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Annual surveys of the Foveaux Strait oyster (*Ostrea chilensis*) fishery (OYU 5) and *Bonamia exitiosa* prevalence, intensity, and disease mortality in February 2022

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EXECUTIVE SUMMARY

Michael, K.P.[1]; Bilewitch, J.; Forman, J.; Smith, L.; Moss, G. (2023). Annual surveys of the Foveaux Strait oyster (*Ostrea chilensis*) fishery (OYU 5) and *Bonamia exitiosa* prevalence, intensity, and disease mortality in February 2022.

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Two-phase, random stratified dredge surveys of Foveaux Strait oysters (OYU 5) have been undertaken since 1990. Since 2012, 26 survey strata have been sampled in stock assessment surveys. The most recent stock assessment survey was in 2017, with the next scheduled for 2023. The introduction of five-yearly stock assessments in 2012 has placed greater onus on annual, smaller surveys to monitor changes in the oyster population in commercial fishery areas, as well as the status of *Bonamia exitiosa*, a pathogen that causes high mortality in Foveaux Strait oysters. These (Bonamia) surveys represent 14 of the 26 stock assessment survey strata (46% of the full survey area). 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. Bonamia surveys update information on:

- oyster densities and population sizes of four size groups of oysters;
- the status of *Bonamia exitiosa* infection (prevalence and intensity);
- estimates of disease mortality (from *B. exitiosa*) in the commercial fishery areas.

The most recent Bonamia survey was carried out from 6 to 21 February 2022, in collaboration with the Bluff Oyster Management Company Ltd, on F.V. *Golden Quest* and sampled 74 stations. The population sizes of commercial (≥ 65 mm diameter) and recruit-sized (≥ 58 mm) oysters decreased by 30.5% and 30.4%, respectively, and pre-recruits (≥ 50 mm to 57 mm) by 24.0%. The decrease of 243.5 million recruit-sized oysters between 2021 and 2022 cannot be accounted for by the 8 million oysters landed by the commercial fishery in 2021, and the estimated 45 million oysters that died from Bonamia between surveys. There is a possibility that inter-survey variation, i.e., whether random stations on average land on high or low density areas in a patchily distributed population in any given year contributed, in part, to the decrease in population sizes. However, sampling methods, sampling effort, and survey designs were consistent with previous surveys. One hypothesis is that there may have been a reduction of dredge efficiency in 2022 due changes in the seabed. Commercial catch rates in March 2022 were also lower than for the 2021 season. The population size of small oysters (10–49 mm) in the Bonamia survey area has been increasing since 2015 and remained high in 2022 (1116.6 million oysters, 95% CI 723.1–1709.9). In the absence of significant mortality, continued rapid increases in pre-recruit sized oysters is likely. Depending on annual growth rates, known to vary considerably, increases to commercial-sized and recruit-sized oysters may be slow. Commercial catch rates for the 2022 season are expected to remain similar or be slightly lower than the 2021 levels.

Future levels of disease mortality in the fishery are uncertain. *Bonamia exitiosa* mortality has been low since 2018. In 2022, non-fatal infections of *B. exitiosa* were 1.0% of the recruit-sized oyster population and mortality from *B. exitiosa* is expected to be low in 2023. The effects of other co-infections, such as the apicomplexan X and *Alcicornis longicornutus* (previously *Bucephalus longicornutus*), are unknown. *Bonamia ostreae* has the potential to cause catastrophic mortality in this naïve oyster population.

A Total Allowable Commercial Catch (TACC) of 15 million oysters represents an exploitation rate of 8.7% of the portion of the commercial population size (portion of recruit-sized oysters occurring at densities above 400 oysters per tow) and 2.7% of the recruit-sized population (across all densities) in core strata. The Bluff Oyster Management Company runs a voluntary quota shelving programme with

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which almost all vessels comply. In 2021, the oyster industry landed 50.8% of the TACC, equating to 7.6 million oysters representing 0.9% of the recruit-sized population in the Bonamia survey area in 2022.

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 (Ministry of Fisheries 2011), 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 (Ngāi Tahu) and recreational fishers, and the then Ministry of Fisheries, now Fisheries New Zealand.

The haplosporidian parasite of flat oysters *Bonamia exitiosa* (Bonamia) was previously thought to be an endemic disease of Foveaux Strait oysters, however, a recent study (Hill-Spanik et al. 2015) found *B. exitiosa* has a broad geographic distribution. Bonamia 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) in the 2014 survey report (referenced in Appendix 6).

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 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. A FSOFPMC review of the SRP and research priorities in Bluff, October 2019, proposed the following, in order of importance:

1. Understanding the roles of shellfish pathogens (especially co-infections) in oyster recruitment, meat condition, and mortality
2. Stock assessment, moving towards an adaptive disease-based model
3. A review of data collected by annual surveys
4. Research on the effects of fishing in the context of natural disturbance
5. The use of processor data to investigate spatio-temporal patterns in meat condition and brooding
6. The use of fishery supplementary surveys and the spreading of fishing effort to increase the spatial coverage of industry data
7. To incorporate the skippers' logbook data into the Integrated Electronic Monitoring and Reporting System (IEMRS)
8. Student, and other, projects to advance the understanding of the oyster population, its habitat and environment, and interaction with the commercial fishery and disease.

Bycatch surveys were introduced in 2019 to provide the information required for the IEMRS regulations. These surveys have been undertaken annually since then (Michael unpublished). Information on oyster catch, bycatch, and oyster discards from the oyster fishery are recorded. Sampling estimates oyster discards above and below the minimum legal size. Live bycatch in the dredge contents is described and quantified in the five categories required for reporting: non-fish non-Quota Management System (QMS) species, QMS commercial species, fish non-QMS, QMS reported bycatch (Porifera), and QMS reported bycatch (Bryozoa).

The unconfirmed detection of *Bonamia ostreae* DNA at a single site in Foveaux Strait in February 2021 has put greater onus on the top three research priorities agreed in 2019. The putative presence of *B. ostreae* raises several questions that are important to both the oyster fishery and the biosecurity of New Zealand marine resources. Overseas experience and the literature are based on a single host pathogen interaction (*Bonamia ostreae* in *Ostrea edulis*) and suggest that *B. ostreae* will spread and cause oyster mortality. The Foveaux Strait oyster fishery differs greatly to other oyster fisheries as oyster habitat is deep, on hard substrates, and the area is highly exposed to storm surges. The magnitude of effects of *B. ostreae* in a new host (*Ostrea chilensis*) and in the different environment of Foveaux Strait are not known. At an individual and at population level, *O. chilensis* in Foveaux Strait can be infected by several pathogens (co-infections) and disease progression is probably determined by the interaction of pathogens and environmental conditions, i.e., oyster mortality is likely to be driven by the effects of *B. ostreae*, *B. exitiosa*, an apicomplexan (APX), and other pathogens, and by hot or cold summers, and the frequency of storms. It is unknown if, to what extent or how quickly, *B. ostreae* would affect the fishery.

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 in the 2016 survey report by Michael et al. (2016) (see Appendix 6). From 1999 to 2006, these surveys sampled the same survey area (1054 km^2) using the same methods and similar strata to ensure data from surveys are comparable. An additional stratum (B1a, 16 km^2, see Figure 1) was introduced by oyster skippers in 2007. Since then, the size of the Foveaux Strait oyster survey area has remained at 1070 km^2. 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. The next stock assessment survey is scheduled for 2023. Annual, smaller surveys (Bonamia surveys) that focus sampling effort on the core commercial fishery area are undertaken in the years between stock assessments (Michael et al. 2015b, see Appendix 6). 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 (15^{th} stratum) thereby sampling the whole stock assessment area. This background stratum receives limited sampling effort (five stations).

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 (Table 1). Clocks are the articulated shells of recently dead oysters which still have 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, while oysters that died up to three years before this are categorised as old clocks.

Table 1: Since 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*

Survey data provide a useful forecast for the following oyster 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. 2015a, referenced in Appendix 6). These surveys incorporate a fully randomised, two-phase sampling design aimed at better estimating oyster densities and population sizes of oysters, new clocks, and oysters fatally and non-fatally infected with Bonamia. 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 throughout 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. 2015a). Surveys since 2015 have achieved CVs well below the survey target of 20%, 6–12% for recruit-sized oysters in the Bonamia survey area, and 6–17% in the stock assessment survey area (Michael et al. 2022, referenced in Appendix 6). 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 Total Allowable Commercial Catch (TACC) for OYU 5 is 15 million oysters. At relatively low levels of catch (fewer than 30 million oysters estimated per year), the trend in the abundance of recruit-sized oysters in the Foveaux Strait fishery is driven by disease mortality from Bonamia (see Large et al. 2021) and by the levels of recruitment to the population (spat settlement). Oyster spat settlement was low between the summers of 2009–10 and 2014–15 despite the population size of spawning-sized oyster densities increasing until 2012. 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 2011 and 2015 (200 million oysters estimated killed between 2012 and 2014), and the continued low replenishment of spat to the oyster population and pre-recruit sized oysters to the fishery, resulted in a substantial decline in the recruit-sized oyster population. All size groups of oysters declined between 2012 and 2017. Low Bonamia mortality and a substantial increase in recruitment to the oyster population since the summer of 2015–16 has increased all size groups of oysters between 2017 and 2021 (Table 2).

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 4.1%) since 2018, as has been the prevalence of fatal infections and non-fatal infections. The low non-fatal infections suggest reduced transmission of Bonamia infection.

This report provides a summary of information from the annual oyster and Bonamia surveys undertaken in February 2022 and updates the series of Foveaux Strait Bonamia surveys. The February 2022 survey estimated oyster population size and the status of Bonamia infection and this report outlines the implications for the future stock status based on the 2017 OYU 5 stock assessment. This survey was undertaken as part of the Fisheries New Zealand research programme OYS2020-01 (Specific objectives 1–4). References for Fisheries Assessment Reports for Foveaux Strait oyster and Bonamia surveys 2010–2021 are given in Table A6.1 (Appendix 6).

Table 2: Changes in population sizes (millions of oysters) between the 2012 and 2017 stock assessment surveys and annual estimates and overall trends since the last stock assessment survey in 2017. Mean population estimates are for commercial-sized, recruit-sized, pre-recruit, and small oysters (millions) in the Bonamia survey area and the stock assessment survey area in 2012, 2017, 2018, 2019, 2020, and 2021. Commercial-sized oyster populations were first estimated in 2019*. The percentage changes in mean population size between 2012 and 2017 and between 2017 and 2021 are given as gains (+) and losses (-), shaded tan for decreases caused by Bonamia mortality, and shaded green for increase in mean population. Estimates of mean population size from the stock assessment survey area in 2018, 2019, 2020, and 2021 should be viewed with caution because of the limited sampling in the large background stratum.

	Mean population size (millions)						% decrease	% increase
	2012	2017	2018	2019	2020	2021	2012–2017	2017–2021
Oyster size: Bonamia survey area								
Commercial*	NA	NA	NA	318.7	316.1	405.6	NA	+127.3
Recruit	688.1	363.6	494.1	542.5	529.9	801.4	-47.2	+220.4
Pre-recruit	297.4	123.1	178.4	216.5	265.3	487.0	-58.6	+395.6
Small	451.3	261.9	401.8	595.8	1 052.4	1 091.2	-42.0	+416.6
Oyster size: Stock assessment survey area								
Commercial *	NA	NA	NA	536.9	504.6	439.3	NA	-81.8
Recruit	918.4	527.4	883.3	868.0	879.3	868.1	-42.6	+164.6
Pre-recruit	414.3	168.2	225.8	309.8	436.6	522.3	-59.4	+310.5
Small	612.2	361.6	552.5	867.8	1 356.7	1 202.1	-40.9	+332.4

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 2020, 2021, and 2022.
2. To evaluate the current status of the prevalence and intensity of Bonamia in the OYU 5 fishery for the fishing years 2020, 2021, and 2022.

1.1.1 Contracted objectives

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 ≤ 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 fishing year's survey.

1.1.2 Specific objectives for the February 2022 survey

1. 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.
2. Estimate the prevalence and intensity of *Bonamia exitiosa* (Bonamia) infection in recruit-sized oysters using ddPCR and heart imprints to maintain the long time series of infection data.
    a. Undertake pre-testing checks on all ddPCR procedures and reagents.
    b. Estimate summer mortality combining two different estimates of mortality:
        i. Pre-survey mortality, the population size of recruit-sized new clocks and gapers.
        ii. Projections of post-survey mortality from oysters with fatal infections (category 3–5 infections).

2. METHODS

Survey strata for the February 2022 survey were the same as for February Bonamia surveys since 2014 (Figure 1). The 2017 stock assessment survey sampled all 26 strata (Michael et al. 2019a, referenced in Appendix 6). The inclusion of a single large background stratum (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.

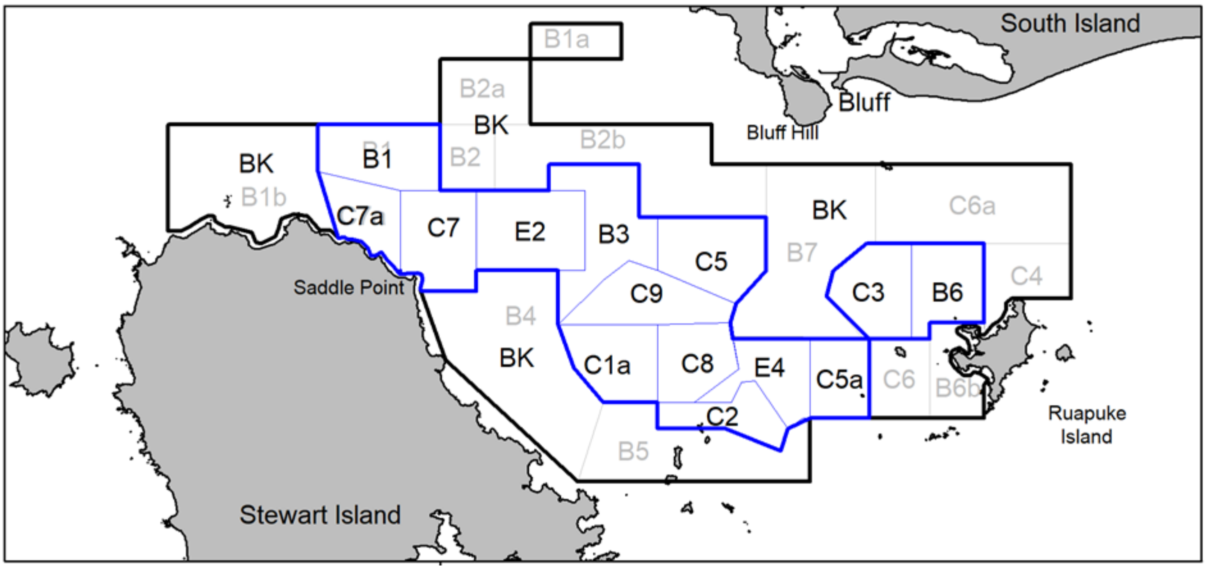

Figure 1: The 2022 survey area with the 2007 survey boundary shown as a heavy, black outer line and the 2022 survey strata representing the core commercial fishery area (Bonamia survey area) shown as blue lines. Bonamia survey strata are labelled with black text. The remaining stock assessment survey strata (shown in grey text), which represent the 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, referenced in Appendix 6). Simulations predicted that 55 stations in the 14 Bonamia survey strata would produce a CV of about 11%. NIWA built software, ALLOCATE (Francis 2006), was used to allocate the numbers of stations to strata in 2022 (Table 3). An R-package, *Random station* (Doonan & Rasmussen 2012), was used to generate the location of 50 random first-phase stations, 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 changes in oyster density and Bonamia status in localised areas. In 2015, the Fisheries New Zealand (then Ministry for Primary Industries) Shellfish Working Group agreed data from the 12 fixed stations added value to the information obtained from surveys. These 12 fixed stations were sampled in February 2022 (Table 3 and Figure 2).

Table 3: The numbers of first-phase, second-phase, and fixed stations sampled, the numbers of foul shots in each stratum, and the area of each stratum for the February 2022 Bonamia survey. 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	Foul shots	Area (km^2)
B1	5	2	1	–	78.2
B3	3	–	–	–	44.7
B6	5	–	–	3	30.0
BK	5	–	2	1	578.3
C1a	4	–	–	–	31.3
C2	3	–	1	1	21.9
C3	3	–	1	–	32.7
C5	4	–	1	–	37.7
C5a	3	–	1	–	23.5
C7	3	–	–	–	36.1
C7a	4	2	–	–	23.6
C8	3	3	1	–	26.8
C9	3	–	–	2	34.5
E2	4	–	2	–	42.8
E4	3	–	2	–	28.0
Total	55	7	12	7	1 070.1

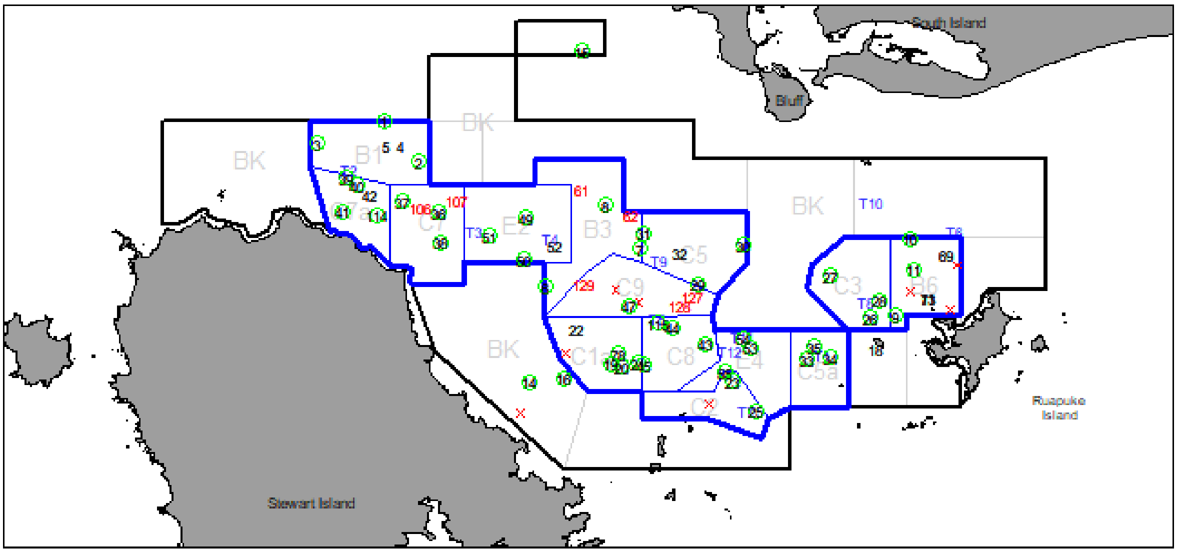

Figure 2: The 2022 survey area with the 2007 survey boundary shown as a heavy, black outer line, the Bonamia survey area as a heavy blue line, and the 2022 Bonamia survey strata shown as fine blue lines. The remaining stock assessment survey strata (fine light grey lines) in the large background stratum were merged into a single stratum (BK). First-phase station numbers are in black text, second-phase in red text, and fixed stations in blue text. Red crosses denote stations that could not be towed because of foul ground (foul shots). Green circles show stations where spat collectors were deployed.

