia* infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and ddPCR heart tissues (ddPCR, open red circles) and stations with no *Bonamia* (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

Between 2012 and 2015, widespread fatal infections caused substantial oyster mortality over the fishery area (Michael et al. 2015a). 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 the south-eastern corner of stratum C7 (Saddle Point) and Bluff Hill (Figure 21). Recruit-sized oyster densities were similarly low in 2017, infection was low and patchy, and fatal infections more widespread, extending into western fishery areas (Figure 22). 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 23). Recruit-sized oyster densities increased further in 2019, and fatal infection was widespread and in similar or higher densities than in 2018 (Figure 24). Non-fatal infection remained relatively low in 2019 (Figure 24).

Patterns in the distribution of prevalence and intensity of infection between 2012 and 2019 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 with low levels of fishing since 2008 because of the low meat quality there.

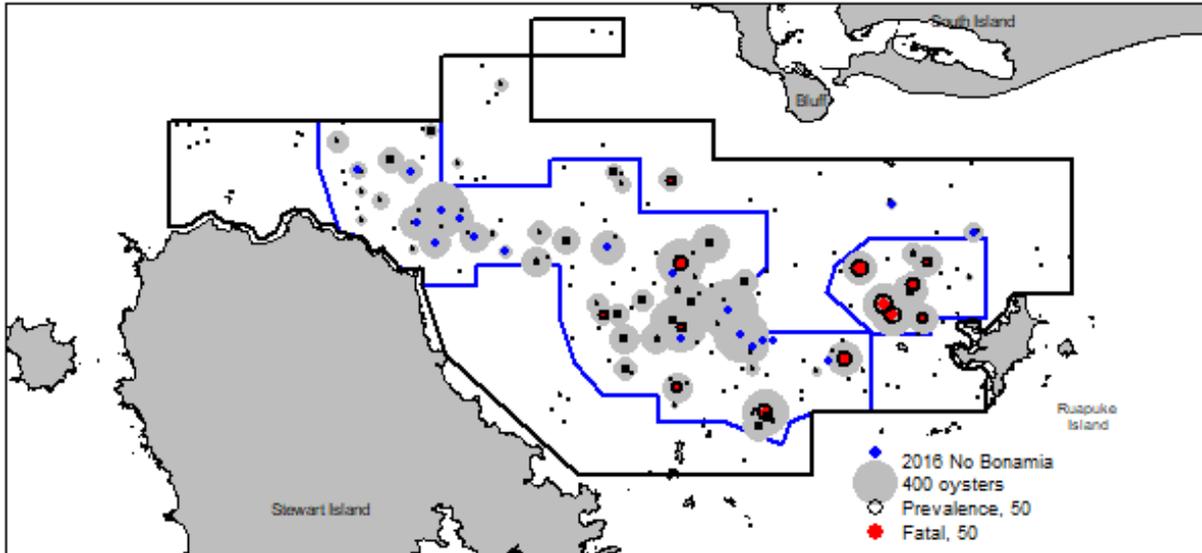


Figure 21: The distributions of oysters and *Bonamia* infection in February 2016: numbers of oysters (filled grey circles), numbers of oysters with *Bonamia* infection (intensity categories 1–5 combined, open black circles), fatal infections (intensity categories 3–5 combined, filled red circles), and stations with no *Bonamia* (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

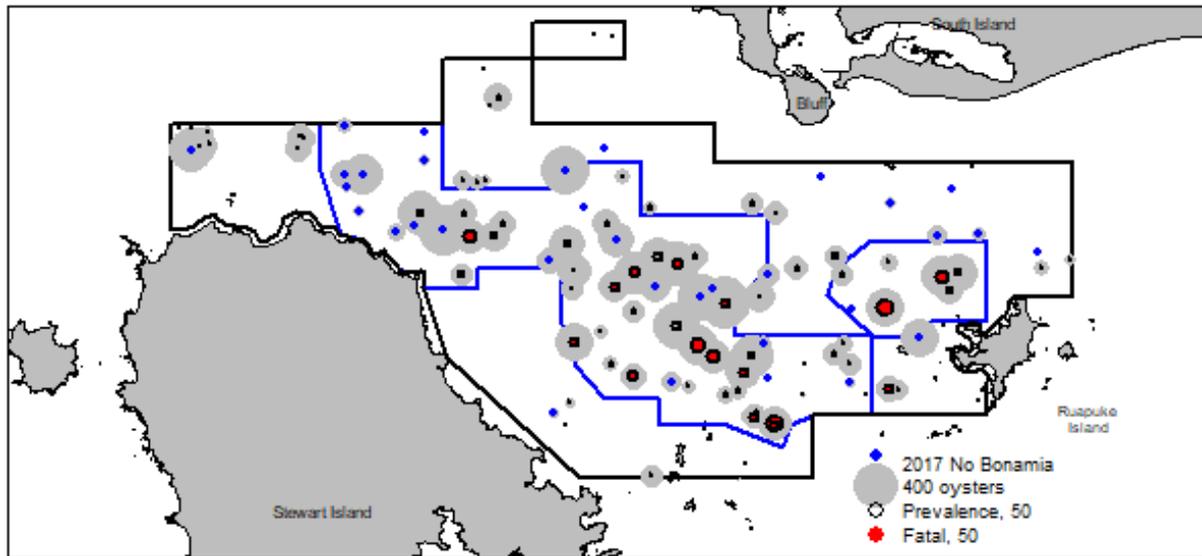


Figure 22: The distributions of oysters and *Bonamia* infection in February 2017: numbers of oysters (filled grey circles), numbers of oysters with *Bonamia* infection (intensity categories 1–5 combined, open black circles), fatal infections (intensity categories 3–5 combined, filled red circles), and stations with no *Bonamia* (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

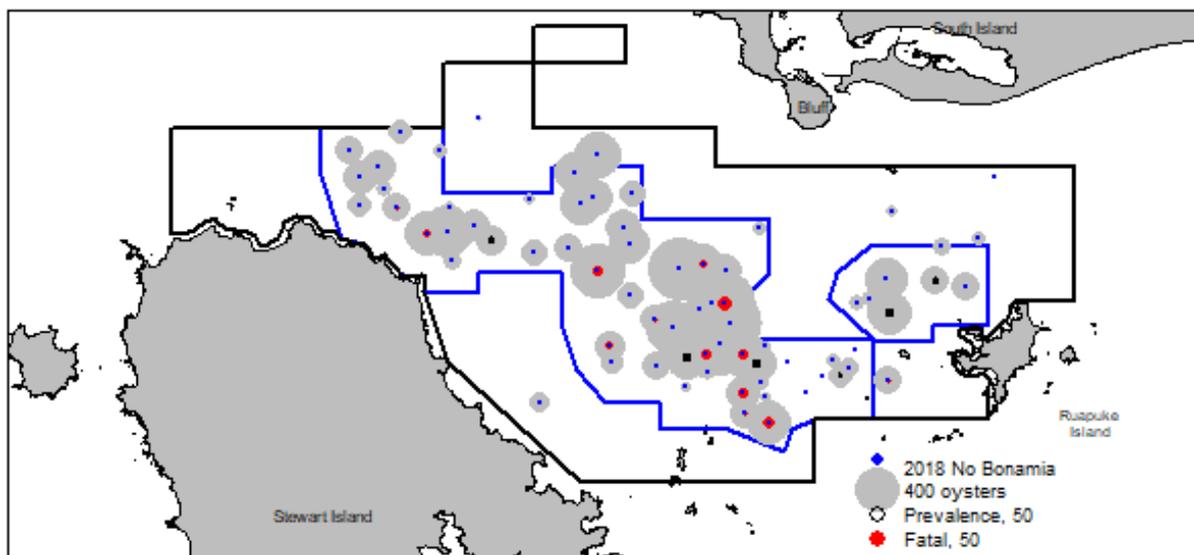


Figure 23: The distributions of oysters and *Bonamia* infection in February 2018: numbers of oysters (filled grey circles), numbers of oysters with *Bonamia* infection (intensity categories 1–5 combined, open black circles), fatal infections (intensity categories 3–5 combined, filled red circles), stations with no *Bonamia* (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

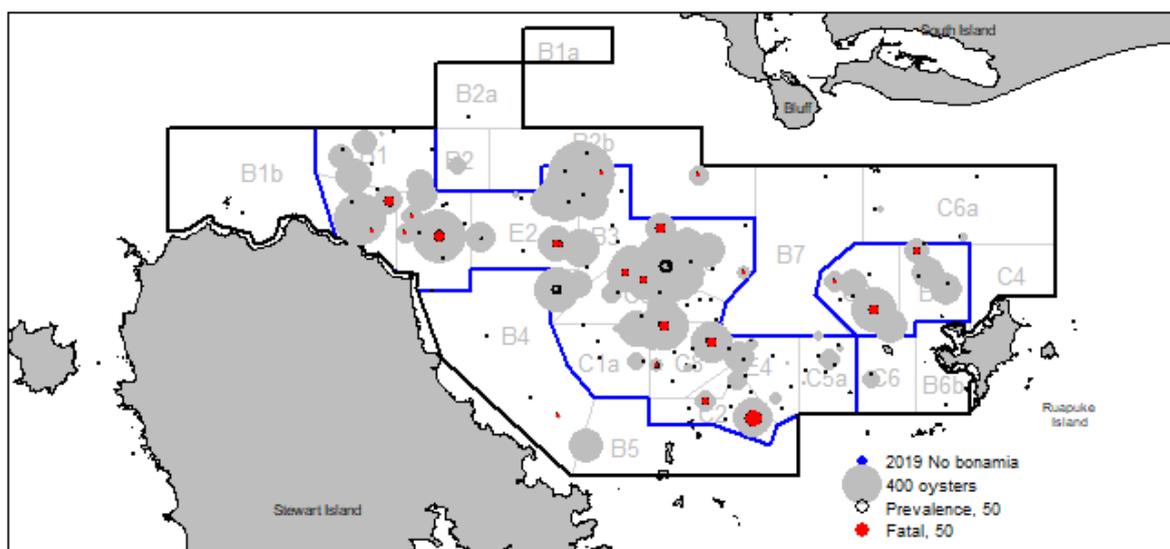


Figure 24: The distributions of oysters and *Bonamia* infection in February 2019: numbers of oysters (filled grey circles), numbers of oysters with *Bonamia* infection (intensity categories 1–5 combined, open black circles), fatal infections (intensity categories 3–5 combined, filled red circles), and stations with no *Bonamia* (filled blue circles). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