2.1 Catch sampling

Except for minor variations, dredge sampling has followed standardised procedures for stock assessment and Bonamia surveys since October 2002, including 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 standardised procedures are given by Michael et al. (2015a, referenced in Appendix 6). Two commercial oyster vessels have been used for surveys since 1999, F.V. *Golden Lea* 1999–2010 and F.V. *Golden Ques*t 2011–2022, 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, except for 2016 when the F.V. *Golden Lea* was skippered by Alan Fowler. 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. In 2019, a fourth commercial size was recorded (see Table 1). Reference rings (65 mm, 58 mm, and 50 mm internal diameter) were used to ensure accurate allocation to each size group. All four size groups were sampled in February 2022.

Clocks and gapers were recorded in two size groups: recruits and pre-recruits. Clocks were further divided into two categories, new and old (see figures 8–10 of Michael et al. 2015a, referenced in Appendix 6). 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 oysters that had died since summer mortality from Bonamia began. Oysters that died before this time 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 one and three 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 in these habitats 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, depth, and 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, Appendix 6 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 2022 and then compared with the estimates from strata sampled in 2016–2021 (Michael et al. 2016, 2019a, 2019b, 2020, 2021, 2022, referenced in Appendix 6). 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, referenced in Appendix 6) 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, small oysters, and new 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 (m^2), and $\bar{x}_i$ is the mean oyster density corrected for dredge efficiency in stratum i. Estimates of population sizes were also presented by stratum separately.

The CV for each stratum was calculated from the standard deviation and mean oyster density and the same calculation was also 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 mean population size ($\bar{x}$) for each stratum and the Bonamia and stock assessment survey areas were estimated by bootstrapping, i.e., resampling with replacement from a normal distribution for which the variance is based on a CV of the population estimate and the error associated with dredge efficiency.

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.

The total error of the estimate of the mean population size ($\bar{x}$) 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.

2.3 Estimates of commercial population size

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.

2.4 Recruitment

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 2021 and February 2022 surveys.

2.4.1 Spat collection

Ostrea chilensis larvae readily settle on manmade substrates, without conditioning, and in the absence of conspecifics. Counts of oyster spat that settled to passive, artificial collectors (November to February) represent densities of competent larvae ready to settle. Settler densities at each site were defined as the cumulative numbers of both living and dead oyster spat that settled on the top and bottom surfaces of the four plates.

Data were recorded at different spatial scales and in different areas: the shell return site (south of Bluff Hill, see Michael et al. 2013) and fishery scale experiments (western, southern, and eastern fishery areas, see Michael et al. 2013), the gradient experiment in the central fishery area (Michael 2019) and fishery scale monitoring (the stock assessment survey area) that began over the summer of 2014–15. 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).

2.5 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 only recruit-sized oysters 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 (Diggles et al. 2003) 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 - 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.

2.5.1 Methods to estimate the prevalence and intensity of *Bonamia exitiosa* 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 *B. 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 (Appendices 2 and 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.

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 and superior levels of sensitivity, detection, and cost-effectiveness has been used since 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), while ddPCR estimates the numbers of *B. exitiosa* positive droplets 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 *O. chilensis* DNA (relative infection levels) in ddPCR samples (see Figure A3.3, Appendix 3).

2.5.2 Review of ddPCR procedures prior to testing and repeat testing

Before the samples from the 2022 survey were analysed, quality control of reagents and methods was undertaken (details in Appendix 3). Each tested 96-well ddPCR plate included positive and negative controls. Reactions with less than 103 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).

2.5.3 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 was 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 was 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), scaled-up to the size of the recruit-sized oyster population by strata and 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 *O. 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.

2.5.4 Heart imprints

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. 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. Fixed stations and stations with less than 15 recruit-sized and pre-recruit oysters were excluded from the analysis of prevalence and intensity of infection.

The categorical score from heart imprints (see Table A3.1, Appendix 3) assumes that category 0 oysters are not infected. The previous study by 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. 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 1 and 2 Bonamia infections represent non-fatal infections and stages 3–5 fatal infections. The differences between non-fatal and fatal infections are corroborated by the corresponding relative infection levels from ddPCR, i.e., the ratio of oyster DNA (β actin) to Bonamia DNA. Relative infection levels for non-fatal infections remain relatively low and increase rapidly from stage 3 in fatal infections (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:

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

from heart imprint samples only

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

2.5.5 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 method in Section 2.2. These estimates are presented by stratum and the Bonamia and stock assessment survey areas. Method 1 used a correction factor from strata with three or more randomly selected stations only, i.e., target stations were not included. The correction factor reduces the estimated population size of oysters by the proportion of fatally infected oysters still alive at the time of survey (Dunn et al. 2002). Method 2 used the numbers of oysters in each Bonamia infection category (stages 1–5) to calculate the numbers of non-fatal and fatal infections in the sample and scaled to the total catch for each station. Population estimates of non-fatal and fatal infections were estimated using the method in Section 2.2. The overall intensity was calculated as the average Bonamia level (stage) in the population. Variance for prevalence and intensity was estimated using standard methods as for population estimates.

2.6 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. 2021), which updated the stock assessment for 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 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; 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) was taken into account, the population size of recruit-sized oysters (837 million) 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 in the stock assessment area decreased by 42.6%, from 918.4 million oysters in 2012 to 527.4 million oysters in 2017, more than expected because recruitment had been very low since 2010.

The most appropriate projection for future stock status is proposed to be 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 predicted population estimates were similar to the estimates of population size from subsequent surveys.

3. RESULTS

Sea conditions were relatively calm over the February 2022 survey period and better than in February 2021. The weather was generally good during sampling. There was a large SW/SE swell on the 14th of February that may have affected dredge efficiency. Tides were mostly swift spring tides, which may also have affected dredge efficiency. Dredge contents were generally fuller and contained more gravel in 2022 than in 2021; an observation corroborated by oyster vessel skippers' experiences at the beginning of the oyster season in March 2022. This suggested there was a change to the seabed between surveys that may have resulted in a lower catchability of oysters during the 2022 survey. Fewer survey tows recorded counts of 500 or more recruit-sized oysters in 2022; 8% of stations in 2022, compared with 10% in 2020 and 26% in 2021. All of the survey tows recorded at least some recruit-sized oysters and the distribution of oysters was widespread. Signs of recent shell growth were few and isolated, suggesting poor growth over the summer. Large numbers of spat and 1–3 year-old oysters were observed on oysters in many areas in 2022, reflecting continued good recruitment to the population and the survival of juveniles. Observations from the survey also suggested some heightened, localised pre-survey mortality at a few stations.

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 C5a (stations 33, 34, 35, and T1). Oyster density was most likely underestimated at these stations.

The efficiency of dredge sampling during the 2022 survey may have been lower than for previous surveys. Dredge tow lengths were mostly about 0.2 nautical miles (371 m). Wind speeds were less than

10 knots and sea conditions mostly good. However, dredge fullness and dredge saturation were higher than in previous surveys (Appendix 4, Figures A4.1–A4.6).

3.1 Survey operational detail

NIWA and BOMC staff began the survey on the 6th of February 2022 and finished on the 21st of February 2022, sampling on seven days during this period. The oyster vessel F.V. *Golden Quest* successfully sampled 74 stations including an additional 2. 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 could not be sampled because of rough ground; first-phase stations 12, 17, 24, 46, and 48, and some of the replacements for station 12 (67 and 68) were replaced by stations 69, 71, 78, 114, and 115.

Target sample size for ddPCR and heart imprints (n = 25 per station) were taken from 72 stations. For samples with fewer than 25 recruit-sized oysters, samples included pre-recruit and small oysters; this included station 45 (mostly pre-recruits, n = 18) and stations T5, 25, and 71, which were mostly recruit-sized with a few pre-recruits.

3.2 Oyster abundance

3.2.1 Changes in oyster densities between 2020 and 2022

Time series of oyster catches adjusted to the standard tow length (0.2 nautical miles) by stratum are shown in Figures 3–6. Catches of all four size classes were spatially patchy and locally variable. Low catches of all four size groups of oysters in stratum C5a may be due to reduced dredge efficiency caused by dense stands of kāeo. Catches of oysters across all groups in the background stratum (BK) were generally lower than the core fishery area.

Mean catches of commercial-sized oysters generally varied by stratum across the fishery area and varied greatly within stratum for any given year (Figure 3), highlighting the localised patchiness of their distribution. Stratum C7a in the western fishery area and strata C5a, C8, E4, and C3 showed generally higher or similar catches in 2022 than in 2021. Strata east of a line between Saddle Point (Stewart Island) and Bluff Hill (E2, C9, C1a, C5, and B6), which had relatively high catches 2020–21, generally had lower catches in 2022. Strata with relatively low catches 2020–21 (B1, C7, C2, C5a, and BK) remained broadly similar; however, the background stratum (BK) had higher catches in 2022 and this probably reflected the effects of interannual survey variation caused by low sampling effort in BK (Figure 3).

At a stratum level, catches of recruit-sized oysters were generally lower across the fishery area in 2022 than in 2021; and there were fewer high catches over 400 oysters per tow in 2022 than in 2020 and 2021 (Figure 4). Few strata (C5a, E4, and BK) trended higher in 2022 than in 2021. Strata with higher catches of recruit-sized oysters in 2020–22 were the same as those strata with high catches of commercial-sized oysters (B6, C3, C5, C7a, C8, C9, E2, and E4) over the same years (Figures 3 and 4).

Catches of pre-recruit oysters were broadly similar across the fishery area in 2020–22, varying without annual pattern (Figure 5). The variability in catches within stratum and amongst years likely reflects small spatial-scale patchiness.

Catches of small oysters were also broadly similar across the fishery area in 2020–22, except for strata C5a and BK, which were generally low (Figure 6). The high catches in BK in 2022 probably reflected sampling variation. Eastern strata C3 and B6, with gravel substrates, showed consistently high catches of small oysters between 2020 and 2022. Only E4, C8, and E2 had higher catches in 2022 than in both 2020 and 2021. The number of small oysters reflected the trend of increasing recruitment to the population indicated by spat monitoring.

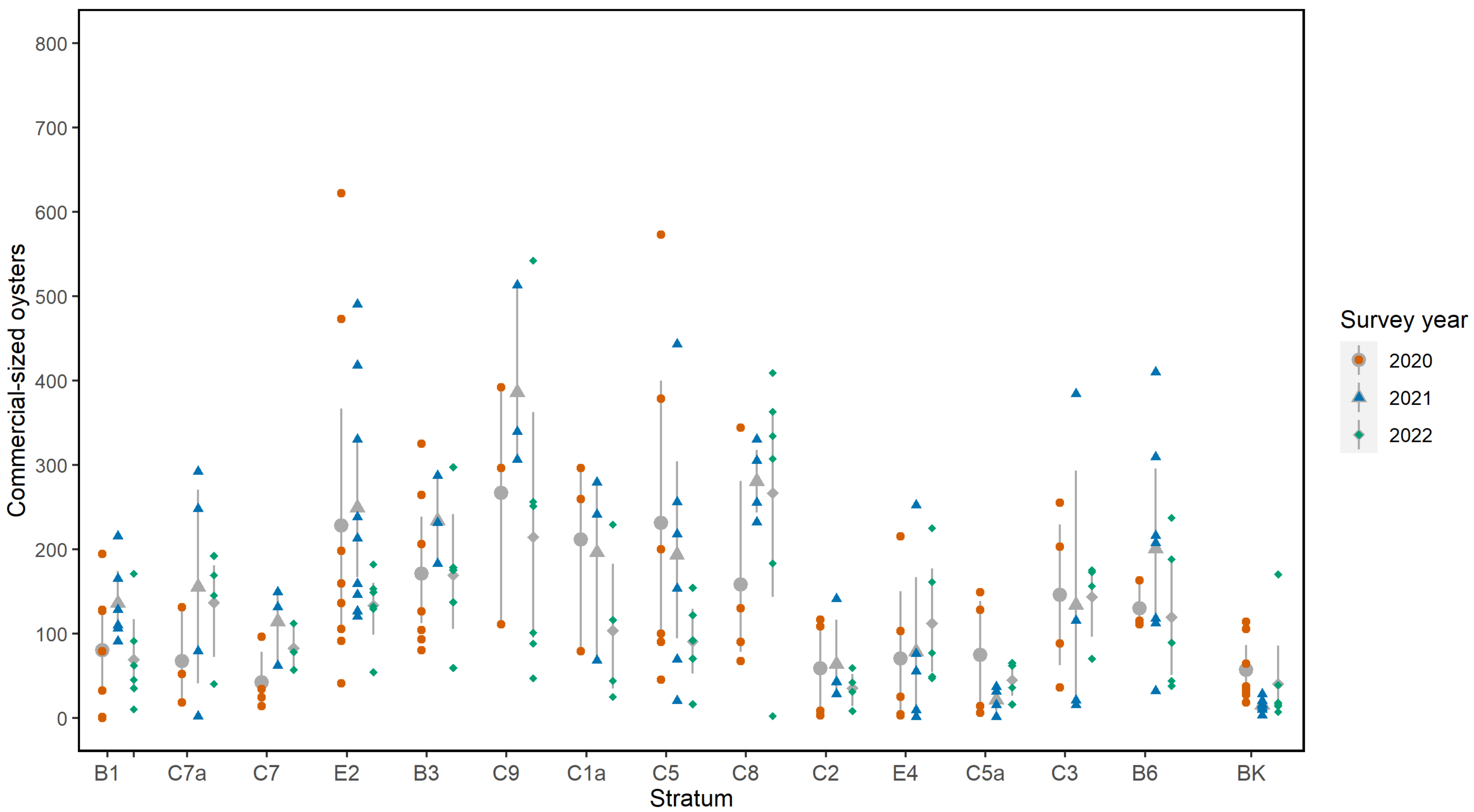

Figure 3: The numbers of commercial-sized oysters (≥ 65 mm in diameter) per tow, with means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines), by stratum for surveys during 2020–2022. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2020 survey are shown as tan filled circles, 2021 as blue filled triangles, and 2022 as green filled diamonds. Bonamia survey strata are arranged west to east, with northern strata at similar longitudes shown first, and the background stratum (BK) furthermost right.

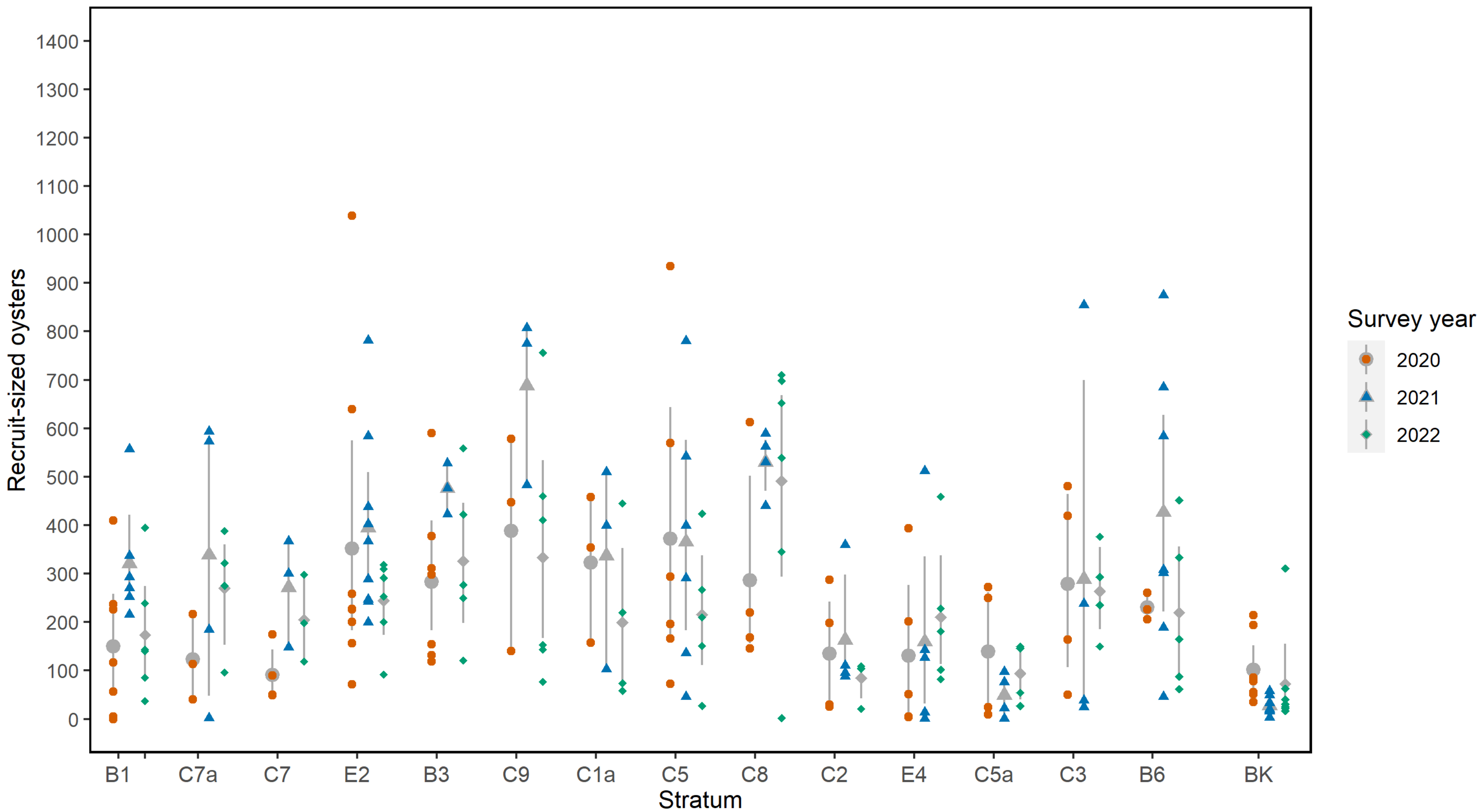

Figure 4: The numbers of recruit-sized oysters (≥ 58 mm in diameter) per tow, with means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines), by stratum for surveys during 2020–2022. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2020 survey are shown as tan filled circles, 2021 as blue filled triangles, and 2022 as green filled diamonds. Bonamia survey strata are arranged west to east, with northern strata at similar longitudes shown first, and the background stratum (BK) furthermost right.