The total numbers of recruit-sized oysters infected with Bonamia

The prevalence of *Bonamia* infections (categories 1–5) in recruit-sized oysters in core strata (*Bonamia* survey area), the background stratum, and the stock assessment survey area estimated from heart imprints for 2019 is shown in Table 16, by ddPCR in Table 17, and non-fatal infections (intensity categories 1 and 2) from heart imprints in Table 18. The prevalence of infection in the *Bonamia* survey area was 1.4% by heart imprints, 7.6 million oysters (95% CI 3.1–13.4, Table 16), and of those infections, only 0.1% were non-fatal (see Table 18). The prevalence by ddPCR was 6.4%, 34.7 million oysters (95% CI 15.0–60.4, Table 17). The infected population of recruit-sized oysters in the *Bonamia* survey area estimated using ddPCR is 4.6 times higher than that estimated using heart imprints.

The prevalence of *Bonamia* infections in the *Bonamia* survey area, estimated from heart imprints declined by 90.1%, from 89.5 million (95% CI 50.8–146.1) in 2014 to 7.6 million (95% CI 3.1–13.4) in 2019 (Table 16). Prevalence over the stock assessment survey area declined by 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, and further declined by 34.4% to 10.2 million (95% CI 4.1–18.4) in 2019 (Table 16). In 2019, 11.0% of the infected oysters (0.8 million oysters, 95% CI 0–2.3, Table 18) in the *Bonamia* survey area had non-fatal infections. There were no non-fatal infections detected in the background stratum (see Table 18).

Table 16: The 2019 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). Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (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, the area of each stratum (Area (km²)) in square kilometres, by stratum, and the 2019 recruit-sized oyster population size (2019 Popn, millions of oysters) and prevalence (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95% CI	Upper 95% CI	Area (km ²)	2019 Popn	Prev (%)
B1	4	0.00	0.00	0.0	0.0	0.0	0.0	78.2	49.9	0.0
B3	7	0.02	0.01	0.9	0.8	0.0	2.2	44.7	97.8	0.8
B6	3	0.02	0.02	1.0	0.7	0.0	2.1	30.0	34.5	2.0
C1a	3	0.00	0.00	0.0	0.0	0.0	0.0	31.3	22.7	0.0
C2	3	0.02	0.02	1.0	0.5	0.0	1.5	21.9	8.9	5.4
C3	3	0.01	0.01	1.0	0.3	0.0	0.9	32.7	34.7	0.8
C5	5	0.02	0.02	0.9	0.8	0.0	2.3	37.7	65.1	1.2
C5a	3	0.00	0.00	0.0	0.0	0.0	0.0	23.5	1.6	0.0
C7	4	0.05	0.04	0.7	1.8	0.0	4.7	36.1	55.7	3.2
C7a	3	0.04	0.03	0.8	0.9	0.0	2.3	23.6	39.1	2.2
C8	4	0.03	0.02	0.7	0.8	0.0	2.1	26.8	27.1	3.1
C9	6	0.02	0.02	0.6	0.8	0.0	2.0	34.5	43.5	1.9
E2	4	0.01	0.01	1.0	0.3	0.0	1.0	42.8	56.7	0.6
E4	3	0.00	0.00	0.0	0.0	0.0	0.0	28.0	5.2	0.0
Core strata	55	0.02	0.00	0.3	7.6	3.1	13.4	491.8	542.5	1.4
BK	5	0.00	0.00	0.8	2.6	0.0	7.3	578.4	325.5	0.8
Survey total	60	0.01	0.00	0.3	10.2	4.1	18.4	1 070.2	868.0	1.2

Table 17: The 2019 estimates of recruit-sized oysters with *Bonamia* infection (prevalence), estimated from ddPCR, scaled to population size in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total). Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (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, the area of each stratum (Area (km²)) in square kilometres, by stratum, and the 2019 recruit-sized oyster population size (2019 Popn, millions of oysters) and prevalence (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95% CI	Upper 95% CI	Area (km ²)	2019 Popn	Prev (%)
B1	4	0.00	0.00	0.0	0.0	0.0	0.0	78.2	49.9	0.0
B3	7	0.13	0.08	0.6	5.8	0.0	13.8	44.7	97.8	5.9
B6	3	0.06	0.06	1.0	1.7	0.0	5.3	30.0	34.5	4.9
C1a	3	0.00	0.00	0.0	0.0	0.0	0.0	31.3	22.7	0.0
C2	3	0.04	0.04	1.0	0.8	0.0	2.5	21.9	8.9	9.0
C3	3	0.11	0.06	0.5	3.6	0.0	8.3	32.7	34.7	10.4
C5	5	0.06	0.05	0.8	2.2	0.0	6.0	37.7	65.1	3.4
C5a	3	0.00	0.00	0.0	0.0	0.0	0.0	23.5	1.6	0.0
C7	4	0.25	0.18	0.7	8.9	0.0	23.3	36.1	55.7	16.0
C7a	3	0.1	0.07	0.6	2.5	0.0	6.1	23.6	39.1	6.4
C8	4	0.13	0.11	0.8	3.6	0.0	10.1	26.8	27.1	13.3
C9	6	0.12	0.08	0.6	4.2	0.0	10.2	34.5	43.5	9.7
E2	4	0.04	0.04	1.0	1.6	0.0	5.0	42.8	56.7	2.8
E4	3	0.00	0.00	0.0	0.0	0.0	0.0	28.0	5.2	0.0
Core strata	55	0.07	0.02	0.3	34.7	15	60.4	491.8	542.5	6.4
BK	5	0.02	0.02	0.9	11.9	0.0	35.6	578.4	325.5	3.6
Survey total	60	0.04	0.01	0.3	46.6	18.4	84.7	1 070.2	868.0	5.4

Table 18: The 2019 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). Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (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, the area of each stratum (Area (km²)) in square kilometres, by stratum, and the 2019 recruit-sized oyster population size (2019 Popn, millions of oysters) and prevalence (Prev (%)).

Stratum	No. stns	Mean density	Density sd	CV	Pop.n	Lower 95% CI	Upper 95% CI	Area (km ²)	2019 Popn	Prev (%)
B1	4	0.00	0.0	0.0	0.0	0.0	0.0	78.2	49.9	0.0
B3	7	0.01	0.0	1.0	0.6	0.0	2.1	44.7	97.8	0.7
B6	3	0.00	0.0	0.0	0.0	0.0	0.0	30	34.5	0.0
C1a	3	0.00	0.0	0.0	0.0	0.0	0.0	31.3	22.7	0.0
C2	3	0.00	0.0	0.0	0.0	0.0	0.0	21.9	8.9	0.0
C3	3	0.00	0.0	0.0	0.0	0.0	0.0	32.7	34.7	0.0
C5	5	0.00	0.0	0.0	0.0	0.0	0.0	37.7	65.1	0.0
C5a	3	0.00	0.0	0.0	0.0	0.0	0.0	23.5	1.6	0.0
C7	4	0.00	0.0	0.0	0.0	0.0	0.0	36.1	55.7	0.0
C7a	3	0.00	0.0	0.0	0.0	0.0	0.0	23.6	39.1	0.0
C8	4	0.01	0.0	0.8	0.2	0.0	0.4	26.8	27.1	0.6
C9	6	0.00	0.0	0.0	0.0	0.0	0.0	34.5	43.5	0.0
E2	4	0.00	0.0	0.0	0.0	0.0	0.0	42.8	56.7	0.0
E4	3	0.00	0.0	0.0	0.0	0.0	0.0	28.0	5.2	0.0
Core strata	55	0.00	0.0	0.8	0.8	0.0	2.3	491.8	542.5	0.1
BK	5	0.00	0.0	0.0	0.0	0.0	0.0	578.4	325.5	0.0
Survey total	60	0.00	0.0	0.8	0.8	0.0	2.3	1 070.2	868.0	0.1

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

Before 2016, the distribution of non-fatal (category 1 and 2) 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 (see Michael et al. 2016) 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 25). The numbers of stations with non-fatal infections were greatly reduced in 2017 (Figure 26) 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 (Figure 27), however, prevalence was higher than in 2017 (Figures 26 & 27) – probably due to the increased sensitivity of ddPCR to detect low-level infections. Non-fatal infections in 2019 were similarly low and confined to a couple of stations in the central fishery area and a couple of smaller isolated patches in stratum C8 (Figure 28).