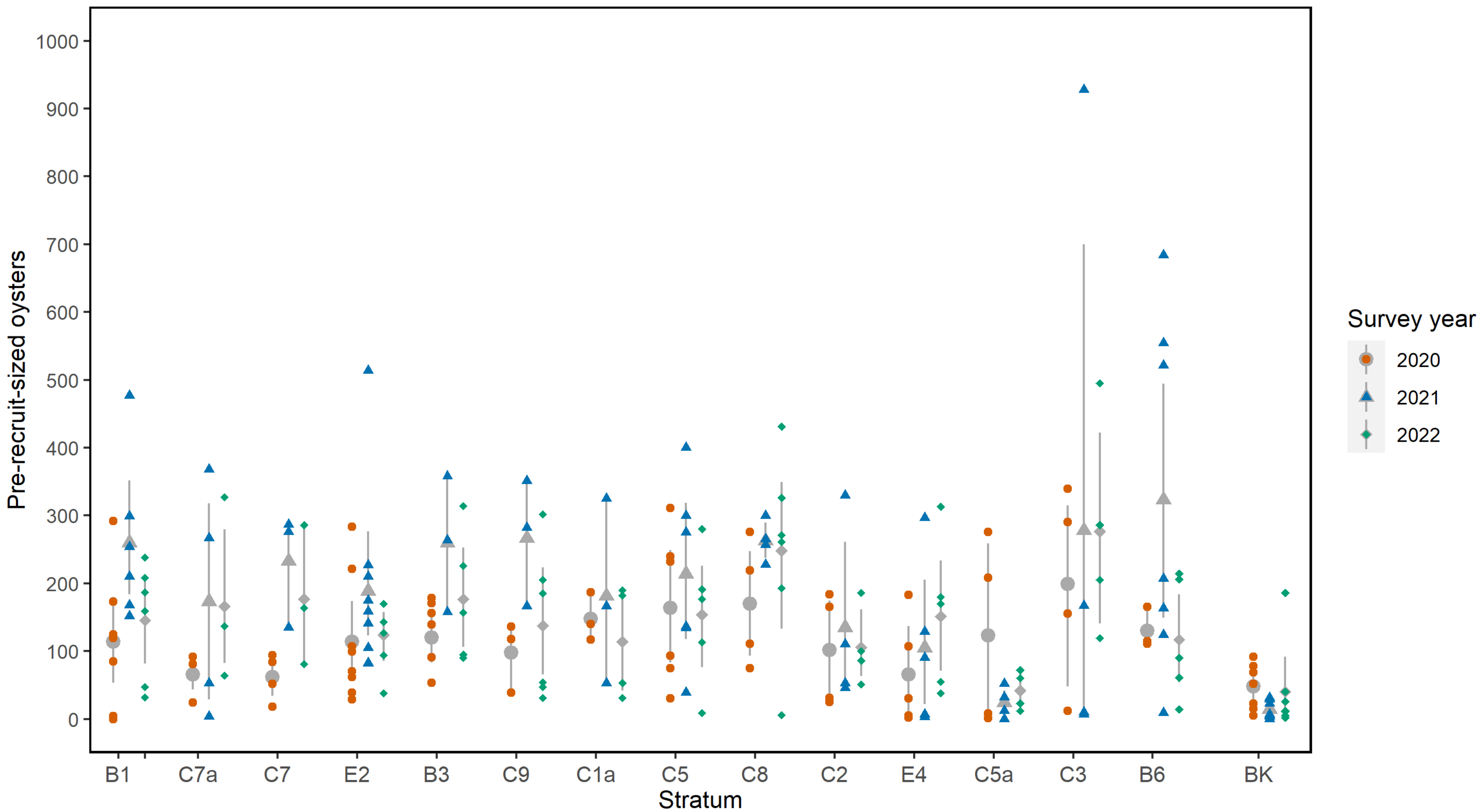

Figure 5: The numbers of pre-recruit oysters (≥ 50–57 mm in diameter) per tow, with means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines), by stratum for surveys during 2020–2022. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2020 survey are shown as tan filled circles, 2021 as blue filled triangles, and 2022 as green filled diamonds. Bonamia survey strata are arranged west to east, with northern strata at similar longitudes shown first, and the background stratum (BK) furthermost right.

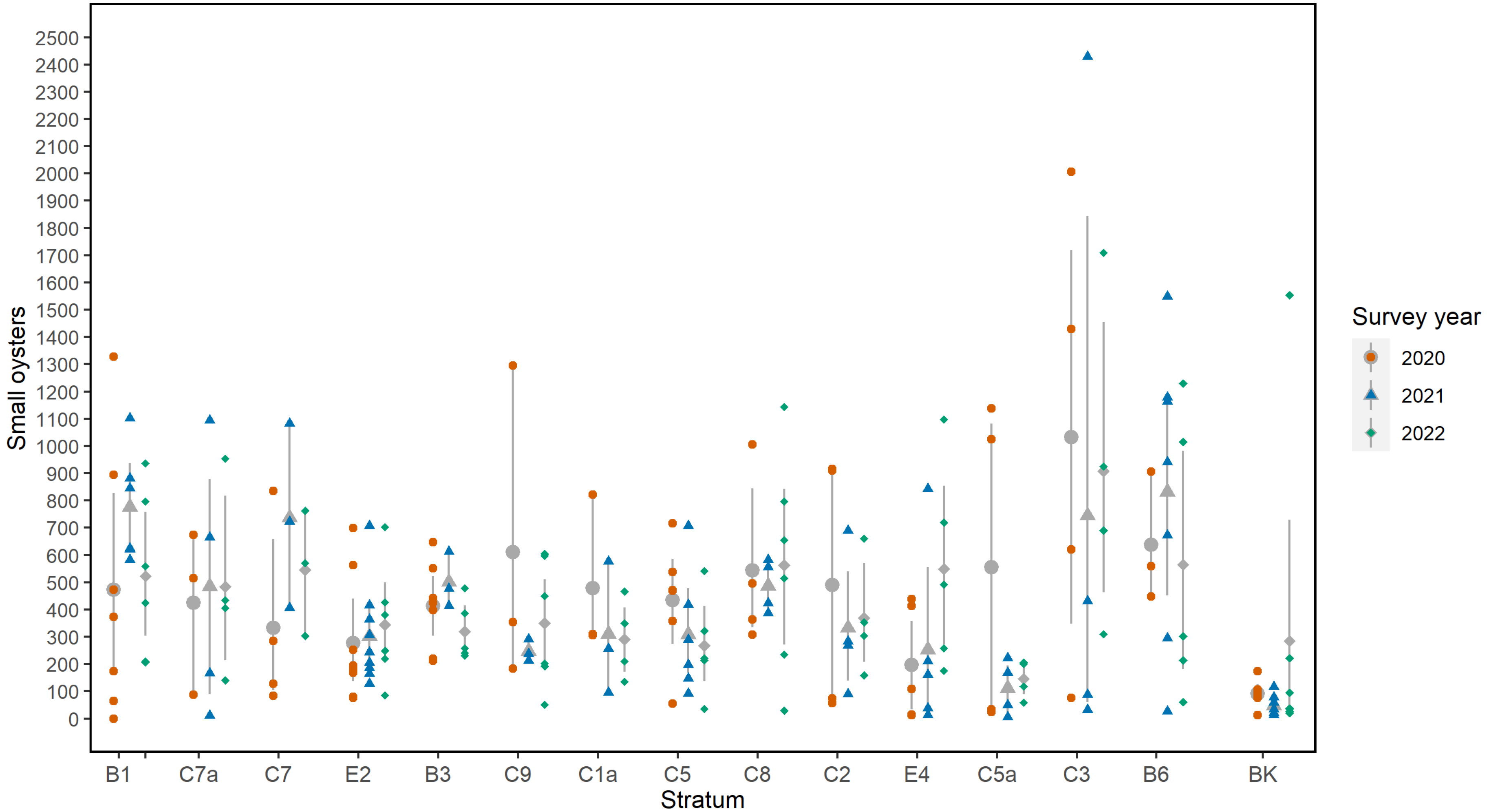

Figure 6: The numbers of small oysters (10–49 mm in diameter) per tow, with means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines), by stratum for surveys during 2020–2022. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2020 survey are shown as tan filled circles, 2021 as blue filled triangles, and 2022 as green filled diamonds. Bonamia survey strata are arranged west to east, with northern strata at similar longitudes shown first, and the background stratum (BK) furthermost right.

3.2.2 Survey estimates of population size

Estimates of absolute population size for commercial-sized, recruit-sized, pre-recruit, and small oysters from the February 2022 survey are given by stratum 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) in Tables 4–7. The population estimates and the percentage change in population size between years in only the Bonamia survey area, for recruit-sized, pre-recruit, and small oysters from the 2012 and 2016–2022 surveys, and for commercial-sized oysters from the 2019–2022 surveys are shown in Table 8. Mean population sizes and 95% confidence intervals for these four size groups for 2012 and 2014–2022 in the Bonamia survey area are shown in Figure 7. Fisheries Assessment Reports for Foveaux Strait oyster and Bonamia surveys 2010–2021 that provided 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. The density of oysters in the background stratum was likely not estimated well by recent surveys (since 2017) due to the low numbers of stations sampled (n=5) over a large area (578.4 km^2).

Fishers high-grade their catches (return the smaller oysters that are above minimum legal size) to maximise the numbers of first grade (referred to here as commercial-sized) oysters. The density and population size of commercial-sized oysters were estimated for the first time in 2019 and represent the size group retained by fishers. The population size of commercial-sized oysters in the core strata decreased 30.5% between 2021 and 2022 (Table 4) after increasing by 28.3% between 2020 and 2021 (Table 8).

Of the recruit-sized population in 2022, commercial sized oysters accounted for 50.6% in the core strata and 52.1% in the stock assessment survey area (Tables 4 and 5). This percentage was similar to 2021 (50.6%) for both the core strata and the stock area (Tables 4 and 5). The percentages of commercial-sized oysters were lower in 2021 and 2022 than in either 2020 or 2019, 59.6% in the core strata and 57.3% in the stock assessment survey area in 2020, and 59.0% in the core strata and 61.9% in the stock assessment survey area in 2019. Most core strata saw declines in population size between 7.4% and 62.4%. Three strata (C3, C5a, and E4) showed substantial increases between 148.3% and 335.1% (Table 4); however, the population sizes for those strata were relatively small. Mean density in the core strata was 0.57 oysters m^{-2} in 2022, lower than in 2021, 2020, or 2019 (0.82 oysters m^{-2}, 0.64 oysters m^{-2}, and 0.65 oysters m^{-2}, respectively). In 2022, mean density ranged from 0.13 oysters m^{-2} in stratum C2 to 1.22 oysters m^{-2} in stratum C8 (Table 4) compared with range of 0.08 oysters m^{-2} in stratum C5a to 1.86 oysters m^{-2} in stratum C9 in 2021. Oyster densities for stratum C5a were likely to be underestimated because this stratum has extensive stands of the stalked ascidian (kāeo, *Pyura pachydermatina*) that substantially reduce oyster catchability.

The population size of recruit-sized oysters in core strata decreased by 30.4% between 2021 and 2022 (Table 5), similar to the decline in commercial sized oysters. This downward trend followed a mostly increasing trend in population size from 2018 (Table 8), with an increase of 51.2% between 2020 and 2021. The mean density in core strata (1.13 oysters m^{-2}) in 2022, is lower than in 2021 (1.63 oysters m^{-2}), but higher than for years 2016–20 (0.74 oysters m^{-2} to 1.10 oysters m^{-2}) (Table 8). Population size in most core strata declined between 8.0% and 57.6% and same three strata as for commercial-sized oysters (C3, C5a, and E4) showed substantial increases, between 132.4% and 243.3% (Table 5). However, the population sizes for those strata were relatively small. Population size declined from 688.1 million oysters in 2012 to 363.6 million oysters in 2017, then increased to 801.4 million oysters in 2021 before declining to 557.9 million oysters in 2022 (Table 8). The CV for the estimate of recruit-sized population in core strata was between 9% and 13% between 2012 and 2022.

The population size of pre-recruit oysters in core strata decreased by 24.0% between 2021 and 2022 (Table 6). This downward trend followed a mostly increasing trend in population size from 2016

(Table 8). Pre-recruits decreased from 297.4 million oysters in 2012 to 89.2 million oysters in 2015 and then increased continually through to 2021. Between 2020 and 2021, the population size increased by 83.6% from 265.3 million oysters in 2020 to 487.0 million oysters in 2021 (Table 8). The population sizes in the background stratum (BK) increased by 329.0% between 2021 and 2022 (Table 6), following a decrease of 79.4% from 171.3 million oysters in 2020 (Michael et al. 2021, referenced in Appendix 6) to 35.3 million oysters in 2021. Pre-recruit mean oyster density in all core strata combined decreased by 24.2% to 0.75 oysters m^{-2} between 2021 and 2022, after an increase of 83.3% between 2020 and 2021 to 0.99 oysters m^{-2} (Table 8). Most core strata saw declines in population size between 5.1% and 64.1%. The same three strata as for commercial-sized and recruit oysters (C3, C5a, and E4) substantially increased between 141% and 346.5% (Table 6); however, the population sizes for those strata were relatively small. Mean pre-recruit oyster density decreased from 0.60 oysters m^{-2} in 2012 to 0.25 oysters m^{-2} in 2017, then continually increased to 0.99 oysters m^{-2} in 2021 and declined to 0.75 oysters m^{-2} in 2022 (Table 8).

The trends in mean densities and population sizes of small oysters differed greatly from those for large size groups of oysters (Figure 7). The mean densities and population sizes of small oysters for all the core strata combined in 2022 remained high and similar to estimates from 2020 and 2021. Mean density of small oysters was 2.14 oysters m^{-2}, 2.22 oysters m^{-2}, and 2.27 oysters m^{-2} in 2020–2022, respectively. Population size increased by 2.3% in 2022 (Table 7) and by 3.7% between 2020 and 2021. The population size of small oysters declined markedly, by 65%, from 451.3 million oysters in 2012 to 156.3 million oysters in 2014 (Michael et al. 2015a, referenced in Appendix 6) but has since increased by 614.4% to 1116.6 million oysters by 2022 (Table 8). Strata B1, C3, C7, E2, B6, and B3 had the highest population sizes (Table 7).

Table 9 shows estimates of commercial population size using the catch of recruit-sized oysters at each station minus 400 oysters for the 2022 core strata (n = 14), all core strata combined, all background strata combined (n= 12), and for the whole 2007 stock assessment survey area sampled. Five core strata (B3, B6, C1a, C8, and C9) supported commercial densities in 2022 compared with nine strata (B1, B3, B6, C1a, C5, C7a, C8, C9, and E2) in 2021 (Table 9). Mean densities in these strata ranged from 0.44 oysters m^{-2} to 1.95 oysters m^{-2} in 2022, compared with a high of 3.33 oysters m^{-2} in 2021. Mean density for all core strata combined was 0.35 oysters m^{-2} in 2022 compared with 1.06 oysters m^{-2} in 2021. Ten core strata supported commercial densities in 2012, six in 2014, two in 2015, six in 2016, three in 2017, five in 2018, six in 2019, and seven in 2020. The proportion of the recruited population above the commercial threshold in core strata was 30.8% in 2022, down from 64.8% in 2021.

Table 4: Absolute population estimates for commercial-sized (≥ 65 mm in diameter) oysters by stratum (Stratum), in the core strata (Core total), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2022. Columns give the numbers of stations sampled (Number stations), mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (Density CV) of the density estimate, the 2022 mean population size in millions of oysters (2022 Pop.n, shaded light blue), lower and upper 95% confidence intervals in millions of oysters where a B prefix denotes the bootstrapped estimates (B.lower and B.upper 95% CI), and the area of each stratum in square kilometres (Area (km^2)). Also given are the 2021 mean population estimates (2021 Pop.n, shaded light grey) and the percentage change in 2022 from the 2021 estimate (Percentage change), shaded green for increases in population size in 2022 and orange for decreases.

Stratum	Number stations	Mean density	Density s.d.	Density CV	2022 Pop.n	B.lower 95% CI	B.upper 95% CI	Area (km^2)	2021 Pop.n	Percentage change
B1	5	0.36	0.14	0.38	28.3	6.6	55.8	78.2	50.2	-43.5
B3	5	0.83	0.19	0.23	37.2	18.9	62.2	44.7	51.6	-28.0
B6	5	0.58	0.19	0.33	17.4	5.6	32.7	30.0	29.5	-40.9
C1a	4	0.51	0.23	0.46	15.8	1.5	33.7	31.3	29.9	-47.0
C2	3	0.13	0.05	0.37	2.9	0.8	5.6	21.9	7.6	-62.4
C3	3	0.67	0.17	0.25	22.0	10.2	37.7	32.7	8.2	167.8
C5	4	0.38	0.11	0.30	14.2	5.3	26.1	37.7	26.3	-45.9
C5a	3	0.19	0.07	0.36	4.5	1.3	8.6	23.5	1.8	148.3
C7	3	0.40	0.08	0.19	14.5	7.9	23.7	36.1	19.8	-26.7
C7a	4	0.67	0.17	0.25	15.7	7.3	27.2	23.6	18.4	-14.7
C8	5	1.22	0.35	0.29	32.8	13.1	59.0	26.8	35.4	-7.4
C9	6	1.04	0.36	0.34	36.0	11.3	68.7	34.5	64.2	-44.0
E2	4	0.65	0.14	0.21	27.7	14.4	46.4	42.8	59.7	-53.6
E4	3	0.47	0.17	0.36	13.1	4.0	25.3	28.0	3.0	335.1
Core total	57	0.57	0.05	0.09	282.1	184.5	428.3	491.8	405.6	-30.5
BK	5	0.25	0.15	0.58	147.2	0.0	350.0	578.4	33.6	338.2
Survey total	62	0.40	0.08	0.21	429.3	221.5	714.9	1 070.2	439.3	-2.3

Table 5: **Absolute population estimates for recruit-sized (≥ 58 mm in diameter) oysters by stratum (Stratum), in the core strata (Core total), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2022 by stratum. Columns give the numbers of stations sampled (Number stations), mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (Density CV) of the density estimate, the 2022 mean population size in millions of oysters (2022 Pop.n, shaded light blue), lower and upper 95% confidence intervals in millions of oysters where a B prefix denotes the bootstrapped estimates (B.lower and B.upper 95% CI), and the area of each stratum in square kilometres (Area (km^2)). Also given are the 2021 mean population estimates (2021 Pop.n, shaded light grey) and the 2022 mean population size represented as a percentage of the 2021 mean population size by stratum (% of 2020). The percentage change from the 2021 estimate (Percentage change) is shaded green for increases in population size in 2022 and orange for decreases.**

Stratum	Number stations	Mean density	Density s.d.	Density CV	2022 Pop.n	B.lower 95% CI	B.upper 95% CI	Area (km^2)	2021 Pop.n	Percentage change
B1	5	0.94	0.30	0.32	73.2	25.0	136.4	78.2	122.1	-40.0
B3	5	1.60	0.37	0.23	71.7	35.9	120.9	44.7	105.3	-31.9
B6	5	1.07	0.36	0.34	32.2	9.9	60.6	30.0	62.8	-48.8
C1a	4	0.97	0.45	0.46	30.5	2.7	65.2	31.3	51.5	-40.8
C2	3	0.38	0.14	0.36	8.3	2.3	16.0	21.9	19.5	-57.6
C3	3	1.37	0.34	0.25	44.9	20.8	77.2	32.7	16.5	172.0
C5	4	0.83	0.26	0.32	31.1	10.7	57.9	37.7	52	-40.2
C5a	3	0.37	0.17	0.47	8.6	0.8	18.5	23.5	3.7	133.8
C7	3	1.00	0.25	0.25	36.1	16.7	63.0	36.1	47.3	-23.6
C7a	4	1.32	0.31	0.23	31.1	15.0	53.2	23.6	40.2	-22.7
C8	5	2.28	0.65	0.28	61.1	24.8	109.2	26.8	66.4	-8.0
C9	6	1.62	0.51	0.31	56.0	20.3	103.7	34.5	114.8	-51.2
E2	4	1.19	0.25	0.21	50.8	26.1	85.0	42.8	92.8	-45.3
E4	3	0.80	0.21	0.27	22.3	9.9	39.4	28.0	6.5	243.3
Core total	57	1.13	0.10	0.09	557.9	366.6	842.9	491.8	801.4	-30.4
BK	5	0.46	0.27	0.59	266.6	0.0	637.5	578.4	66.7	299.6
Survey total	62	0.77	0.15	0.20	824.4	437.1	1 359.3	1 070.2	868.1	-5.0

Table 6: Absolute population estimates for pre-recruit (50–57 mm in diameter) oysters by stratum (Stratum), in the core strata (Core total), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2022 by stratum. Columns give the numbers of stations sampled (Number stations), mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (Density CV) of the density estimate, the 2022 mean population size in millions of oysters (2022 Pop.n, shaded light blue), lower and upper 95% confidence intervals in millions of oysters where a B prefix denotes the bootstrapped estimates (B.lower and B.upper 95% CI), and the area of each stratum in square kilometres (Area (km^2)). Also given are the 2021 mean population estimates (2021 Pop.n, shaded light grey) and the 2022 mean population size represented as a percentage of the 2021 mean population size by stratum (% of 2021). The percentage change from the 2021 estimate (Percentage change) is shaded green for increases in population size in 2022 and orange for decreases.

Stratum	Number stations	Mean density	Density s.d.	Density CV	2022 Pop.n	B.lower 95% CI	B.upper 95% CI	Area (km^2)	2021 Pop.n	Percentage change
B1	5	0.80	0.18	0.22	62.9	32.3	105.8	78.2	101.6	-38.1
B3	5	0.87	0.22	0.25	39.0	18.6	66.8	44.7	57.6	-32.3
B6	5	0.57	0.19	0.34	17.1	5.2	32.3	30	47.6	-64.1
C1a	4	0.55	0.20	0.37	17.3	4.7	33.8	31.3	27.6	-37.3
C2	3	0.54	0.19	0.35	11.9	3.7	22.7	21.9	17.7	-32.6
C3	3	1.38	0.59	0.43	45.1	6.9	92.8	32.7	10.1	346.7
C5	4	0.62	0.21	0.34	23.3	7.1	44.5	37.7	32.5	-28.3
C5a	3	0.17	0.09	0.51	4.1	0.0	9.0	23.5	1.7	138.8
C7	3	0.86	0.29	0.34	31.2	10.2	58.9	36.1	40.5	-23.0
C7a	4	0.81	0.28	0.34	19.1	5.9	36.0	23.6	20.7	-7.7
C8	5	1.15	0.33	0.29	30.9	12.3	55.5	26.8	32.5	-5.1
C9	6	0.67	0.22	0.32	23.1	8.0	43.2	34.5	44.5	-48.1
E2	4	0.65	0.16	0.24	27.7	13.1	47.9	42.8	46	-39.7
E4	3	0.63	0.22	0.36	17.6	5.4	34.2	28	6.4	175.7
Core total	57	0.75	0.07	0.09	370.3	241.5	563.8	491.8	487.0	-24.0
BK	5	0.26	0.17	0.64	151.4	0.0	377.4	578.4	35.3	329.0
Survey total	62	0.49	0.10	0.20	521.8	277.9	858.9	1 070.2	522.3	-0.1

Table 7: Absolute population estimates for small oyster (1049 mm in diameter) oysters by stratum (Stratum), in the core strata (Core total), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2022 by stratum. Columns give the numbers of stations sampled (Number stations), mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (Density CV) of the density estimate, the 2022 mean population size in millions of oysters (2022 Pop.n, shaded light blue), lower and upper 95% confidence intervals in millions of oysters where a B prefix denotes the bootstrapped estimates (B.lower and B.upper 95% CI), and the area of each stratum in square kilometres (Area (km^2)). Also given are the 2021 mean population estimates (2021 Pop.n, shaded light grey) and the 2022 mean population size represented as a percentage of the 2021 mean population size by stratum (% of 2021). The percentage change from the 2021 estimate (Percentage change) is shaded green for increases in population size in 2022 and orange for decreases.

Stratum	Number stations	Mean density	Density s.d.	Density CV	2022 Pop.n	B.lower 95% CI	B.upper 95% CI	Area (km^2)	2021 Pop.n	Percentage Change
B1	5	2.86	0.65	0.23	224.0	111.6	380.2	78.2	297.3	-24.7
B3	5	1.57	0.25	0.16	70.3	41.7	110.8	44.7	111	-36.6
B6	5	2.75	1.14	0.41	82.4	14.0	166.5	30	122.3	-32.6
C1a	4	1.40	0.35	0.25	44.0	20.6	76.1	31.3	47.2	-6.8
C2	3	1.89	0.70	0.37	41.5	11.1	81.0	21.9	38.1	8.8
C3	3	4.57	2.17	0.47	149.3	10.0	320.3	32.7	30.1	396.1
C5	4	1.40	0.53	0.38	52.9	12.2	105.5	37.7	57.4	-7.9
C5a	3	0.61	0.20	0.32	14.4	5.0	26.5	23.5	8.4	71.0
C7	3	2.66	0.65	0.24	96.2	45.8	166.1	36.1	128.2	-25.0
C7a	4	2.35	0.83	0.35	55.6	15.9	106.3	23.6	57.9	-4.0
C8	5	2.97	0.85	0.29	79.6	32.0	142.6	26.8	66.2	20.2
C9	6	1.71	0.46	0.27	58.9	25.6	104.3	34.5	41.3	42.5
E2	4	1.98	0.63	0.32	84.8	29.6	157.9	42.8	73.8	14.9
E4	3	2.25	0.77	0.34	63.0	20.7	120.3	28	12	425.1
Core total	57	2.27	0.23	0.10	1 116.6	723.1	1 709.9	491.8	1 091.2	2.3
BK	5	1.83	1.47	0.80	1 060.1	0.0	2 988.4	578.4	111	855.0
Survey total	62	2.03	0.80	0.39	2 176.7	488.3	4 354.1	1 070.2	1 202.1	81.1

Table 8: The population estimates and the percentage change in population size between years in the Bonamia survey area only, for recruit-sized, pre-recruit, and small oysters from the 2012, and 2016–2022 surveys and for commercial-sized oysters for the 2019–2022 surveys. Percentage changes in the population size of recruit-sized, pre-recruit, and small oysters quantify recent trends in increases or decreases in population size. Columns give the mean oyster density per square metre (Mean density), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n), bootstrapped lower and upper 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	Mean density	CV	Pop.n	B.lower 95% CI	B.upper 95% CI	% change
2012						
Recruit	1.40	0.09	688.1	449.2	1 046.7	
Pre-recruit	0.60	0.10	297.4	192.6	454.4	
Small	0.92	0.16	451.3	261.5	731.7	
2016						**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						**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						**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						**2018-2019**
Commercial	0.65	0.13	318.7	198.0	500.1	–
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
2020						**2019-2020**
Commercial	0.64	0.12	316.1	198.8	492.5	-0.8
Recruit	1.08	0.12	529.9	333.2	825.7	-2.3
Pre-recruit	0.54	0.11	265.3	169.1	410.7	+22.5
Small	2.14	0.14	1 052.4	644.4	1 665.9	+76.6
2021						**2020-2021**
Commercial	0.82	0.07	405.6	271.0	606.6	+28.3
Recruit	1.63	0.06	801.4	536.2	1 196.7	+51.2
Pre-recruit	0.99	0.08	487.0	320.9	733.6	+83.6
Small	2.22	0.07	1 091.2	726.3	1 637.0	+3.7
2022						**2021-2022**
Commercial	0.57	0.09	282.1	184.5	428.3	-30.5
Recruit	1.13	0.09	557.9	366.6	842.9	-30.4
Pre-recruit	0.75	0.09	370.3	241.5	563.8	-24.0
Small	2.27	0.10	1 116.6	723.1	1 709.9	+2.3

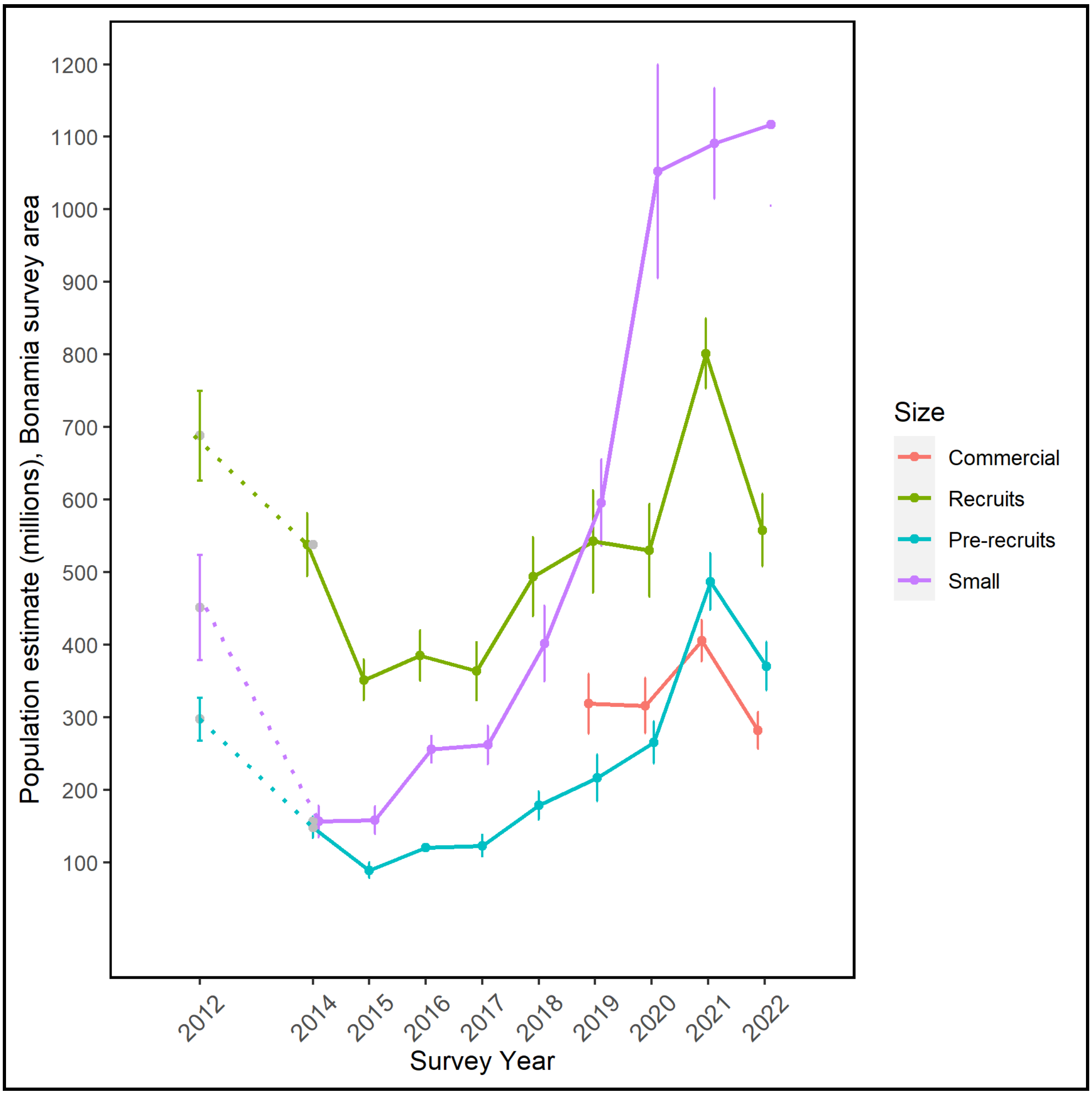

Figure 7: Mean population sizes and 95% confidence intervals for commercial-sized, recruit-sized, pre-recruit, and small oysters in the Bonamia survey area between 2012 and 2022. The survey data for February 2013 are not comparable (sampling does not cover all the Bonamia survey area) and the trends in mean population sizes between 2012 and 2014 have been interpolated (dotted lines).

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) by stratum in the core strata (Stratum), the Bonamia survey area (Core total), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2022. Columns give the numbers of stations sampled (Number stations), mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (Density CV) of the density estimate, the 2022 mean population size in millions of oysters (2022 Pop.n, shaded light blue), lower and upper 95% confidence intervals in millions of oysters where a B prefix denotes the bootstrapped estimates (B.lower and B.upper 95% CI), and the area of each stratum in square kilometres (Area (km^2)). Also given are the 2021 mean population estimates (2021 Pop.n, shaded light grey) and the percentage change in 2022 from the 2021 estimate (Percentage change), shaded green for increases in population size in 2022 and orange for decreases.

Stratum	Number stations	Mean density	Density s.d.	Density CV	2022 Pop.n	B.lower 95% CI	B.upper 95% CI	Area (km^2)	2021 Pop.n	Percentage change
B1	5	0	0	0	0	0	0	78.2	39.4	-100.0
B3	5	0.97	0.60	0.62	43.5	0	105.9	44.7	105.3	-58.7
B6	5	0.44	0.44	1.00	13.2	0	41.5	30	45.4	-70.9
C1a	4	0.55	0.55	1.00	17.4	0	55.4	31.3	25.7	-32.3
C2	3	0	0	0	0	0	0	21.9	0	–
C3	3	0	0	0	0	0	0	32.7	0	–
C5	4	0	0	0	0	0	0	37.7	20	-100.0
C5a	3	0	0	0	0	0	0	23.5	0	–
C7	3	0	0	0	0	0	0	36.1	0	–
C7a	4	0	0	0	0	0	0	23.6	34.9	-100.0
C8	5	1.95	0.80	0.41	52.2	9.8	106.1	26.8	66.4	-21.4
C9	6	1.32	0.63	0.48	45.4	3.3	97.9	34.5	114.8	-60.5
E2	4	0	0	0	0	0	0	42.8	67.4	-100.0
E4	3	0	0	0	0	0	0	28	0	–
Core total	57	0.35	09	0.27	171.6	75.1	304.0	491.8	519.3	-67.0
BK	5	0	0	0	0	0	0	578.4	0	–
Survey total	62	0.16	0.04	0.27	171.6	75.1	304.0	1 070.2	519.3	-67.0

3.2.3 Changes in the distribution of live oysters

All 74 stations were used to describe oyster distribution. Because sampling effort was focused on core strata and the background strata received 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 2022. Hence the survey was unlikely 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 8–12).