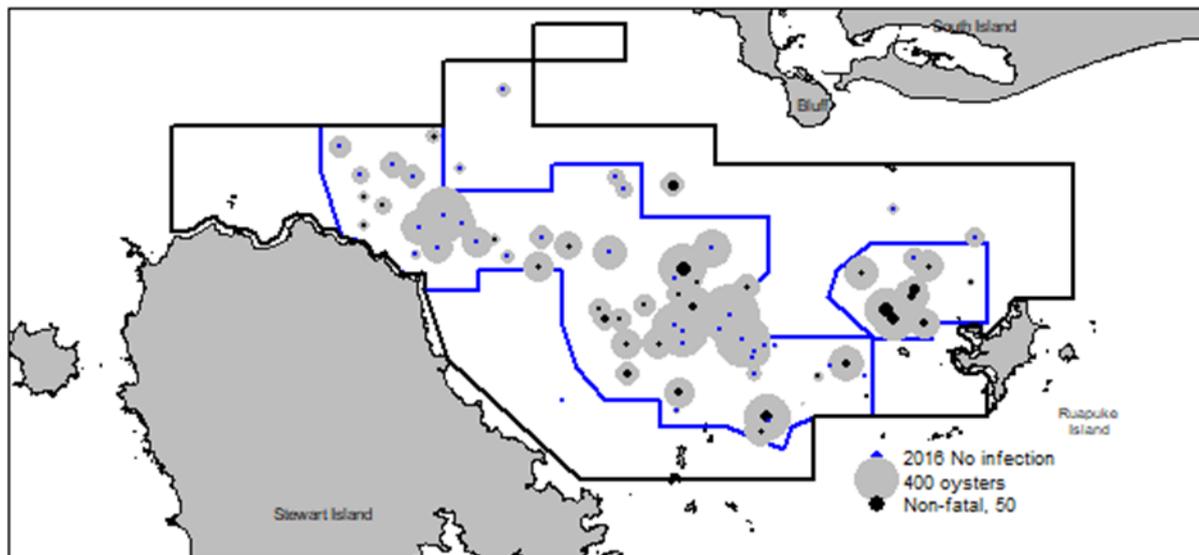


Figure 25: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2016. Stations with no *Bonamia* infection are shown by blue circles.

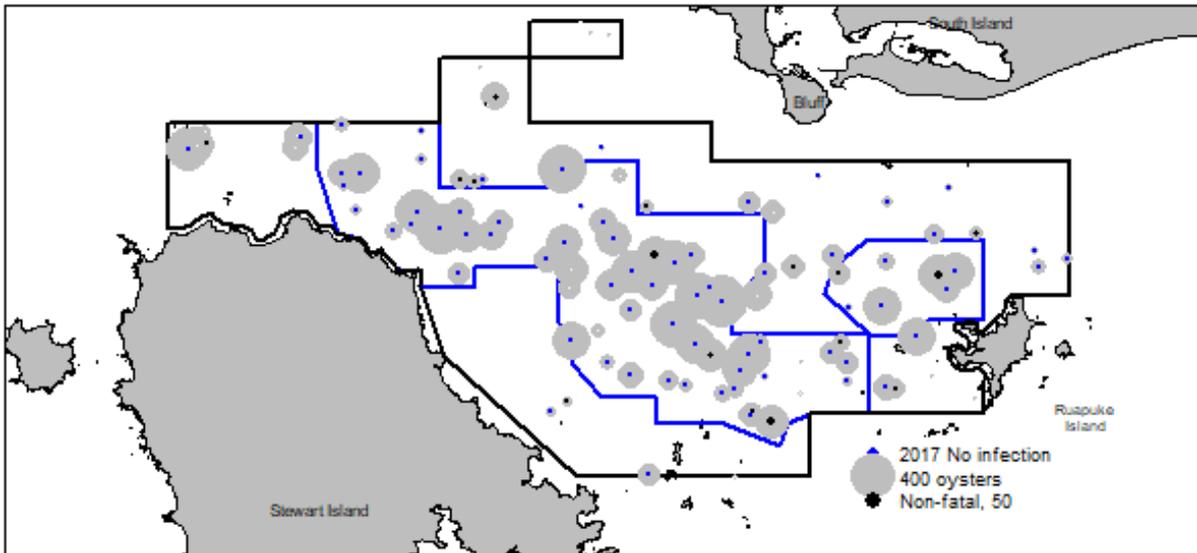


Figure 26: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2017. Stations with no *Bonamia* infection are shown by blue circles.

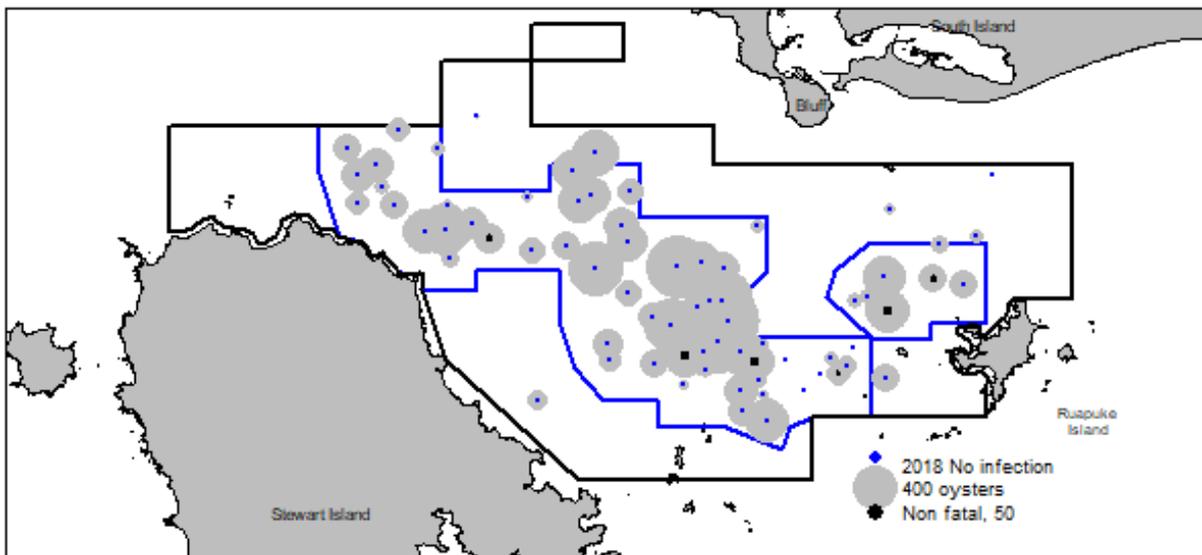


Figure 27: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2018. Stations with no *Bonamia* infection are shown by blue circles.

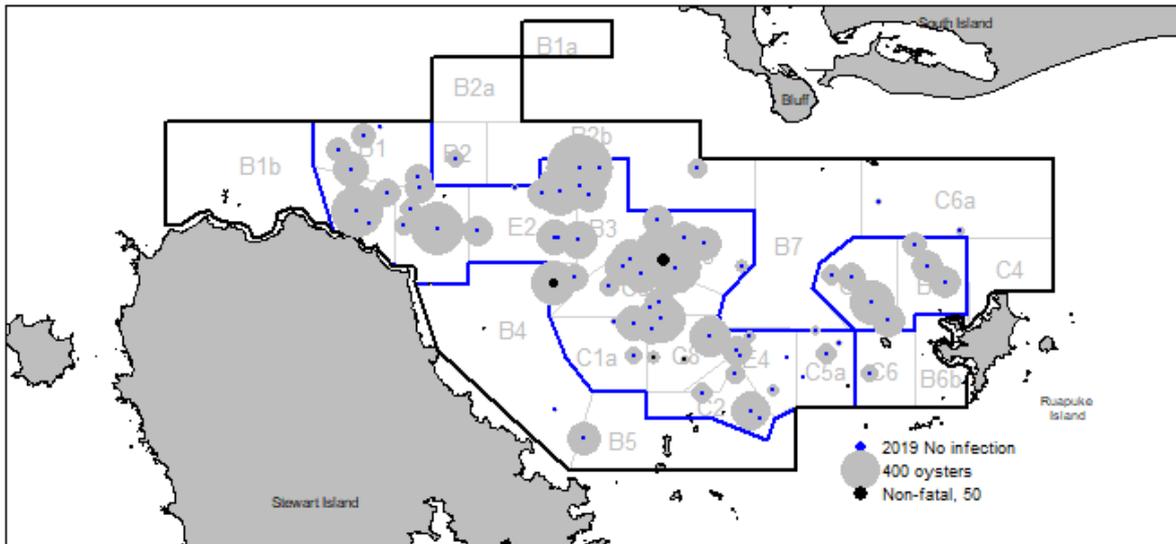


Figure 28: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2019. Stations with no *Bonamia* infection are shown by blue circles.

3.5 Summer mortality from *Bonamia* in the commercial fishery area

Pre-survey mortality was estimated from the population size of recruit-sized new clocks and gapers (see Table 10). In 2019, pre-survey mortality in all core strata combined was estimated to be 4.1 million recruit-sized oysters (95% CI 2.5–6.4), 0.8% 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 in Tables 19–21. 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 with category 3 and higher infections in the catch was used to calculate a correction factor (Method 1) for each stratum (1 (the total catch) less the mean proportion of oysters infected with *Bonamia*, Table 19) and this correction factor was applied to the mean oyster density estimated from all random tows. Using this method, post-survey mortality of oysters was projected to reduce the recruit-sized oyster population in core strata by 6.8 million oysters (1.3%) from 542.5 million oysters at the time of the survey (February 2018) to 535.7 million oysters (Table 19) by early March 2019 (the beginning of the new oyster season). Post-survey mortality of recruit-sized oysters by stratum (Table 19) ranged from no mortality to 5.4% in C2. In core strata, post-survey mortality in 2019 (6.8 million, 1.3%) was similar to 2018 (1.5%), and lower than for 2015–2017: 8.7%, 3.8%, and 3.0% respectively.

The estimates of post-survey mortality in core strata from fatally infected oysters scaled to the size of the catch (Method 2) were the same as those estimated using averaged correction factors (Method 1),

6.8 million oysters (1.3%) (Table 20). Three of the fourteen strata in 2019 (C7, C7a, and C9) accounted for 51% of the projected mortalities.

The speed at which 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). In 2019, this summer mortality was 2.0% of the recruit-sized population in core strata (Table 21). Summer mortality since 2016 has been below 5.1% in the stock assessment survey and *Bonamia* survey areas, and substantially less than in 2012 (9.2–13.1%) (Table 21).

Table 19: 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 stock assessment survey area (Survey total) sampled in February 2019. Columns give the number of randomly selected stations sampled (No. stns), the correction factor applied to each stratum (Correction factor), the mean oyster density per square metre (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 light grey), mean post survey mortality population size (Pop.n2, filled medium grey) in millions of oysters, lower and upper 95% confidence intervals (CI) for the post-mortality estimate, the area of each stratum (Area (km²)), losses of oysters (Losses, millions), and the percentage mortality (% Mortality, shaded dark grey) 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)	Losses	% Mortality
B1	4	1.00	0.64	0.20	0.32	49.9	49.9	17.2	92.7	78.2	0.0	0.0
B3	7	1.00	2.19	0.74	0.34	97.8	97.7	32.0	183.9	44.7	0.1	0.1
B6	3	0.98	1.13	0.15	0.13	34.5	33.8	20.8	52.3	30.0	0.7	2.0
C1a	3	1.00	0.73	0.49	0.68	22.7	22.7	0.0	57.5	31.3	0.0	0.0
C2	3	0.95	0.38	0.10	0.27	8.9	8.4	3.7	14.7	21.9	0.5	5.4
C3	3	0.99	1.05	0.26	0.25	34.7	34.4	15.9	60.0	32.7	0.3	0.8
C5	5	0.99	1.71	0.68	0.40	65.1	64.3	13.8	128.4	37.7	0.8	1.2
C5a	3	1.00	0.07	0.04	0.55	1.6	1.6	0.0	3.6	23.5	0.0	0.0
C7	4	0.97	1.49	0.76	0.51	55.7	54.0	0.0	118.7	36.1	1.8	3.2
C7a	3	0.98	1.62	0.88	0.54	39.1	38.2	0.0	87.4	23.6	0.8	2.2
C8	4	0.97	0.99	0.51	0.51	27.1	26.4	0.0	58.9	26.8	0.7	2.5
C9	6	0.98	1.24	0.29	0.23	43.5	42.7	20.7	72.9	34.5	0.8	1.9
E2	4	0.99	1.32	0.70	0.53	56.7	56.4	0.0	128.7	42.8	0.3	0.6
E4	3	1.00	0.19	0.10	0.52	5.2	5.2	0.0	11.8	28.0	0.0	0.0
Core total	-	-	-	-	-	542.5	535.7	-	-	-	6.8	1.3
BK	5	0.99	0.56	0.23	0.41	325.5	322.9	58.7	659.1	578.4	2.6	0.8
Survey total	60	-	0.80	0.14	0.17	868.0	858.6	483.9	1380.1	1070.2	9.4	1.1

Table 20: 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 2019. Columns give the number of stations sampled (No. stns), the mean oyster density of oysters per square metre expected to die (Mean density), the standard deviation of the mean density (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size at the time of survey (Pop.n, filled light grey) mean population size of the millions of oysters estimated to die (Losses, shaded medium grey), lower and upper 95% confidence intervals (95% CI) in millions of oysters, the area of each stratum (Area (km²)) in square kilometres, and the percentage mortality (% Mortality, shaded dark grey) by stratum.

Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Losses	Lower 95% CI	Upper 95% CI	Area (km ²)	% Mortality
B1	4	0.00	0.00	0.00	49.9	0.0	0.0	0.0	78.2	0.0
B3	7	0.00	0.00	1.00	97.8	0.1	0.0	0.3	44.7	0.1
B6	3	0.02	0.02	1.00	34.5	0.7	0.0	2.1	30.0	2.0
C1a	3	0.00	0.00	0.00	22.7	0.0	0.0	0.0	31.3	0.0
C2	3	0.02	0.02	1.00	8.9	0.5	0.0	1.5	21.9	5.4
C3	3	0.01	0.01	1.00	34.7	0.3	0.0	0.9	32.7	0.8
C5	5	0.02	0.02	0.89	65.1	0.8	0.0	2.3	37.7	1.2
C5a	3	0.00	0.00	0.00	1.6	0.0	0.0	0.0	23.5	0.0
C7	4	0.05	0.04	0.72	55.7	1.8	0.0	4.7	36.1	3.2
C7a	3	0.04	0.03	0.78	39.1	0.9	0.0	2.3	23.6	2.2
C8	4	0.03	0.02	0.87	27.1	0.7	0.0	2.0	26.8	2.5
C9	6	0.02	0.02	0.63	43.5	0.8	0.0	2.0	34.5	1.9
E2	4	0.01	0.01	1.00	56.7	0.3	0.0	1.0	42.8	0.6
E4	3	0.00	0.00	0.00	5.2	0.0	0.0	0.0	28.0	0.0
Core total	55	0.01	0.00	0.30	542.5	6.8	2.6	12.3	491.8	1.3
BK	5	0.00	0.00	0.83	325.5	2.6	0.0	7.3	578.4	0.8
Survey total	60	0.01	0.00	0.31	868.0	9.4	3.5	17.2	1070.2	1.1

Table 21: Summer mortality in 2012 and for 2015–2019 in the stock assessment survey area and for 2015–2019 in the Bonamia survey area. Summer mortality is 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 assessment survey area						Bonamia survey area				
	2012	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019
Pre-survey mortality											
Recruit-sized new clocks (NC, millions)	30.0	23.7	3.6	7.8	3.4	9.2	13.5	1.4	5.3	2.9	4.1
Post-survey mortality											
Correction factor (millions of oysters)	81.1	49.0	24.3	18.3	23.1	9.4	34.4	14.8	13.4	15.7	6.8
Scaled catch (millions of oysters)	56.9	50.9	24.8	*12.9	10.7	9.4	31.6	14.8	10.9	7.3	6.8
Combined summer mortality											
Correction factor +NC (millions of oysters)	111.1	72.7	27.9	26.1	26.5	18.6	47.9	16.2	18.7	18.6	10.9
Scaled catch +NC (millions of oysters)	86.9	74.6	28.4	*20.7	14.1	18.6	45.1	16.2	16.2	10.2	10.9
Population before summer mortality											
Recruit-sized oysters +NC (millions of oysters)	948.4	533.6	564.7	535.2	886.7	868.0	364.9	386.6	368.9	497.0	542.5
Percent summer mortality											
Correction factor +NC (%)	11.7	13.6	4.9	4.9	3.0	2.1	13.1	4.2	5.1	3.7	2.0
Scaled catch +NC (%)	9.2	14.0	5.1	*3.9	1.6	2.1	12.4	4.2	4.4	2.1	2.0

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

3.6 The status of the OYU 5 fishery in 2019 and future trends

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 suggested 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 29).

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 (consistent 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 29).

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, 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 assessment area, and 47.2% in the *Bonamia* survey area (688.1 million oysters in 2012 to 363.6 million oysters in 2017). *Bonamia* mortality declined over the stock assessment area to about 5% of the recruit-sized population in 2016 and 2017 (see Table A6.1 (Appendix 6) for references). 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. This upward trend in recruit-size stock abundance and recruitment, and low *Bonamia* mortality, continued into the summer of 2018–19. The numbers of spat per collector were similar to the previous summer, small oysters per recruit continued to increase, and the recruit-sized population increased by around 9.8% between 2018 and 2019, as did pre-recruit sized oysters (around 21.4%) and small oysters (around 48.3%).

The future status of the fishery is best represented by series “a” in Figure 29 which assumes no *Bonamia* mortality; actual mortality was 2%. Moreover, non-fatal infections have declined to about 0.1% of the recruit-sized population, suggesting low *Bonamia* mortality in 2020. Relatively low oyster densities and low non-fatal infections suggest reduced transmission of disease.