The distributions of oyster densities of all sizes were widespread, covering most of the fishery area and with the highest densities in core fishery strata, as would be expected by the distribution of sampling effort (Figures 8–12). At most sites across the core fishery area, the highest densities of recruit-sized oysters comprised substantial proportions of commercial-sized oysters (Figures 8 and 9). The distributions of commercial and recruit-sized oysters were widespread throughout the fishery area; however, there was a noticeable decrease in the size of high-density patches between 2021 and 2022 (Figures 8 and 9). The distribution of localised areas of relatively high recruit-sized oyster densities were patchy, often interspersed amongst lower density patches. The patches of relatively high recruit-sized oyster densities were widespread in 2022 as they were in 2021 (Figure 10). The increasing patches of relatively high recruit-sized oyster densities were probably the result of relatively low (less than 3%) Bonamia mortality and relatively high recruitment over the last five summers.

The densities of pre-recruit oysters were patchily distributed across the fishery area (Figure 11). High-density patches occurred in three clusters: western (strata B1, C7, and C7a), central (strata C1a, C5, C8, and C9), and eastern (strata C2 and B6).The sizes of relatively high-density patches have decreased between 2021 and 2022, especially in strata C3 and B6 in the eastern fishery area (Figure 11). Densities were still relatively low in some central fishery areas.

Relatively high densities of small oyster were widespread and consistent in 2022 as they were in 2021 (Figure 12) and in 2020. Densities have increased markedly across the entire fishery since 2018 and have remained similar between 2020 and 2022. The distributions of small oysters showed small scale spatial patchiness across all fishery regions (Figure 12). These distributions of small oysters showed consistent recruitment across the oyster fishery, irrespective of habitat type, and may demonstrate the settlement and survival of spat on live oysters. Small oysters are less vulnerable to Bonamia mortality. The increasing densities reflected increased recruitment to the oyster population, consistent with increased spat settlement since 2015.

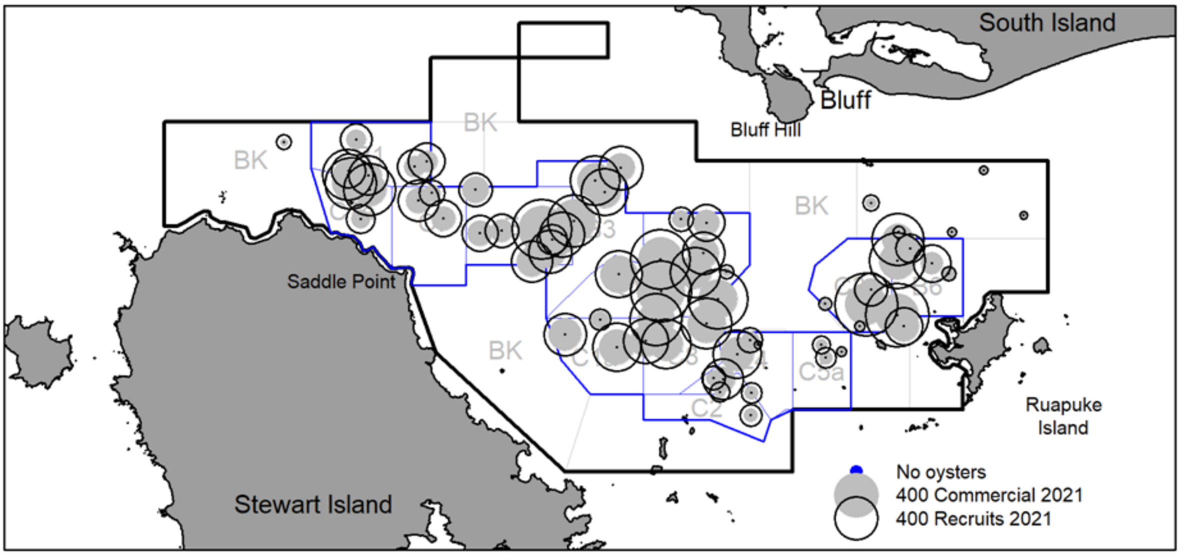

Figure 8: Density (numbers of oysters per standard tow representing an area swept of 1221 m^2) of commercial-sized (filled grey circles) and recruit-sized (open black circles) oysters sampled during the February 2021 survey. Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

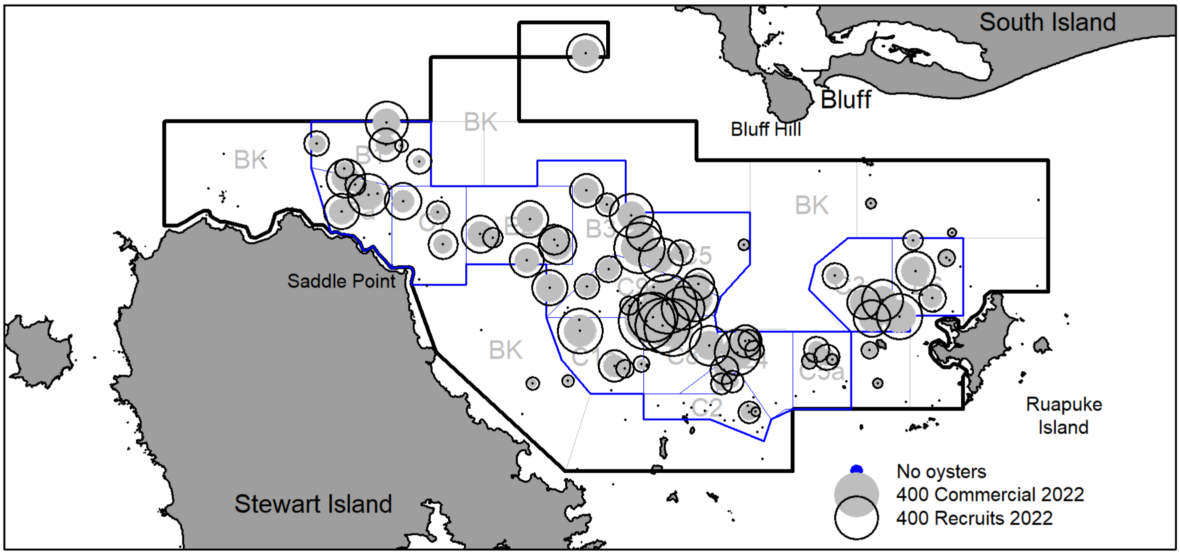

Figure 9: Density (numbers of oysters per standard tow representing an area swept of 1221 m^2) of commercial-sized (filled grey circles) and recruit-sized (open black circles) oysters sampled during the February 2022 survey. Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

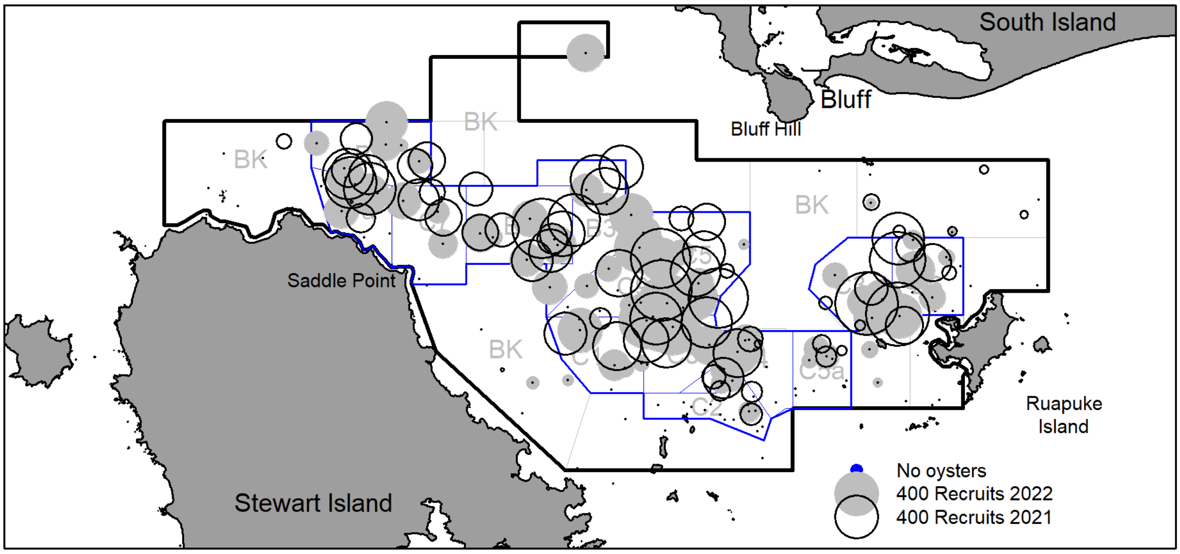

Figure 10: Density (numbers of oysters per standard tow representing an area swept of 1221 m^2) of recruit-sized oysters sampled during the February surveys in 2022 (filled grey circles) and in 2021 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

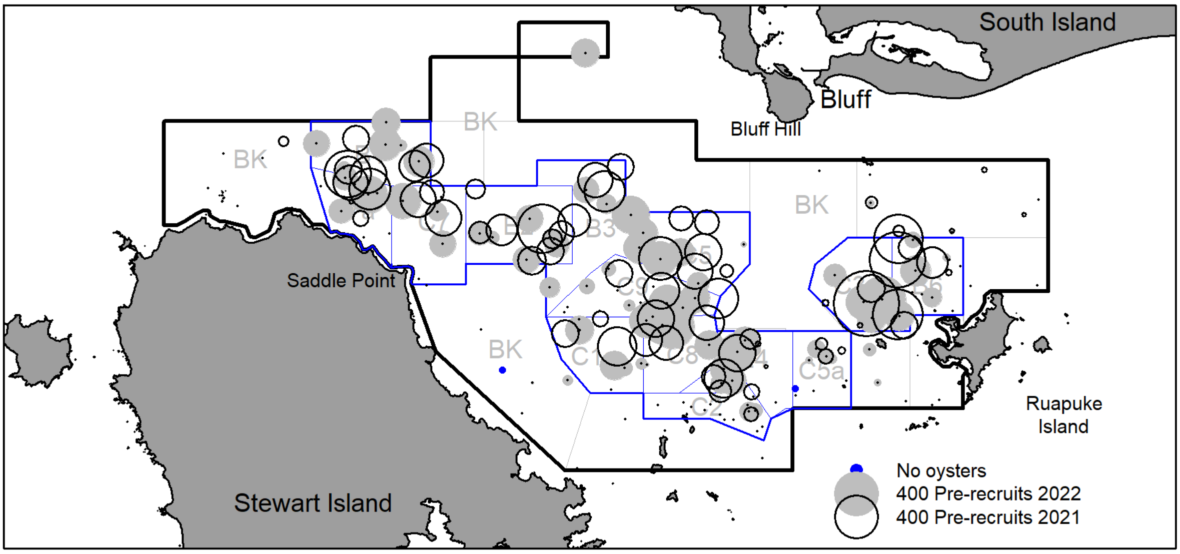

Figure 11: Density (numbers of oysters per standard tow representing an area swept of 1221 m^2) of pre-recruit sized oysters sampled during the February surveys in 2022 (filled grey circles) and in 2021 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

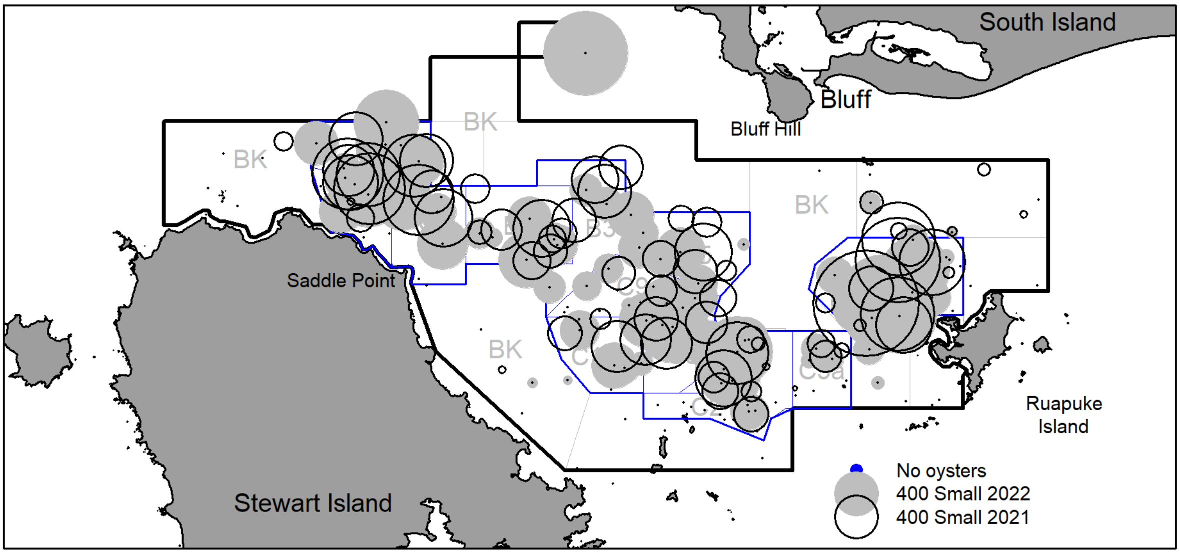

Figure 12: Density (numbers of oysters per standard tow representing an area swept of 1221 m^2) of small oysters sampled during the February surveys in 2022 (filled grey circles) and in 2021 (open black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

3.3 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, except on *Astraea heliotropium*, an ubiquitous and abundant large gastropod. 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 showed large fluctuations in a broadly cyclic trend between 1993 and 2022 (Figure 13). 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 13). 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-sized oyster population was increasing. The numbers of spat per recruit increased substantially between 2016 and 2020 (Figure 13). The numbers of spat have remained similarly high in 2021 and 2022; however, the numbers of recruits increased by 51.2% in 2021 and the numbers of spat per recruit declined slightly, and in 2022 the numbers of recruits decreased by about 30%, resulting in an upward trend in the numbers of spat per recruit (Figure 13). 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 (Fu et al. 2013) and the numbers of settlers recorded on spat collectors (Figure 14).

The total numbers of spat per collector sampled over the summers from 2005–06 to 2021–22 is shown in Figure 14. Settler densities were high in 2020–21 and in 2021–22 (Figure 14).

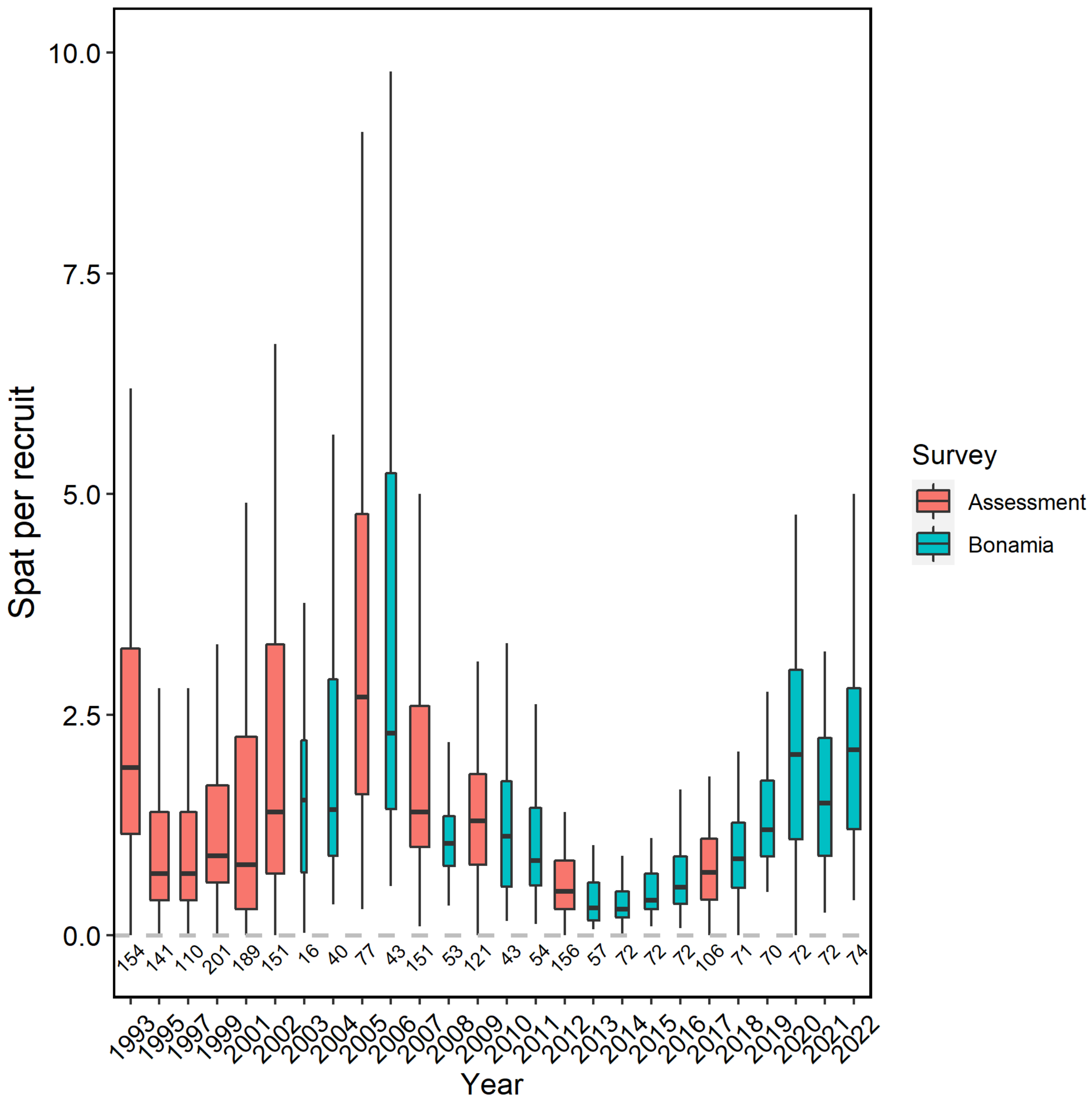

Figure 13: The numbers of small oysters per recruited oysters sampled between 1993 and 2022 on the stock assessment surveys (Assessment) and Bonamia surveys (Bonamia). Medians are shown as solid lines, boxes represent 50th percentiles (25–75%), and whiskers 90th percentiles (5–95%). Outliers smaller than 5% and greater than 95% have not been plotted for ease of visualisation. The number of stations sampled each year varied (16 lowest and 201 highest; shown below boxes as black text).