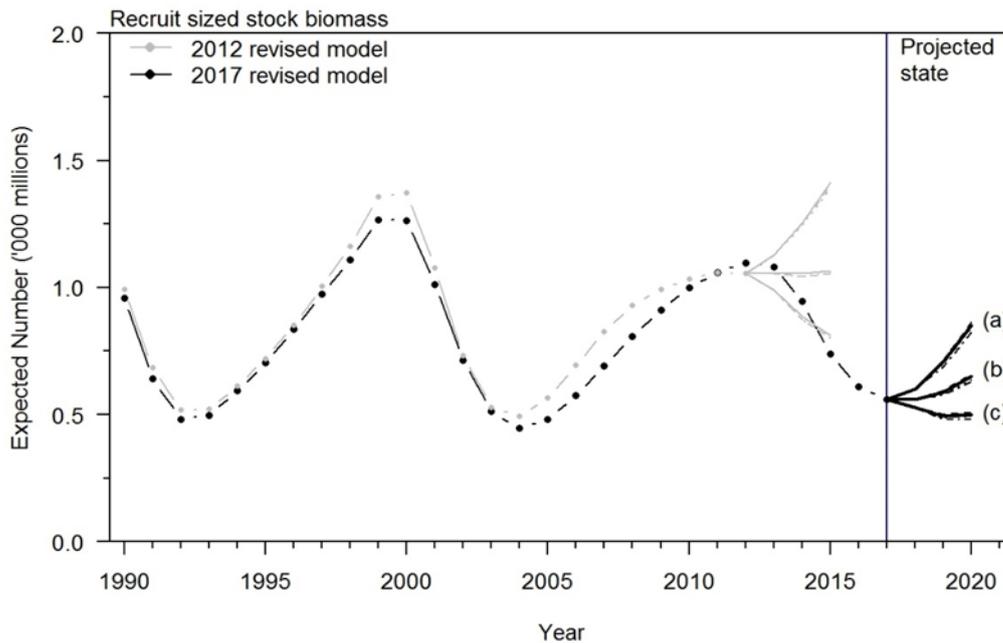


Figure 29: Model estimates of recent recruit-sized stock abundance and projected recruit-sized stock abundance with catches of 7.5 (solid line), 15 (dash dot), and 30 million oysters (dash line) under assumptions of (a) no disease mortality, (b) disease mortality of 0.10 y^{-1} , and (c) disease mortality 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. 2018a).

4. 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 populations 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 CVs for population estimates, well below the 20% target set by Fisheries New Zealand for stock assessment surveys. A CV of 13% was obtained for estimates of recruit-sized oysters in the *Bonamia* survey area in 2019.

The objectives of *Bonamia* surveys have changed over time (see Michael et al. 2016). A new time series of *Bonamia* and oyster surveys, incorporating a fully randomised, two-phase sampling design and a standard *Bonamia* survey area, was established in 2014 to make these surveys comparable from year to year. The February 2019 survey is the fifth 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.

4.1 Survey results

This survey used the same vessel, skipper and crew, and standard sampling methods as for previous Foveaux Strait surveys. Sampling conditions during the 2019 survey were not expected to have affected dredge efficiency. Additionally, the CVs obtained for population estimates from the survey were a little higher (13%) than those predicted for recruit-sized oysters (CV of 11%), probably because the rebuilding of localised, relatively high-density populations have increased the variation in oyster densities. The timing of *Bonamia* surveys coincides with a period of peak seasonal mortality from *Bonamia* and the shedding of infective particles. In 2019, some *Bonamia* mortality had occurred before the survey (estimated as new clocks), but most of the mortality was expected shortly after the survey (category 3 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. This 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 and 2019 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 6.4% of recruit-sized oysters in the *Bonamia* survey area.

Stock assessment and *Bonamia* surveys estimate oyster densities and mean population sizes by stratum, 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. Strata with high density patches (“oyster beds”) are best represented by those with recruit-sized oyster densities greater than 1.0 oysters m⁻². In 2019 there were six strata (B3, C5, C7, C7a, C8, and E2) that had recruit-sized oyster densities greater than 400 oysters per tow (1.0 oysters m⁻²). In the *Bonamia* survey area, the recruit-sized oyster population above this threshold was 202.1 million oysters (95% CI 46.7–399.1) and, at a TACC of 15 million oysters, this represents an exploitation rate of 7.4% of this portion of the stock.

4.2 Outlook for the 2019 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 2014), 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 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 2.4 S/H (95% CI 2.4–2.5) in 2018. Although the size of the recruit-sized population continues to increase, catch rates of commercial-sized oysters are expected to remain similar to 2018 levels for the 2019 season. Oysters growing to recruit size in 2019 may not grow to “commercial size” for another 1–2 years.

In the medium-term, all the key indicators for the future rebuilding of the fishery are strongly positive. The three size groups of oysters continued to increase between 2018 and 2019. Spat monitoring, catch

sampling, and the survey data show significant recruitment to the oyster population, and increases in pre-recruit and small oysters will support future increases in recruit-sized oysters. *Bonamia* mortality was low in 2018 and 2019, and it is expected to be low in 2020. 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.

5. ACKNOWLEDGEMENTS

We thank Graeme Wright (BOMC) for his support and for facilitating the survey and David Skeggs for the support of BOMC, Stephen Hawke and his crew (Josh Tarrant, Victoria Pearsey, Rex Ryan, Terry Cleaver, and Keith Lovett), and Graeme Moss (NIWA) for the long days at sea and tremendous effort put in to complete the survey to a high standard.

Thanks also to Sadie Mills, Kate Neil, Dean Stotter, Anna Kilimnik, Diana MacPherson, Reyn Naylor, and others who helped process oyster samples at NIWA Wellington (Greta Point). We thank Alistair Dunn who developed scripts for the analyses of these data, and Reyn Naylor and Marine Pomarède (Fisheries New Zealand) for reviewing this report and comments on the manuscript. This investigation was funded by the Ministry for Primary Industries under Project OYS2017/01, objectives 1–4.

6. REFERENCES

- Andrew, N.L.; Hine, P.M.; Cranfield, H.J.; Dunn, A.; Michael, K.P. (2000). Foveaux Strait dredge oyster strategic research plan for the period 2000-2004. Final Research Report to the Ministry of Fisheries. (Unpublished report held by Fisheries New Zealand, Wellington. 10 p.)
- Bilewitch, J.; Sutherland, J.; Hulston, D.; Forman, J.; Michael, K.P. (2018). Genetic quantification of *Bonamia exitiosa* infection levels in the flat oyster *Ostrea chilensis*: qPCR & ddPCR quantification of *B. exitiosa*. Prepared for Seafood Innovations Ltd. NIWA Client Report: 2018056WN (Project SIL15304). 51 p.
- Cranfield, H.J.; Doonan, I.J.; Michael, K.P. (1991) Assessment of the effects of mortality due to *Bonamia* on the oyster population of Foveaux Strait in 1990 and the outlook for management in 1991. New Zealand Fisheries Assessment Research Document 91/18. (Unpublished report held in the NIWA Wellington library). 24 p.
- Cranfield, H.J.; Dunn, A.; Doonan, I.J.; Michael, K.P. (2005). *Bonamia exitiosa* epizootic in *Ostrea chilensis* from Foveaux Strait, southern New Zealand between 1986 and 1992. *ICES Journal of Marine Science* 62(1): 3–13.
- Cranfield, H.J.; Michael, K.P.; Doonan, I.J. (1999) Foveaux Strait oyster (*Tiostrea chilensis*) assessment, 1997. New Zealand Fisheries Assessment Research Document 99/11. (Unpublished report held by the NIWA Wellington library) 31 p.
- Diggles, B.K.; Cochenec-Laureau, N.; Hine, P.M. (2003). Comparison of diagnostic techniques for *Bonamia exitiosus* from flat oysters *Ostrea chilensis* in New Zealand. *Aquaculture*. 220(1–4): 145–156.
- Doonan, I.J.; Cranfield, H.J.; Michael, K.P. (1994). Catastrophic reduction of the oyster, *Tiostrea chilensis* (Bivalvia: Ostreidae), in Foveaux Strait, New Zealand, due to infestation by the protistan *Bonamia* sp. *New Zealand Journal of Marine and Freshwater Research*, 28(4): 335–344.
- Doonan, I.J.; Rasmussen, S. (2012). Random Station User Manual (RandonStation v1.00-2012-06-13). NIWA unpublished report. 47 p.
- Dunn, A. (2005). Stock assessment of Foveaux Strait dredge oysters (*Ostrea chilensis*) for the 2003–04 fishing year. *New Zealand Fisheries Assessment Report 2005/25*. 63 p.
- Francis, R.I.C.C. (1984). An adaptive survey for stratified random trawl surveys. *New Zealand Journal of Marine and Freshwater Research* 18: 59–71.

- Francis, R.I.C.C. (2006). Optimum allocation of stations to strata in trawl surveys. *New Zealand Fisheries Assessment Report 2006/23*. 50 p.
- Fu, D. (2013). An updated stock assessment for Foveaux Strait dredge oysters (*Ostrea chilensis*) for the 2012 fishing year. Final Research Report to the Ministry for Primary Industries for project OYS2009/01C, objective 1. (Unpublished report held by Fisheries New Zealand, Wellington). 56 p.
- Fu, D.; Dunn, A. (2009). An updated stock assessment for Foveaux Strait dredge oysters (*Ostrea chilensis*) for the 2008–09 fishing year. *New Zealand Fisheries Assessment Report 2009/53*. 71 p.
- Fu, D.; Dunn, A.; Michael, K.P.; Hills, J. (2016). The development and performance of a length-based stock assessment of Foveaux Strait oysters (*Ostrea chilensis*, OYU 5) in southern New Zealand, and application to management. *Fisheries Research 183*: 506–517.
- Hill-Spanik, K.M.; McDowell, J.R.; Stokes, N.A.; Reece, K.S.; Burreson, E.M.; Carnegie, R.B. (2015). Phylogeographic perspective on the distribution and dispersal of a marine pathogen, the oyster parasite *Bonamia exitiosa*. *Marine Ecology Progress Series 536*: 65–76.
- Hine, P.M. (1991). The annual pattern of infection by *Bonamia* sp in New Zealand flat oysters, *Tiostrea chilensis*. *Aquaculture 93*(3): 241–251.
- Jolly, G.M.; Hampton, I. (1990). A stratified random transect design for acoustic surveys of fish stocks. *Canadian Journal of Fisheries and Aquatic Sciences 47*:1282–1291.
- Large, K.; Doonan, I.J.; Michael, K.P. (2018a). Foveaux Strait oyster (OYU 5) stock assessment. An updated stock assessment for Foveaux Strait dredge oysters (*Ostrea chilensis*) for the 2017 fishing year. Prepared for Bluff Oyster Management Company Ltd. *NIWA Client Report: 2018196WN* (Project BOM17302). 117 p.
- Large, K.; Michael, K.P.; Wright, G. (2018b). OYU 5 Catch Sampling 2016. Estimates of the length frequency distribution of Foveaux Strait dredge oysters (*Ostrea chilensis*) from catch sampling in 2016. Prepared for Bluff Oyster Management Company Ltd (BOMC). *NIWA Client Report: 2018197WN* (Project BOM17302). 41 p.
- Maas, E.; Michael, K.P.; Mackay, J.; Hulston, D.; Forman, J. (2013). Developing tools to monitor *Bonamia*. Final Research Report to the Ministry for Primary Industries, for project OYS201101. (Unpublished report held by Fisheries New Zealand, Wellington.) 42 p.
- Michael, K.P. (2010). A strategic research plan (2010–2015) to underpin management goals of the 2009 Fisheries Plan for Foveaux Strait oysters (*Ostrea chilensis*, OYU 5). *New Zealand Fisheries Assessment Report 2010/21*. 35 p.
- Michael, K.P.; Bilewitch, J.; Forman, J.; Hulston, D.; Sutherland, J.; Moss, G.; Large, K. (2019a). 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. *New Zealand Fisheries Assessment Report 2019/02*. 61 p.
- Michael, K.P.; Dunn, A. (2005). Foveaux Strait dredge oyster (*Ostrea chilensis*) OYU 5 Strategic Research Plan. Unpublished report held by NIWA, Wellington. 41 p.
- Michael, K.P.; Forman, J.; Hulston, D. (2015a). A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU5) in commercial fishery areas and the status of *Bonamia* (*Bonamia exitiosa*) in February 2015. *New Zealand Fisheries Assessment Report 2015/73*. 86 p.
- Michael, K.P.; Forman, J.; Hulston, D.; Bilewitch, J.; Moss, G. (2019b). Foveaux Strait oyster and *Bonamia* surveys, February 2017. *New Zealand Fisheries Assessment Report 2019/46*. 83 p.
- Michael, K.P.; Forman, J.; Hulston, D.; Fu, D.; Maas, E. (2015b). A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU 5) in commercial fishery areas and the status of *Bonamia* (*Bonamia exitiosa*) in February 2014. *New Zealand Fisheries Assessment Report 2015/40*. 107 p.
- Michael, K.P., Forman, J., Hulston, D., Sutherland, J. (2016). A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU5) in commercial fishery areas and the status of *Bonamia* (*Bonamia exitiosa*) in February 2016. *New Zealand Fisheries Assessment Report 2016/67*. 95 p.

- Michael, K.P.; Fu, D.; Forman, J.; Hulston, D. (2013). The Foveaux Strait oyster (*Ostrea chilensis*, OYU5) stock assessment survey and status of bonamia infection and mortality, February 2012. *New Zealand Fisheries Assessment Report 2013/09*. 64 p.
- Michael, K.P., Forman, J., Maas, E., Hulston, D., Fu, D. (2014). The status of infection by bonamia (*Bonamia exitiosa*) in Foveaux Strait oysters (*Ostrea chilensis*) in February 2013, estimates of summer disease mortality, and implications for the projections of future stock status made in the 2012 stock assessment for OYU 5. *New Zealand Fisheries Assessment Report 2014/49*. 63 p.
- Ministry of Fisheries (2009). Foveaux Strait Dredge Oyster Fisheries Plan, May 2009. Ministry of Fisheries. www.fish.govt.nz. 15 p.

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 Longitude S E Depth (m) Speed (knots)

Finish position Latitude Longitude S E

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

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

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

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

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

Sediment type

Circle the main type (one only)

Weed Shell Shell/sand Shell/gravel Pea gravel Sand Silt Sponges Bryozoa

0 1 2 3 4 5 6 7 8

Comments: _____

1 Nautical mile = 1.853 km

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

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

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

APPENDIX 2: BONAMIA SAMPLING 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												
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Site description/comments:					Recorder	Checker	Heart/Gill plate 25th	Extra Gillx2 plate25th

Size category: 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

APPENDIX 3: PROCESSING OF OYSTER TISSUES AND TESTING FOR *BONAMIA EXITIOSA*

Sampling of oyster tissues

Samples of up to 30 randomly selected recruit-sized oysters are required from each station to determine the status of *Bonamia* infection. When there were insufficient recruit-sized oysters in the catch, pre-recruit and small oysters were used to fill the sample size, or the whole catch was retained for processing. Samples were bagged, labelled with station number, date, and time on waterproof labels and the sacks tied securely. The oysters for *Bonamia* samples were kept cool and damp in oyster sacks, transferred to poly bins, and flown to NIWA Wellington for processing. Oyster samples generally arrived in Wellington within 36 hours of capture and were mostly processed on the day of arrival. The samples were held in poly bins under cool conditions (about 8–12 °C) in the aquarium. Oyster samples not processed the day they arrived were processed the following day.

For each sample, station and sample data were recorded on *Bonamia* sampling forms (an example given in Appendix 2), and the total numbers of live and dead oysters in the samples noted. A subsample of up to 25 recruit-sized oysters from each station was taken for heart imprints and droplet digital polymerase chain reaction (ddPCR) analysis to estimate the prevalence and intensity of *Bonamia*. Each oyster in the sample was assigned a unique number from 1 to 25, assigned a size category using oyster size rings, and measured for length and height using callipers (Figure A3.1), and the measurement was truncated to the lower whole millimetre. If samples contained insufficient recruit-sized oysters, pre-recruits were used in preference to small oysters. Recruit-size oysters were denoted with an R, pre-recruit oysters with P, and small oysters with an O. Gaping oysters with valves of the shell apart, but which closed when tapped, were marked with an asterisk alongside the corresponding oyster number. Oysters incubating larvae were recorded as either white (early-stage) larvae, grey (late-stage) larvae, yellow (almost ready to settle) larvae, or with no larvae present (coded NA).

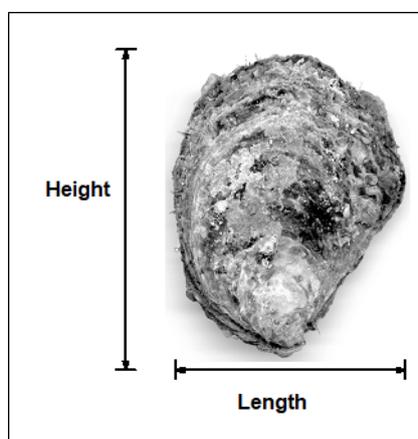


Figure A3.1: An oyster showing length (anterior-posterior axis) and height (dorsal-ventral axis) dimensions.

Droplet digital polymerase chain reaction (ddPCR) testing method

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

Review of ddPCR procedures prior to testing

Before the samples from the 2019 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. Less than 1 copy/ μl 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.

Estimates of prevalence and intensity of infection using ddPCR

In 2019, the prevalence of infection was first determined by ddPCR methods and then by heart imprints (see below). All ddPCR-positive samples and a random subsample of 3–5 ddPCR-negative samples were screened for *Bonamia* infection using heart imprints. These oysters were also scored for intensity of infection using the categorical methods of Diggles et al. (2003) (see below).

Laboratory work sheets recorded sampling data including date, name of sampler, plate number, station number, and the date and time the sample was collected. Heart tissues from 25 oysters at each station were analysed for *Bonamia* infection using ddPCR. The oyster samples were tested using an assay 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. 4 μl of 1:20-diluted tissue digests were combined with BioRad ddPCR SuperMix, primers and probes in a total volume of 23 μl . 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.

The ddPCR data from tested survey samples were 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 103 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 103 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. The repeated ddPCR scorings were used in the analysis for presence/absence and quantification.

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 the final quantification value was relative, because 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, the ddPCR tests 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.

Heart imprint methods

Heart imprints were made by removing the heart (dark organ adjacent to adductor muscle, see Figure A3.2) with fine forceps, draining excess water and fluid on filter paper, and lightly dabbing the heart on a slide to deposit a small amount of haemolymph. Three rows of 8 to 10 imprints were made on

labelled slides. Slides were placed in slide racks to air dry for at least 5 minutes. The slides were stained with Hemacolor © and oven dried at 60 °C.

Analysis of oyster heart imprint data

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

The prevalence and intensity of *Bonamia* infection was determined from heart imprints in all oyster samples that had tested positive by ddPCR from all 72 stations. A further 3 or more randomly selected samples from each station that tested negative with ddPCR were also examined. Oyster heart imprints were examined under a microscope using a times 50 objective lens under oil and scored for intensity of infection using the criteria listed in Table A3.1. Three good heart imprints containing oyster haemocytes were located and examined on each slide, and the number of *Bonamia* cells counted for each. If no *Bonamia* cells were found, further imprints were examined to confirm the absence of *Bonamia*. In 2019, heart imprints were examined by a single experienced reader. A review of scoring protocols was undertaken before screening samples.