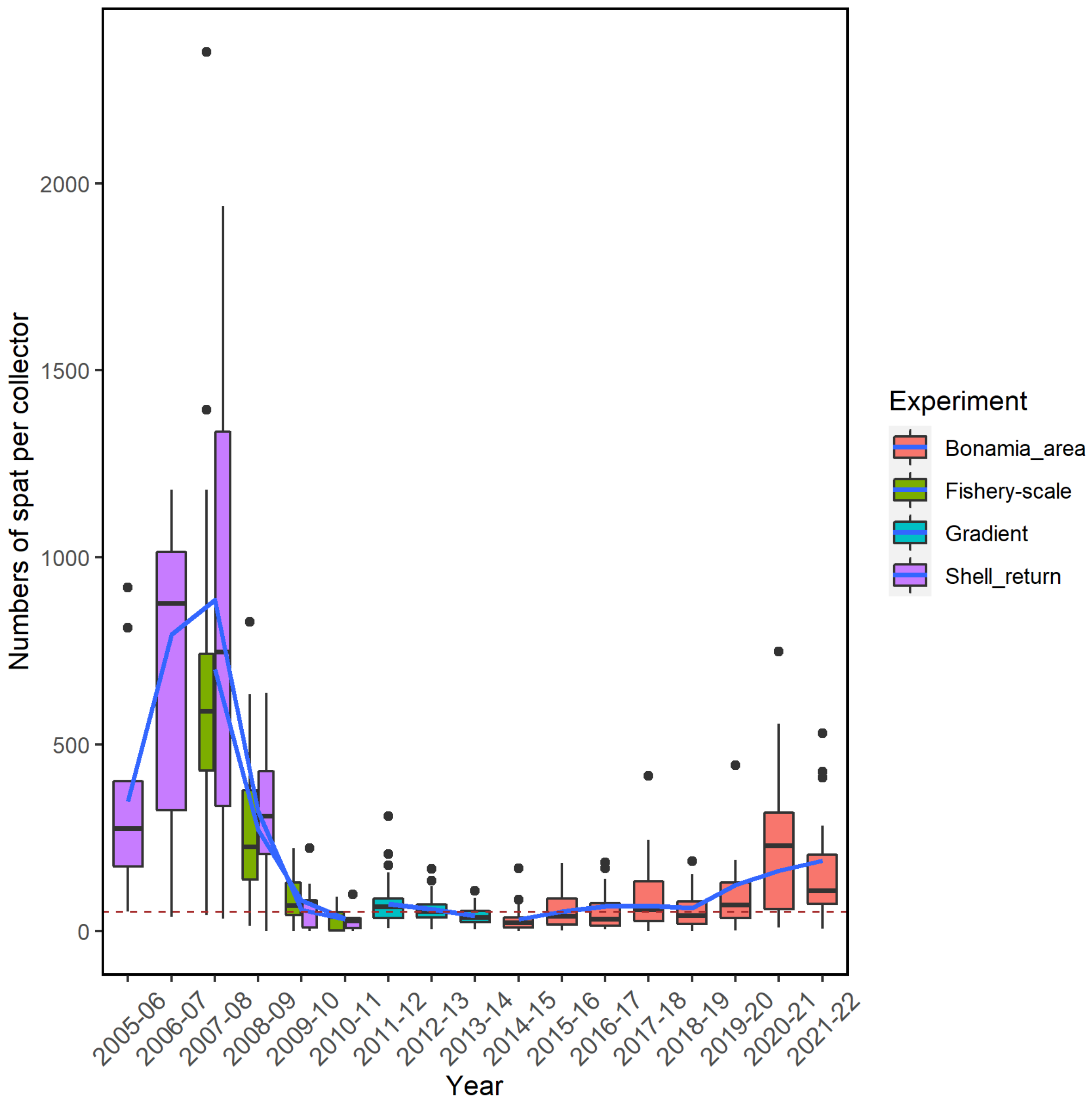

Figure 14: The total numbers of spat per collector sampled over the summers of 2005–06 to 2021–22. Spat settlement shows the success of spawning and indicates the levels of replenishment to the oyster population. Data represent four different experiments and different areas: the shell return site (south of Bluff Hill), fishery-scale experiments (western, southern, and eastern fishery areas), the gradient experiment in the central fishery area, and fishery-scale monitoring (the stock assessment survey area) that began over the summer of 2014–15. Brown dashed horizontal line denotes mean recruitment during the low period between 2009–10 and 2014–15.

3.4 Status of Bonamia infection and mortality

3.4.1 Estimates of oyster mortality before and during the February 2022 survey

Descriptive statistics for the percentages of recruit-sized and pre-recruit new clocks and gapers combined sampled from survey stations with more than 50 live recruit-sized and pre-recruit oysters between 2018 and 2022 are given in Table 10. There were few gapers observed during the February 2022 survey. Over all stations sampled in 2022, those with recruit-sized gapers represented 21.9% (14 stations), fewer than in 2020 and 2021, and more than in 2018 and 2019 (Table 10). Pre-recruit gapers accounted for 5.0% (3 stations) in 2022, fewer than in 2020 and 2021, and more than in 2018 and 2019 (Table 10) (see Appendix 6 for a list of survey reports).

Counts of recruit-sized new clocks ranged from 1–18, and for pre-recruits 1–15 in 2022, similar to 2021. The percentages of new clocks were low, a median of 0.91% and 0% for recruit and pre-recruit size groups, respectively. These low percentages suggest that pre-survey mortality has remained low.

Table 10: The number of stations with more than 50 live recruit-sized and pre-recruit oysters combined for surveys 2018–2022 (No. stations), the percentage of stations with new clocks and gapers (Stns (%), NC & G), 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 live oysters combined. Also shown are the number (No. zero stations) and percentage (% zero stations) of stations with no new clocks and gapers by year and size.

	Percentage new clocks and gapers									
	Recruit-sized					Pre-recruits				
Year	2018	2019	2020	2021	2022	2018	2019	2020	2021	2022
No. stations	55	57	62	57	64	40	40	53	54	60
Stns (%), NC & G	10.9	10.9	22.0	34.0	21.9	1.8	1.4	12.5	13.0	5.0
Median	0	0.01	0.01	0.01	0.91	0	0	0	0	0
Minimum	0	0	0	0	0	0	0	0	0	0
Maximum	0.09	0.04	0.04	0.03	5.71	0.03	0.05	0.10	0.02	2.38
5th percentile	0	0	0	0	0	0	0	0	0	0
95th percentile	0.04	0.03	0.03	0.02	4.87	0.03	0.02	0.04	0.02	0.56
No. zero stations	23	11	19	9	12	33	21	34	24	34
% zero stations	41.8	19.3	30.6	15.8	18.7	82.5	52.5	64.2	44.4	56.7

The numbers of recruit-sized new clocks sampled in survey tows between 2020 and 2022 are compared in Figure 15. The numbers of recruit-sized new clock have generally remained low and consistent within strata; however, in 2022, there were noticeably higher individual counts in strata B1, B3, C7, C3, C5, C8, C5a, E2, and BK (Figure 15).

The distribution of pre-survey mortality of recruit-sized oysters increased in localised patches in 2022. It was otherwise widespread, relatively low, and locally variable between 2020 and 2022 (Figures 16 and 17). Stations with pre-survey mortality were interspersed among stations with no detectable mortality.

The population size of recruit-sized new clocks in core strata has continued to increase to be 35.1% higher in 2022 (estimate of 7.4 million) compared with 2021 (Table 11). This increase was in addition to the 57.6% increase between 2021 (5.5 million new clocks) and 2020 (3.5 million) (see Table 13). All but six strata in the core area showed increases in recruit-sized new clocks (Table 11).

The population size of pre-recruit new clocks in core strata increased by 29% (2.6 million, Table 12) in 2022 and 300.0% between 2021 (2.0 million) and 2020 (0.5 million, Table 13). Pre-recruit oysters increased substantially over the same period. Pre-recruit new clocks increased in half the core strata, while the rest remained similar or decreased (Table 12).

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 in 2022 (1.3%), but higher than in 2021 (0.7%) and similar to mortality between 2016 and 2020, which ranged between 0.4% and 1.4% (Table 13). Mortality was higher before 2016, 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 and followed similar trends to the core area (Table 13).

Pre-survey mortality of pre-recruit oysters in core strata also remained low in 2022 (0.7%), had increased from 2021 (0.4%), and was similar to that for years 2016 to 2020, which ranged between 0.2% and 0.7% (Table 13). Pre-survey mortality of pre-recruit oysters was higher before 2016. In the stock assessment survey area, pre-survey mortality of pre-recruit oysters was 0.6% in 2022, slightly higher than in 2021, and similar to mortality between 2016 and 2020 (range 0.4%–1.1%, Table 13). Recent estimates were lower than pre-survey mortality prior to 2016 (Table 13).

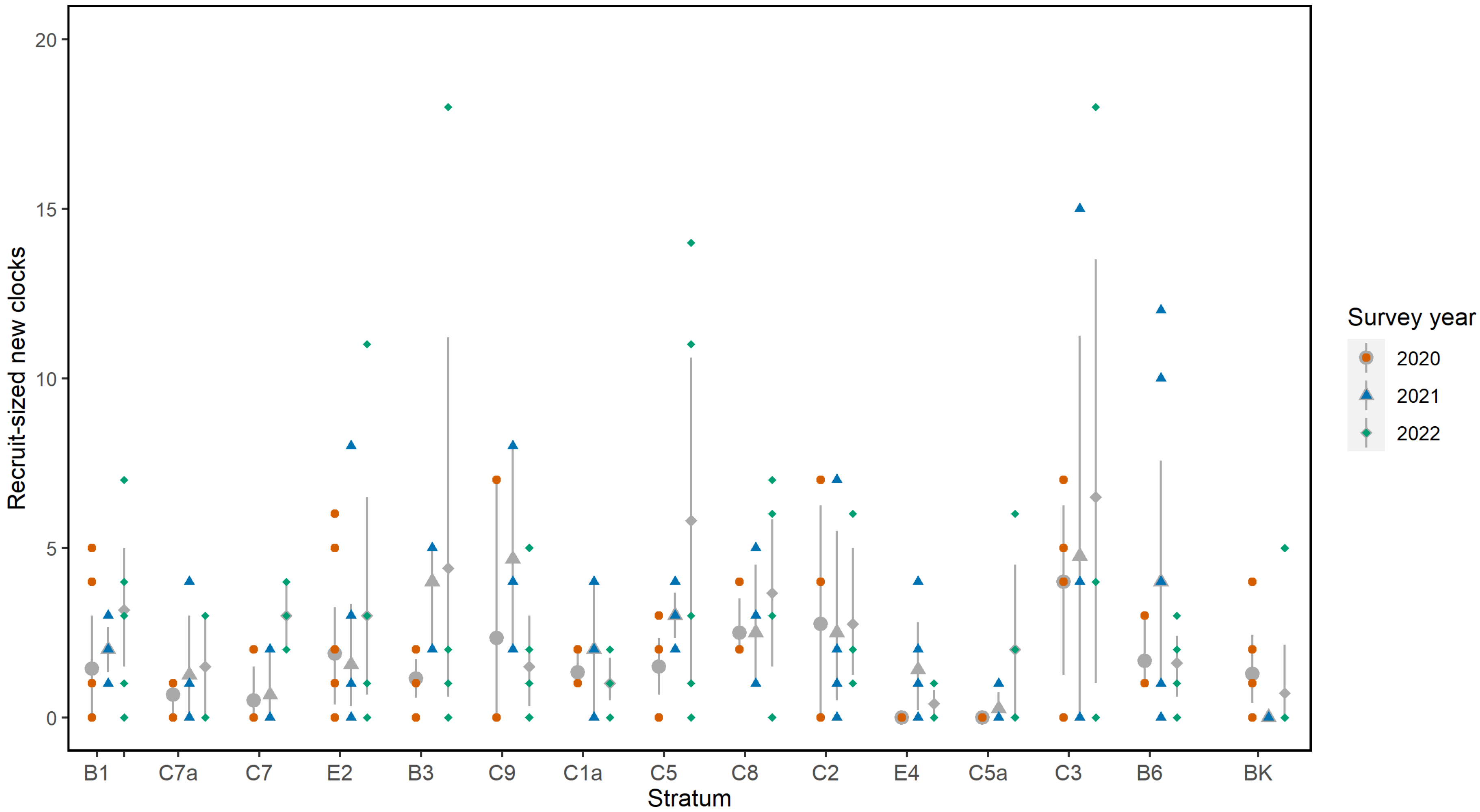

Figure 15: The numbers of recruit-sized new clocks (≥ 58 mm in diameter) per tow, means (grey symbols matching shape showing survey year), and 95% confidence intervals (grey lines) by stratum for surveys 2020–22. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2020 survey are shown as tan filled circles, 2021 as blue filled triangles, and 2022 as green filled diamonds. Bonamia survey strata are arranged west to east, with northern strata at similar longitudes shown first, and the background stratum (BK) furthermost right.

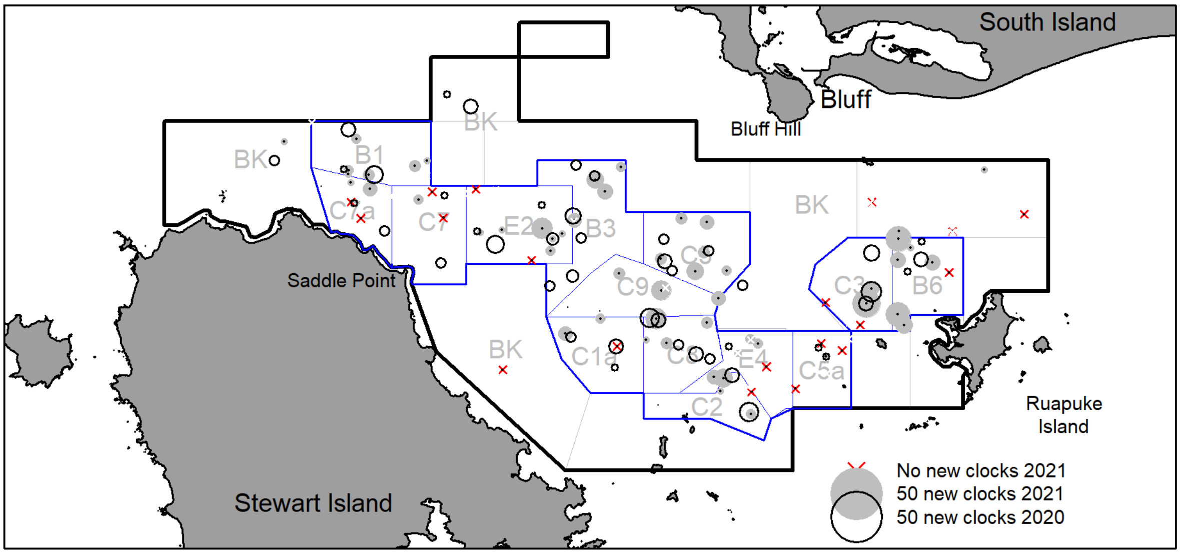

Figure 16: The distribution of recruit-sized new clocks and gaper densities combined in 2021 (filled grey circles) and 2020 (open black circles), which indicates the pre-survey mortality in February 2021 and 2020. Stations with no recruit-sized new clocks and gapers are shown as red crosses.

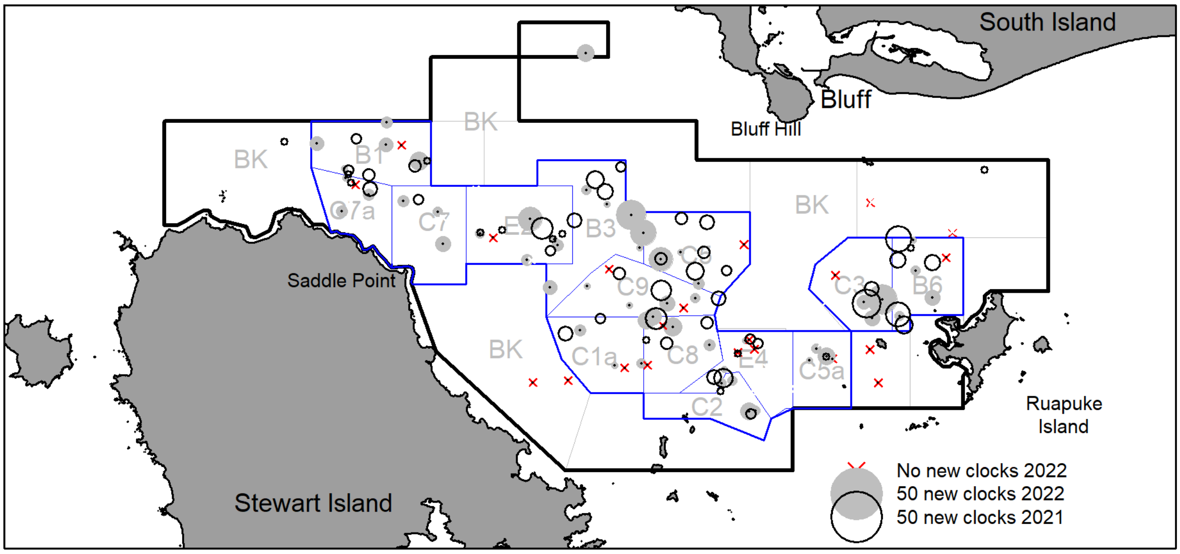

Figure 17: The distribution of recruit-sized new clocks and gaper densities combined in 2022 (filled grey circles) and 2021 (open black circles) , which indicates the pre-survey mortality in February 2022 and 2021. Stations with no recruit-sized new clocks and gapers are shown as red crosses.