Table A3.1: Criteria used to stage intensity of bonamia infection in oysters.

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

Histology

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

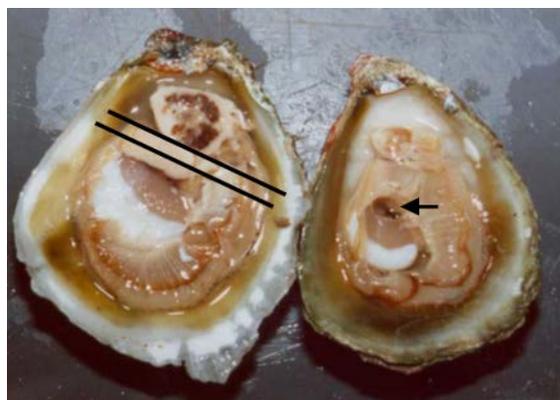


Figure A3.2: Lines on left oyster show location of 5 mm thick standard section taken for histology. The arrow on the oyster on the right shows the heart, a black organ adjacent to the adductor muscle.

The comparison of estimates of the intensity of infection estimated from heart imprints and ddPCR

Estimates of the intensity of *Bonamia exitiosa* infection from heart imprints and ddPCR are not directly comparable because heart imprints score the numbers of *B. exitiosa* parasites in haemocytes using the methods of Diggle et al. (2003) and ddPCR estimates the numbers of *B. exitiosa* gene copies in the sample. However, there is a good relationship between the increasing intensity of infection shown by heart imprints and an increase in the ratio of *B. exitiosa* DNA to *Ostrea chilensis* DNA in standard ddPCR samples (Figure A3.3). ddPCR is much more sensitive in detecting low, non-fatal infections, shown by the positive levels of infection shown in Figure A3.3 where heart imprints were not able to detect infection (score 0). At intensifying levels of infection (heart imprint score 3) and fatal infections (scores 4 & 5), the relative level of infection from ddPCR increases rapidly (Figure A3.3).

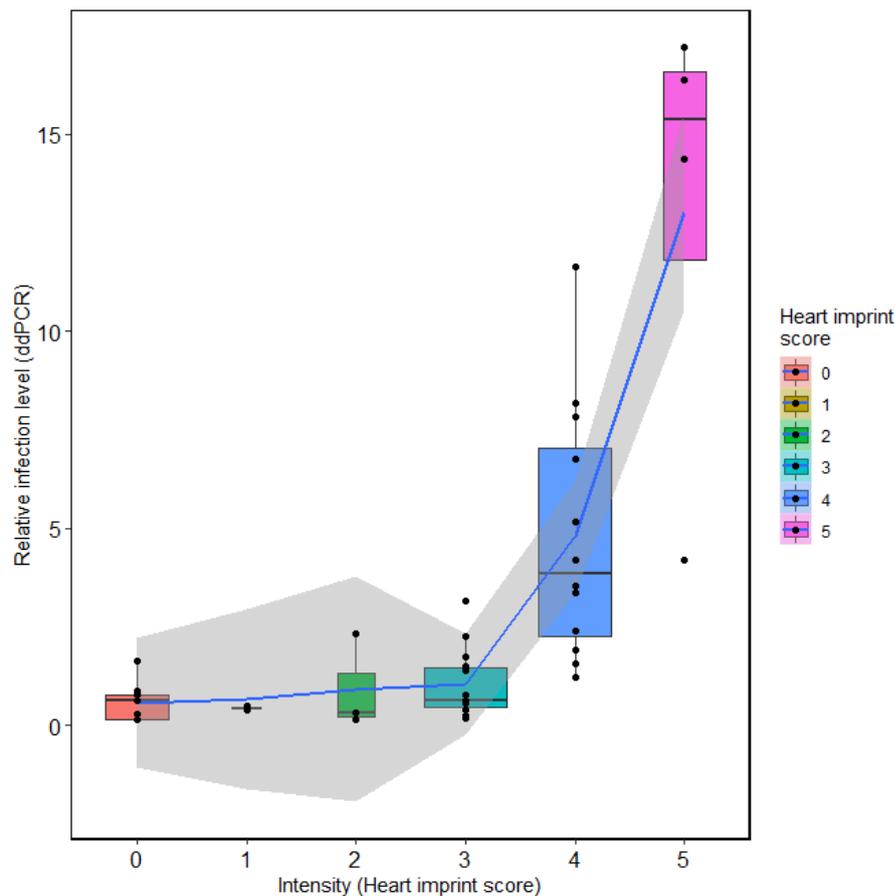


Figure A3.3: Boxplots of the intensity of *Bonamia exitiosa* from ddPCR calculated as the ratio of the concentration of *Bonamia* targets to the concentration of β -actin targets in each sample plotted by the categorical intensity of infection from heart imprints using the methods of Diggle et al. (2003). Medians shown as solid lines, boxes represent 50th percentiles and whiskers 95th percentiles, and outliers as filled black circles. Loess smoothing shown as a blue line and $1\pm SE$ shaded grey.

APPENDIX 4: SURVEY CONDITIONS AND COMPARABILITY

Dredge tow lengths were almost all 0.2 nautical miles (371 m, 95% CI 0.197–0.206) in length (Figure A4.1). 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 A4.2). 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. Operational limits for dredge sampling of 20 knots and Figure A4.2 show the February 2019 survey was undertaken in similar conditions to previous February surveys.

Oyster dredges are considered saturated and cease fishing before the end of tow when they are more than 80% full on landing (J. 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 70% with a median fullness of 40%, the same as in 2015–2018, 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 2019 survey (Figure A4.3). Observations and anecdotal evidence from video data recorded during dredge trials suggest that dredge saturation may occur in dredges landed less than 80% full; however, when this occurred, 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 2019 survey data; future surveys will identify stations with this pattern in the distribution of catch. No stations were landed over 70% full in 2019.

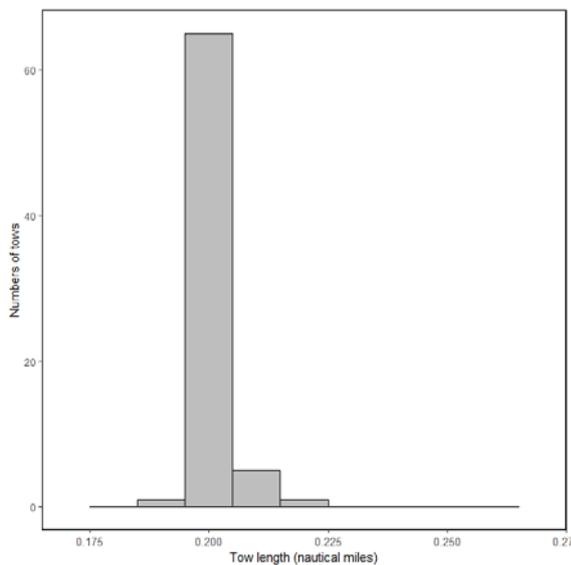


Figure A4.1: Distribution of dredge tow lengths from the February 2019 survey. The standard tow length was 0.2 nautical mile (371 m).

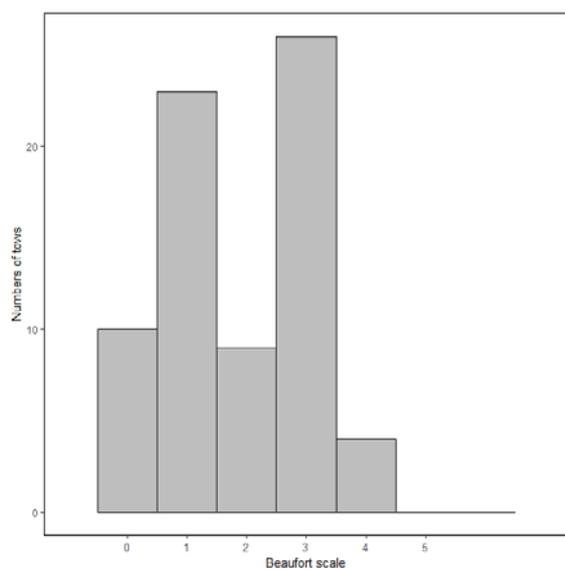


Figure A4.2: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2019. Beaufort scale: 0, < 1 knot; 1, 1–2 knots; 2, 3–6 knots; 3, 7–10 knots; 4, 11–15 knots; 5, 16–20 knots; and 6, 21–26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

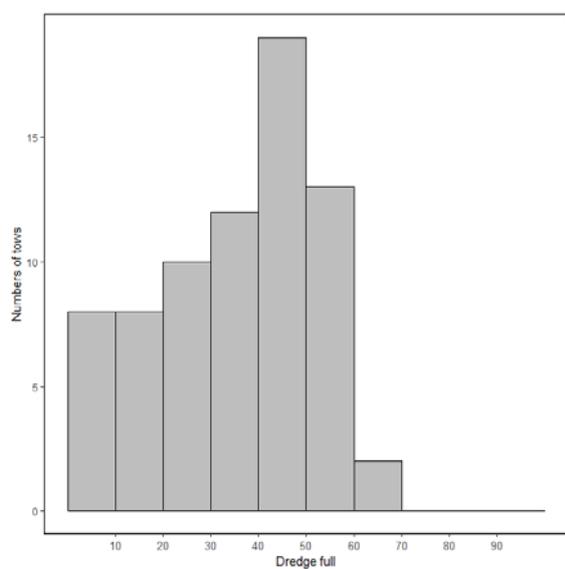


Figure A4.3: Distribution of dredge fullness (%) recorded for survey tows in February 2019. No tows were landed with a dredge fullness of greater than 80%. Unpublished video data suggests that dredge saturation may occur below 80% full.

APPENDIX 5: 2019 SURVEY CATCH AND INFECTION DETAILS

Table A5.1: 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 2019 survey.

Str	Stn	Recruits	Density	Total	Un.inf	NF.inf	Fat.inf	%Prev	Int	% ddP
B1	1	167	0.14	25	25	0	0	0	NA	0
B1	2	6	0.00	13	13	0	0	0	NA	0
B1	3	190	0.16	25	25	0	0	0	NA	0
B1	4	161	0.13	25	25	0	0	0	NA	0
B1	T2	354	0.29	25	25	0	0	0	NA	0
B3	5	1 317	1.08	25	25	0	0	0	NA	0
B3	6	523	0.43	25	24	1	0	4	1	0.2
B3	7	314	0.26	24	24	0	0	0	NA	0
B3	8	201	0.16	25	25	0	0	0	NA	0
B3	62	388	0.32	25	25	0	0	0	NA	0
B3	63	83	0.07	25	24	0	1	4	4	0.2
B3	64	326	0.27	25	25	0	0	0	NA	0.2
B6	9	176	0.14	25	23	0	2	8	3.5	0.2
B6	10	263	0.21	25	25	0	0	0	NA	0
B6	11	267	0.22	25	25	0	0	0	NA	0
BK	52	88	0.07	25	25	0	0	0	NA	0
BK	53	7	0.01	21	19	0	2	10	4.5	0.2
BK	54	98	0.08	25	24	0	1	4	3	0.2
BK	140	294	0.24	25	25	0	0	0	NA	0
BK	144	90	0.07	25	25	0	0	0	NA	0
BK	T6	20	0.02	25	25	0	0	0	NA	0
BK	T10	12	0.01	25	25	0	0	0	NA	0
C2	15	39	0.03	25	25	0	0	0	NA	0
C2	16	98	0.08	25	25	0	0	0	NA	0
C2	84	115	0.09	25	22	0	3	12	3.5	0.2
C2	T7	429	0.35	25	21	0	4	16	3	0.2
C3	18	212	0.17	25	25	0	0	0	NA	0.2
C3	19	129	0.11	25	24	0	1	4	3	0.2
C3	20	314	0.26	25	25	0	0	0	NA	0
C3	T8	552	0.45	25	24	0	1	4	4	0.2
C5	21	49	0.04	25	24	0	1	4	3	0.2
C5	22	875	0.71	25	25	0	0	0	NA	0
C5	23	306	0.25	25	25	0	0	0	NA	0
C5	92	282	0.23	25	25	0	0	0	NA	0
C5	93	247	0.20	25	23	0	2	8	2	0.2
C5	T9	1 133	0.93	25	24	1	0	4	2	0.2
C7	27	103	0.08	25	24	0	1	4	3	0.2
C7	29	117	0.10	25	24	0	1	4	4	0.2
C7	30	796	0.65	25	24	0	1	4	3	0.2
C7	104	254	0.21	25	25	0	0	0	NA	0
C8	34	51	0.04	25	22	2	1	12	2	0.2
C8	35	286	0.23	25	25	0	0	0	NA	0
C8	36	19	0.02	25	24	1	0	4	2	0.2
C8	37	472	0.39	25	24	0	1	4	4	0.2
C8	T5	609	0.50	25	24	0	1	4	3	0.2

Table A5.1: continued

Str	Stn	Recruits	Density	Total	Un.inf	NF.inf	Fat.inf	%Prev	Int	% ddP
C8	T5	609	0.50	25	24	0	1	4	3	0.2
C9	38	366	0.30	25	24	0	1	4	5	0.2
C9	39	238	0.19	25	25	0	0	0	NA	0
C9	40	398	0.33	25	25	0	0	0	NA	0
C9	41	109	0.09	25	25	0	0	0	NA	0
C9	42	60	0.05	25	25	0	0	0	NA	0
C9	43	370	0.30	25	24	0	1	4	5	0.2
E2	44	238	0.19	25	25	0	0	0	NA	0
E2	45	17	0.01	25	25	0	0	0	NA	0
E2	46	677	0.55	25	25	0	0	0	NA	0
E2	47	151	0.12	25	24	0	1	4	4	0.2
E2	T3	238	0.19	25	25	0	0	0	NA	0
E2	T4	343	0.28	25	24	0	1	4	3	0.2
E4	48	0	0.00	25	25	0	0	0	NA	0
E4	49	67	0.05	25	25	0	0	0	NA	0
E4	50	47	0.04	25	25	0	0	0	NA	0
E4	T11	46	0.04	25	25	0	0	0	NA	0.2
E4	T12	259	0.21	25	25	0	0	0	NA	0
C1a	12	11	0.01	18	18	0	0	0	NA	0
C1a	13	346	0.28	25	25	0	0	0	NA	0
C1a	14	90	0.07	25	25	0	0	0	NA	0
C5a	24	0	0.00	25	25	0	0	0	NA	0
C5a	26	15	0.01	25	25	0	0	0	NA	0
C5a	98	26	0.02	25	25	0	0	0	NA	0
C5a	T1	123	0.10	25	25	0	0	0	NA	0
C7a	31	233	0.19	25	23	0	2	8	4.5	0.2
C7a	32	697	0.57	25	25	0	0	0	NA	0
C7a	33	92	0.08	25	24	0	1	4	3	0.2

APPENDIX 6: REFERENCES FOR SURVEYS 2010–2018

Table A6.1: Fisheries Assessment Reports for Foveaux Strait oyster and Bonamia surveys 2010–2018. Reports include estimates of oyster densities, population sizes, and CVs by stratum, core strata combined, background stratum, and the whole survey area.

Survey year	Citation
2010	Michael, K.P.; Forman, J.; Hulston, D.; Fu, D. (2011). The status of infection by bonamia (<i>Bonamia exitiosa</i>) in Foveaux Strait oysters (<i>Ostrea chilensis</i>), changes in the distributions and densities of recruit, pre-recruit, and small oysters in February 2010, and projections of disease mortality. <i>New Zealand Fisheries Assessment Report 2011/5</i> . 51 p.
2011	Michael, K.P.; Forman, J.; Hulston, D.; Fu, D. (2012). The status of infection by bonamia (<i>Bonamia exitiosa</i>) in Foveaux Strait oysters (<i>Ostrea chilensis</i>) in February 2011, estimates of pre-survey and projections of post-survey disease mortality, and implications for the projections of future stock status made in the 2009 stock assessment for OYU 5. <i>New Zealand Fisheries Assessment Report 2012/37</i> . 57 p.
2012	Michael, K.P.; Fu, D.; Forman, J.; Hulston, D. (2013). The Foveaux Strait oyster (<i>Ostrea chilensis</i> , OYU5) stock assessment survey and status of bonamia infection and mortality, February 2012. <i>New Zealand Fisheries Assessment Report 2013/09</i> . 64 p.
2013	Michael, K.P.; Forman, J.; Maas, E.; Hulston, D.; Fu, D. (2014). The status of infection by bonamia (<i>Bonamia exitiosa</i>) in Foveaux Strait oysters (<i>Ostrea chilensis</i>) in February 2013, estimates of summer disease mortality, and implications for the projections of future stock status made in the 2012 stock assessment for OYU 5. <i>New Zealand Fisheries Assessment Report 2014/49</i> . 63 p.
2014	Michael, K.P.; Forman, J.; Hulston, D.; Maas, E.; Fu, D. (2015). A survey of the Foveaux Strait oyster (<i>Ostrea chilensis</i>) population (OYU5) in commercial fishery areas and the status of bonamia (<i>Bonamia exitiosa</i>) in February 2014. <i>New Zealand Fisheries Assessment Report 2015/40</i> . 107 p.
2015	Michael, K.P.; Forman, J.; Hulston, D. (2015). A survey of the Foveaux Strait oyster (<i>Ostrea chilensis</i>) population (OYU5) in commercial fishery areas and the status of bonamia (<i>Bonamia exitiosa</i>) in February 2015. <i>New Zealand Fisheries Assessment Report 2015/73</i> . 86 p.
2016	Michael, K.P.; Forman, J.; Hulston, D.; Sutherland, J. (2016). A survey of the Foveaux Strait oyster (<i>Ostrea chilensis</i>) population (OYU5) in commercial fishery areas and the status of bonamia (<i>Bonamia exitiosa</i>) in February 2016. <i>New Zealand Fisheries Assessment Report 2016/67</i> . 95 p.
2017	Michael, K.P.; Forman, J.; Hulston, D.; Bilewitch, J.; Moss, G. (2019). Foveaux Strait oyster and Bonamia surveys, February 2017. <i>New Zealand Fisheries Assessment Report 2019/46</i> . 83 p.
2018	Michael, K.P.; Bilewitch, J.; Forman, J.; Hulston, D.; Sutherland, J.; Moss, G.; Large, K. (2019). A survey of the Foveaux Strait oyster (<i>Ostrea chilensis</i>) population (OYU 5) in commercial fishery areas and the status of Bonamia (<i>Bonamia exitiosa</i>) in February 2018. <i>New Zealand Fisheries Assessment Report 2019/02</i> . 61 p.