Table 11: Absolute population estimates for recruit-sized (≥ 58 mm in diameter) new clocks in the core strata by stratum (Stratum), total core area (Core total), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2022. Columns indicate the numbers of stations sampled (Number stations), mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (Density CV) of the density estimate, the 2022 mean population size in millions of oysters (2022 Pop.n, shaded light blue), lower and upper 95% confidence intervals in millions of oysters where a B prefix denotes the bootstrapped estimates (B.lower and B.upper 95% CI), and the area of each stratum in square kilometres (Area (km^2)). Also given are the 2021 mean population estimates (2021 Pop.n, shaded light grey) and the 2022 mean population size represented as a percentage of the 2021 mean population size by stratum (% of 2021). The percentage change from the 2021 estimate (Percentage change) is shaded green for a decrease in population size in 2022 and orange for an increase.

Stratum	Number stations	Mean density	Density s.d.	Density CV	2022 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2021 Pop.n	Percentage change
B1	5	0.02	0.01	0.31	1.4	0.5	2.5	78.2	0.7	95.7
B3	5	0.02	0.02	0.78	1.0	0.0	2.7	44.7	0.9	10.5
B6	5	0.01	0.00	0.32	0.2	0.1	0.4	30	0.6	-61.2
C1a	4	0.00	0.00	0.40	0.2	0.0	0.3	31.3	0.3	-49.9
C2	3	0.01	0.00	0.19	0.2	0.1	0.3	21.9	0.3	-41
C3	3	0.04	0.03	0.75	1.2	0.0	3.3	32.7	0.2	510.6
C5	4	0.02	0.02	0.72	0.9	0.0	2.3	37.7	0.6	43
C5a	3	0.00	0.00	1.00	0.1	0.0	0.2	23.5	0	-
C7	3	0.01	0.00	0.19	0.5	0.3	0.9	36.1	0.1	428.4
C7a	4	0.01	0.00	0.58	0.2	0.0	0.4	23.6	0.2	-14
C8	5	0.01	0.01	0.45	0.4	0.0	0.8	26.8	0.2	99.3
C9	6	0.01	0.00	0.50	0.3	0.0	0.6	34.5	0.8	-68.7
E2	4	0.02	0.01	0.56	0.9	0.0	2.1	42.8	0.4	127.4
E4	3	0.00	0.00	0.50	0.1	0.0	0.2	28	0.3	-69.7
Core total	57	0.02	0.00	0.21	7.4	3.9	12.5	491.8	5.5	35.1
BK	5	0.00	0.00	1.00	2.9	0.0	9.2	578.4	0	–
Survey total	62	0.01	0.00	0.32	10.3	3.7	19.1	1070.2	5.5	87.0

Table 12: Absolute population estimates for pre-recruit (50–57 mm in diameter) new clocks in the core strata by stratum (Stratum),), total core area (Core total), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2022. Columns indicate the numbers of stations sampled (Number stations), mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (Density CV) of the density estimate, the mean population size in millions of oysters 2022 (Pop.n, shaded light blue), lower and upper 95% confidence intervals in millions of oysters where a B prefix denotes the bootstrapped estimates (B.lower and B.upper 95% CI), and the area of each stratum in square kilometres (Area (km^2)). Also given are the 2021 mean population estimates (2021 Pop.n, shaded light grey) and the 2022 mean population size represented as a percentage of the 2021 mean population size by stratum (% of 2021). The percentage change from the 2021 estimate (Percentage change) is shaded green for a decrease in population size in 2022 and orange for an increase.

Stratum	Number stations	Mean density	Density s.d.	Density CV	2022 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2021 Pop.n	Percentage change
B1	5	<0.01	<0.01	0.67	0.2	0.0	0.6	78.2	0.3	-22.4
B3	5	<0.01	<0.01	0.67	0.3	0.0	0.8	44.7	0.2	57.8
B6	5	<0.01	<0.01	0.61	0.1	0.0	0.1	30	0.1	-40.8
C1a	4	<0.01	<0.01	1.00	0.0	0.0	0.1	31.3	0.1	-62.7
C2	3	<0.01	<0.01	1.00	0.0	0.0	0.1	21.9	0.1	-64.7
C3	3	0.01	0.01	1.00	0.4	0.0	1.3	32.7	0.1	291.2
C5	4	0.02	0.02	1.00	0.7	0.0	2.3	37.7	0.2	257.9
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0	–
C7	3	<0.01	<0.01	0.50	0.1	0.0	0.3	36.1	0.2	-41.0
C7a	4	<0.01	<0.01	0.38	0.1	0.0	0.3	23.6	0	–
C8	5	<0.01	<0.01	0.65	0.2	0.0	0.4	26.8	0.2	-12.7
C9	6	<0.01	<0.01	1.00	0.0	0.0	0.1	34.5	0.2	-85.5
E2	4	<0.01	<0.01	0.80	0.3	0.0	0.9	42.8	0.1	221.9
E4	3	0.00	0.00	0.00	0.0	0.0	0.0	28	0.1	0.0
Core total	57	0.01	<0.01	0.35	2.6	0.8	4.9	491.8	2.0	28.7
BK	5	<0.01	<0.01	1.00	0.6	0.0	1.8	578.4	0.6	-4.8
Survey total	62	<0.01	<0.01	0.34	3.1	1.0	6.1	1070.2	2.6	21.0

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, 2014–2022 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					
	Recruit-sized			Pre-recruit		
Year	Oysters	New clocks	% mort	Oysters	New clocks	% mort
2012	688.1	22.4	3.2	297.7	8.9	2.9
2014	538.0	39.4	6.8	148.4	3.6	2.4
2015	351.4	13.5	3.7	89.2	2.2	2.4
2016	385.2	1.4	0.4	120.5	0.2	0.2
2017	363.6	5.3	1.4	123.1	0.9	0.7
2018	494.1	2.9	0.6	178.4	0.4	0.2
2019	542.5	4.1	0.8	216.5	1.0	0.5
2020	529.9	3.5	0.7	265.3	0.5	0.2
2021	801.4	5.5	0.7	487.0	2.0	0.4
2022	557.9	7.4	1.3	370.3	2.6	0.7

	Stock assessment survey area					
	Recruit-sized			Pre-recruit		
Year	Oysters	New clocks	% mort	Oysters	New clocks	% mort
2012	918.4	30	3.2	414.3	12.0	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
2020	879.3	8.5	1.0	436.6	5.0	1.1
2021	868.1	5.5	0.6	522.3	2.6	0.5
2022	824.4	10.3	1.2	521.8	3.1	0.6

3.4.2 The prevalence and intensity of Bonamia infection

3.4.2.1 Sampling effectiveness for the prevalence and intensity of infection by Bonamia

Samples of 25 recruit-sized and pre-recruit oysters were collected from all stations in 2022; this included 70 stations of recruits only, two stations of recruits and pre-recruits, and two stations of recruits, pre-recruits, and small oysters. In all, 1800 heart imprint slides were sampled and archived. This sample comprised 1758 recruit-sized oysters (97.7% of oysters sampled), 20 pre-recruits, and 22 small oysters. In previous years, a similarly high proportion of recruit-sized oysters were sampled. Only a subsample of these samples was screened (n= 342).

Matching heart and gill tissue samples were taken for ddPCR from 1768 oysters. Replicate gill tissue samples were also taken and archived for future reference. Only heart tissues were processed with ddPCR.

3.4.2.2 ddPCR detection of Bonamia in oyster heart tissues

In 2022, 32 of the 1800 oyster samples could not be tested for *Bonamia exitiosa*. As in previous years, a simple proteinase digestion method was used for high-throughput extraction of DNA from heart tissues. Many samples produced low concentrations (< 5 cp/ul) for the oyster beta-actin internal ddPCR control reaction, indicating insufficient tissue was extracted. These reactions were either repeated from heart digests using 1ul of undiluted digest or using 2 ul of a 1:20 dilution of gill tissue digested in the same way. The ddPCR test results for gill tissues typically produced internal control concentrations that were an order of magnitude higher than the corresponding test on heart tissue digests.

A summary of ddPCR samples tested and the corresponding heart imprint slides examined in 2022 is given in Table 14. Of the 1768 slides taken from random stations with more than 15 recruit and pre-recruit sized oysters in 2022, a subset of 342 heart imprint slides were examined for Bonamia infection; 89 were positive for *Bonamia exitiosa* using ddPCR and the remaining 253 were random samples of negative slides (3–5 from each station). The remaining 1396 slides were from oysters screened using ddPCR and were not infected. In 2022, 97.3% of oysters had no detectable infection using histology, similar to 2019–2021 (97.0%–97.3%), but higher (less infection) than in previous years (see Table A6.1, Appendix 6 for references). ddPCR, as expected, showed high sensitivity in the detection of low-level infections. Unscaled prevalence of Bonamia by ddPCR was 19.0% higher (5.0%) than for heart imprints (4.2%).

Table 14: The numbers of oyster heart tissue samples screened for Bonamia using ddPCR and heart imprints in 2022. The total numbers of samples tested (Sample (N)) and the numbers of samples that tested negative (ddPCR-) or positive (ddPCR+) using ddPCR and from heart imprint slides are summarised. For each station, the sample of heart imprint slides screened (Slides read (N)) included all ddPCR positives (Heart imprint +ve) and three or more randomly selected ddPCR negative samples (Heart imprint -ve).

ddPCR samples	Recruits	Recruits	Recruits
Bonamia infection	Sample (N)	ddPCR-	ddPCR+
Heart	1768	1679	89
Heart imprints			
Slides read (N)	342		
Heart imprint -ve	268		
Heart imprint +ve	74		

3.4.2.3 Prevalence and intensity of infection from heart imprints

Heart imprints underestimated the true prevalence of Bonamia infection and were lower than ddPCR estimates (Table 15). The mean prevalence from heart imprints in 2022 (8.2%) was higher than in 2021 and 2020 (Table 15), but lower than in previous surveys (2009–2017, 1.9–15.3%, referenced in Appendix 6). ddPCR detection of *B. exitiosa* in heart tissues is more sensitive than heart imprints. Mean prevalence from ddPCR in 2022 was 10.8%, lower than in 2021 (4.8%), 2020 (5.7%) and in 2019 (7.4%). Mean prevalence from ddPCR in 2022 was 32% higher than from heart imprints in 2022. Differences in the prevalence of infection between ddPCR and heart imprints were, in part, determined by the intensity of infection.

Table 15: Comparisons of infection levels (prevalence (Prev %) and intensity (Inten)) in survey tows between 2020 and 2022. Number of samples for each method (N), mean and median prevalence and intensity estimated by heart imprints (Hist.), prevalence from ddPCR, standard deviation (s.d.), and 5% and 95% percentiles (5% and 95%) are reported. Data are from random stations with more than 15 recruit and pre-recruit oysters in the sample.

	2020			2021			2022		
	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)
N	55	27	55	53	23	53	35	35	36
Mean	3.2	3.0	5.7	3.0	3.4	4.8	8.2	3.1	10.8
Median	0	3.0	4.0	0	3.4	4.0	4.5	3.0	9.0
s.d.	3.9	1.0	5.7	4.2	1.0	5.7	5.9	1.0	8.1
5%	0	1.0	0	0	1.6	0.0	2.7	1.0	0.0
95%	12.0	5.0	17.2	12.0	5.0	16.0	23.9	5.0	28.6

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 4.2% of oysters with detectable infections in 2022, mean light (category 1 and 2) infection was 1.3% (0.1–5% in 2010–2021) and 2.9% had category 3 or higher infections (1.2–11% in 2010–2020), 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 was similar between 2018 and 2022 and was the lowest since 2007 (Figure 18). During periods of relatively high prevalence (2012–2015), qPCR showed higher prevalence than heart imprints. Prevalence from ddPCR decreased from 2018 to 2019 and then increased in 2020 and remained similar until 2022; ddPCR was higher than for heart imprints, reflecting the increased sensitivity of PCR methods.

The 2022 proportion of infected oysters with non-fatal infections (less than category 3, Diggles et al. 2003) was similar to 2020 and 2021 (Figure 19). In 2022, the proportions of infected oysters with non-fatal infections (less than category 3, Diggles et al. 2003) and fatal infections (category 3 and higher) were similar to 2020 and 2021 (Figure 19). The median intensity of infection in 2022 was similar to the long-term average (2007–2021). The timing of the intensification of infections may vary from year to year and patterns observed in Figure 19 likely reflect this variation.

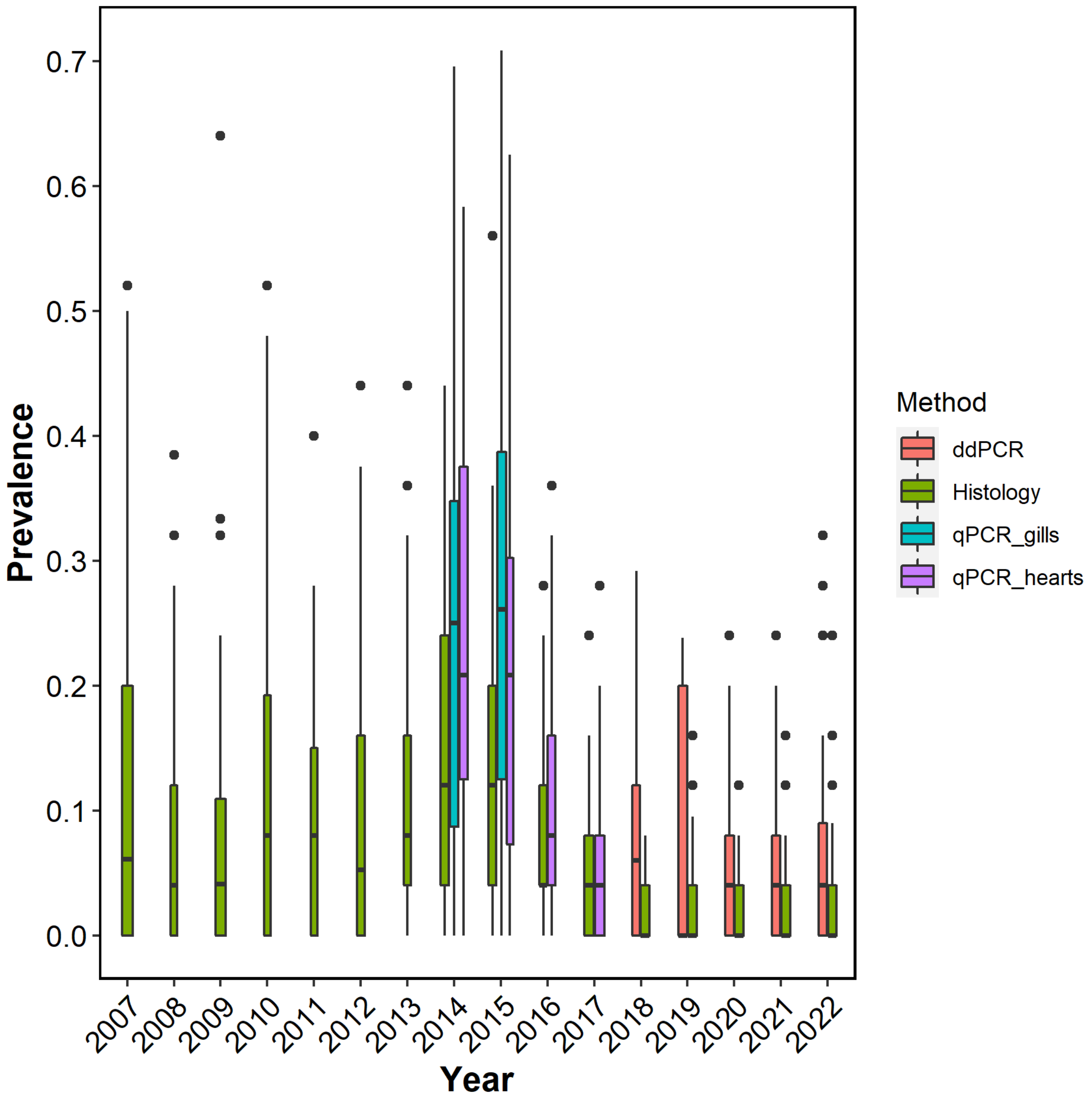

Figure 18: Boxplots of the median prevalence of Bonamia infection, 2007–2022, including the median prevalence of infection at all stations determined from heart tissue (ddPCR) 2018–2022, heart imprints (Histology) 2007–2022, gill tissues (qPCR_gills) in 2014 and 2015, and qPCR heart tissues (qPCR_hearts) 2014-2017. Heart tissues have been tested with ddPCR since 2018. Medians shown as solid lines, boxes represent 50th percentiles, whiskers 95th percentiles, and outliers as filled black circles.

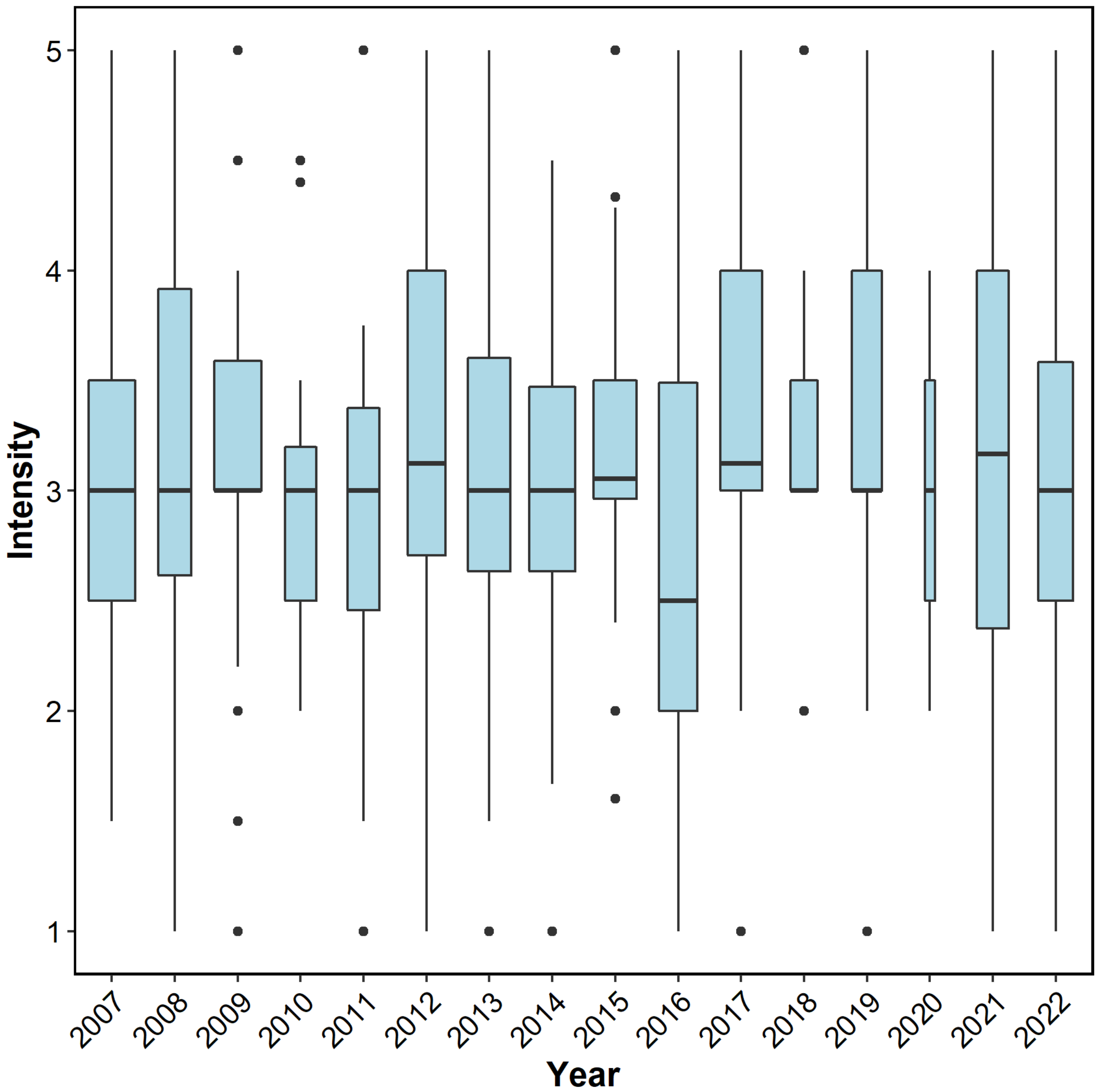

Figure 19: Boxplots of the mean intensity of Bonamia infection between 2007 and 2022. The mean intensity of infection at all stations was determined from histology. Medians are shown as solid lines, boxes represent 50th percentiles, whiskers 95th percentiles, and outliers are shown as filled black circles. The width of boxes scaled by the number of stations with Bonamia infection.

3.4.3 Changes in the distribution of prevalence and intensity of Bonamia infection

The distribution of the prevalence of Bonamia infection, estimated from heart imprints and ddPCR analyses between 2020 and 2022, is widespread but patchy, both spatially and temporally (Figures 20–22). Sites with Bonamia infection were interspersed with sites with no Bonamia infection across the fishery area and these sites varied between years (Figures 20–22). Some of the variability was due to detection method; ddPCR detected infection at some sites, presumably low intensity infections, where there was no infection detected by heart imprints.

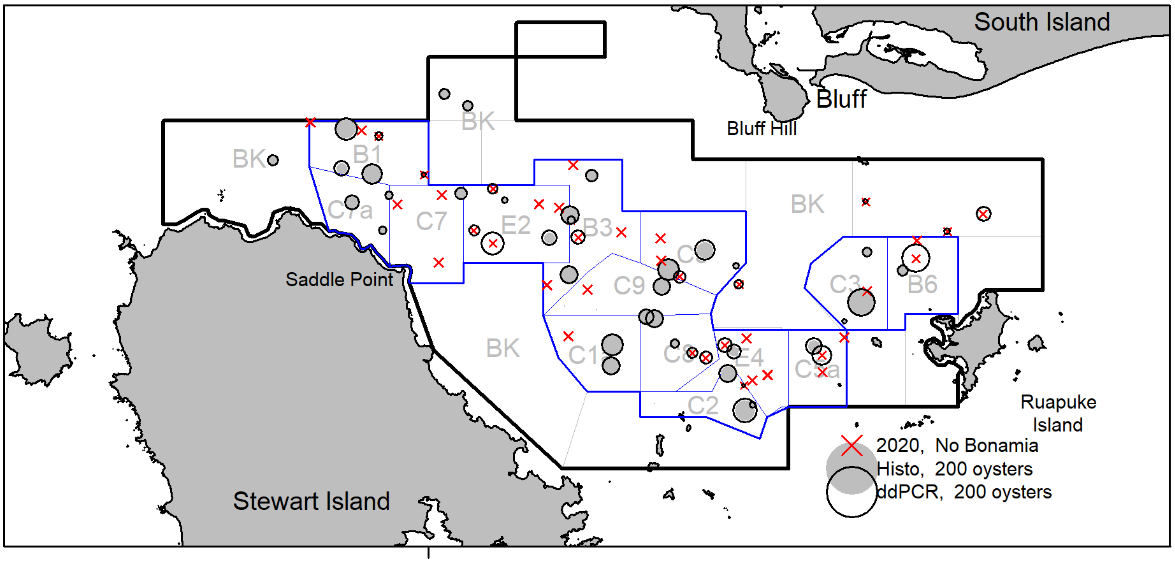

Figure 20: **The distributions of Bonamia infection (intensity categories 1–5 combined) in February 2020 estimated from heart imprints (Histo, filled grey circles) and ddPCR analysis of heart tissues only (ddPCR, open black circles). Stations with no Bonamia are indicated by red crosses. Open black circles with red crosses only indicate infection detected by ddPCR, but not by heart imprints. The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.**

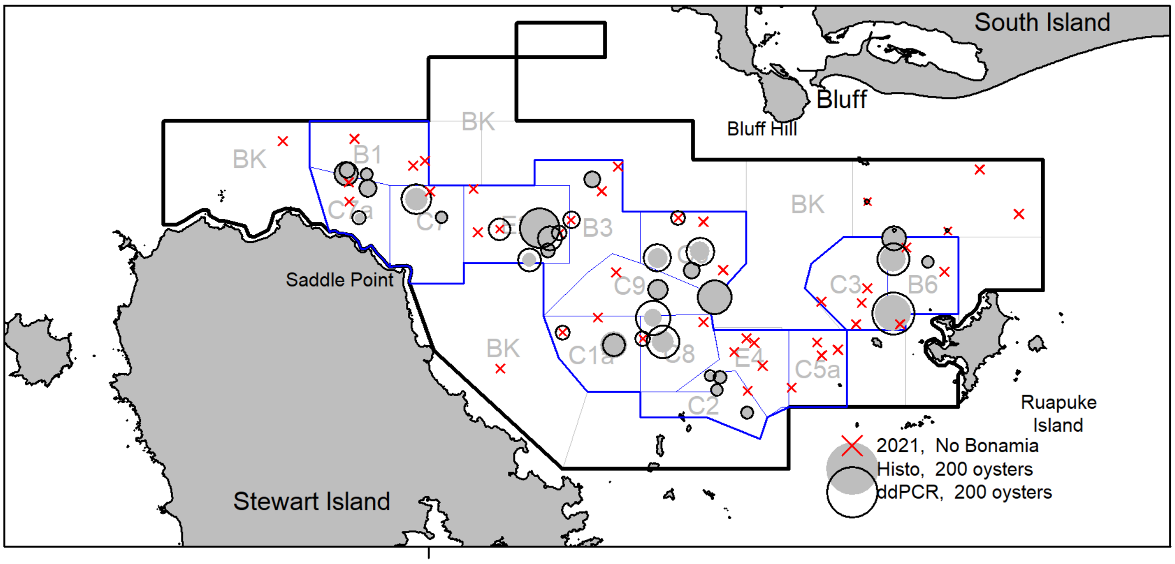

Figure 21: **The distributions of Bonamia infection (intensity categories 1–5 combined) in February 2021 estimated from heart imprints (Histo, filled grey circles) and ddPCR analysis of heart tissues only (ddPCR, open black circles). Stations with no Bonamia are indicated by red crosses. Open black circles with red crosses only indicate infection detected by ddPCR, but not by heart imprints. The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.**

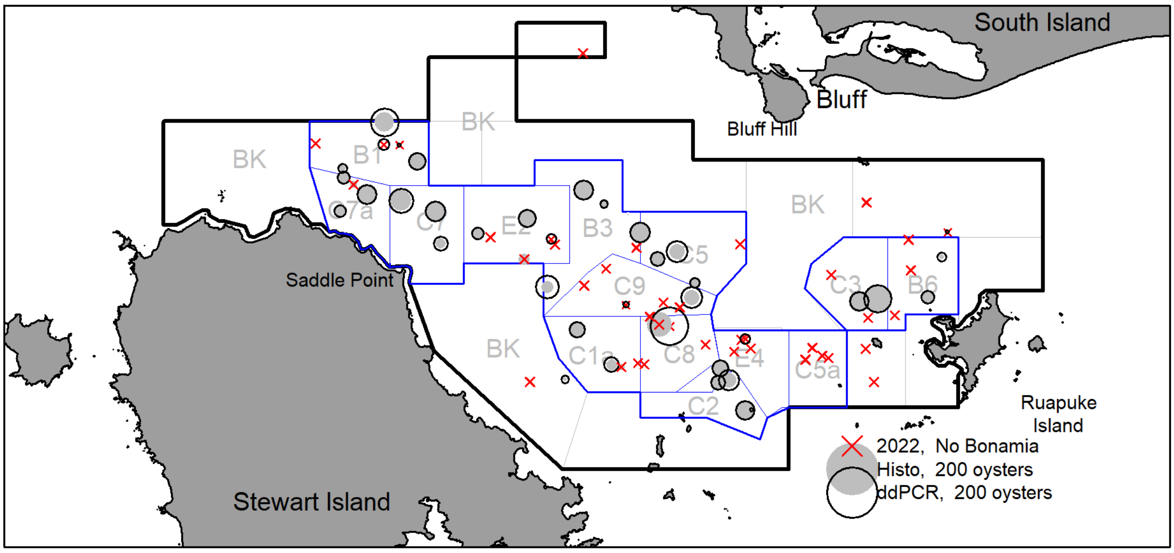

Figure 22: The distributions of Bonamia infection (intensity categories 1–5 combined) in February 2022 estimated from heart imprints (Histo, filled grey circles) and ddPCR analysis of heart tissues only (ddPCR, open black circles). Stations with no Bonamia are indicated by red crosses. Open black circles with red crosses only indicate infection detected by ddPCR, but not by heart imprints. 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, referenced in Appendix 6). 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. Recruit-sized oyster densities were similarly low in 2017, infection was low and patchy, and fatal infections were more widespread, extending into western fishery areas. 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 (Michael et al. 2019, referenced in Appendix 6). Recruit-sized oyster densities increased further in 2019 and fatal infection was widespread, patchy, and in similar or higher densities than in 2018. Non-fatal infection remained relatively low in 2019 (Michael et al. 2019, referenced in Appendix 6). In 2020, fatal infection remained widespread, patchy, and densities of fatal infections were similar to 2019 (Figure 23). In 2021, fatal infection became more widespread and increased slightly compared with previous years (Figure 24). In 2022, the distribution of fatal infections remained widespread; however, distribution of infections had become patchier, especially in central, southern, and eastern fishery areas and the densities of fatal infections had decreased (Figure 25).

Patterns in the distribution of prevalence and intensity of infection between 2012 and 2022 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.

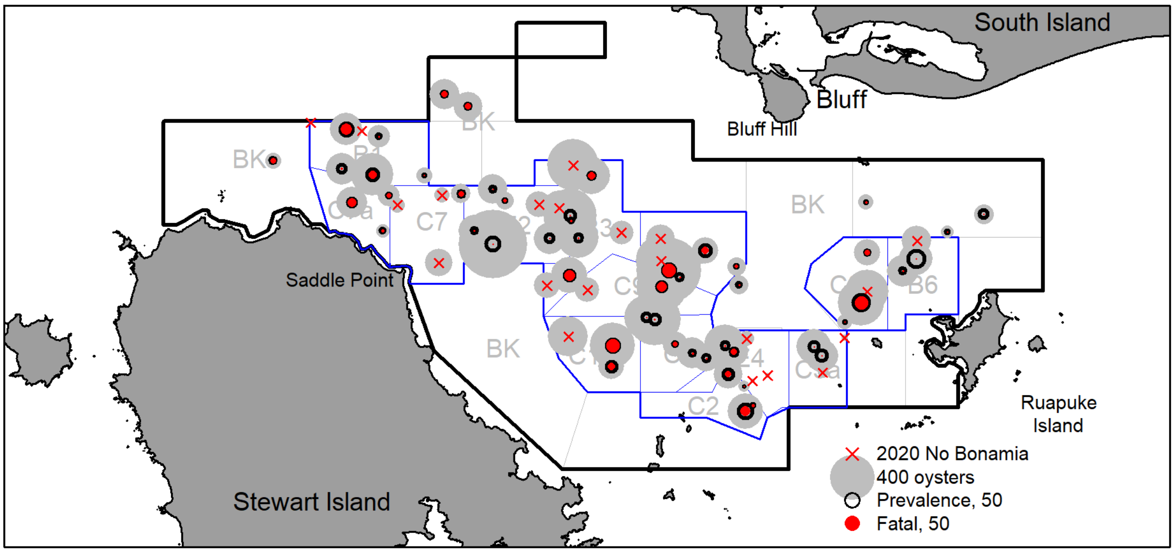

Figure 23: The distributions of recruit-sized oysters and Bonamia infection in February 2020: numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles), fatal infections (intensity categories 3–5 combined, filled red circles), and stations with no Bonamia (red crosses). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

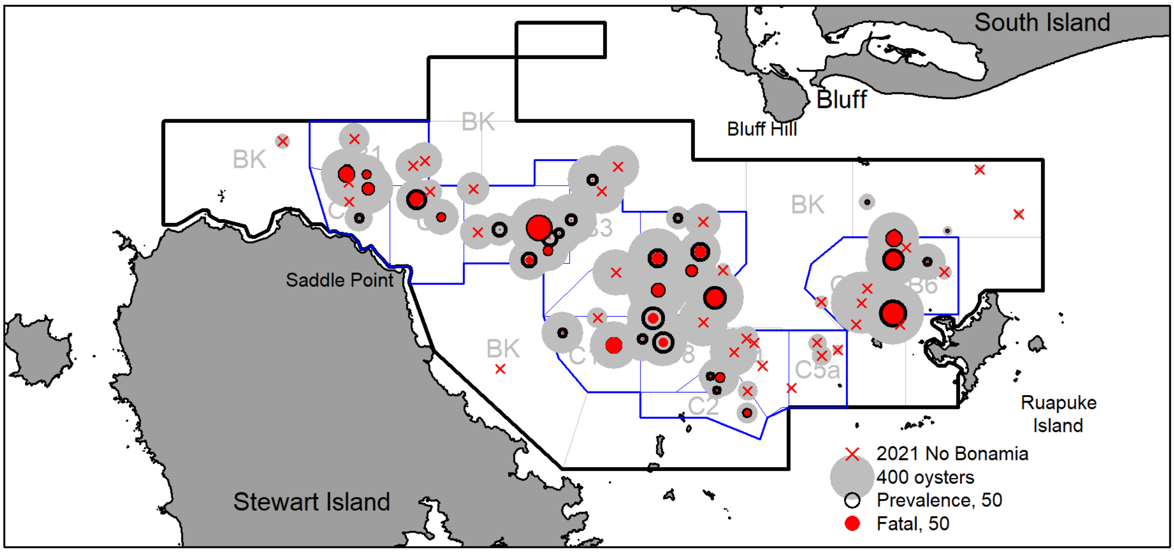

Figure 24: The distributions of recruit-sized oysters and Bonamia infection in February 2021: numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles), fatal infections (intensity categories 3–5 combined, filled red circles), and stations with no Bonamia (red crosses). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

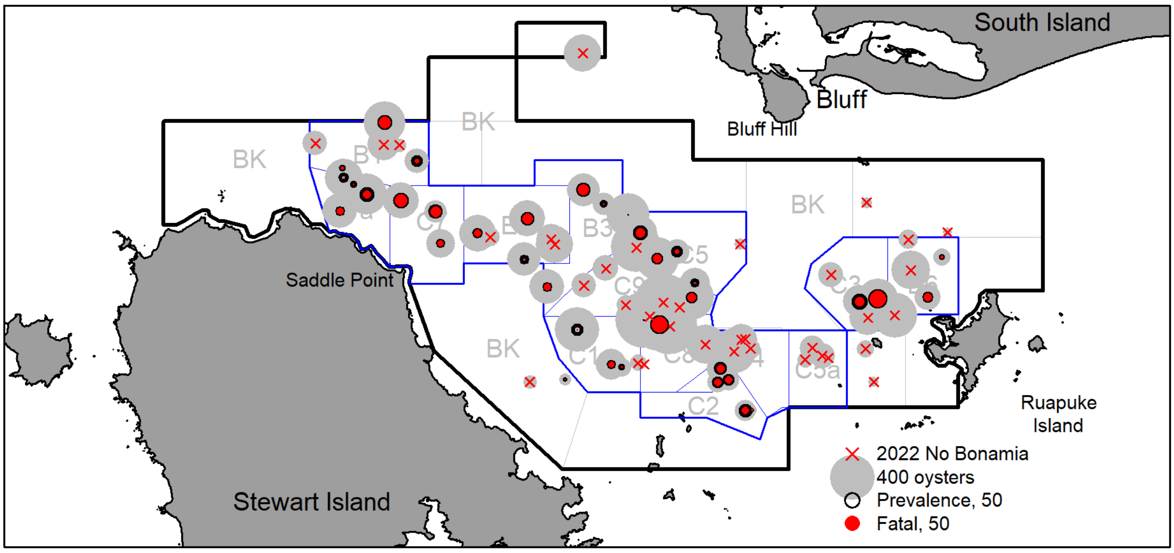

Figure 25: The distributions of recruit-sized oysters and Bonamia infection in February 2022: numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, open black circles), fatal infections (intensity categories 3–5 combined, filled red circles), and stations with no Bonamia (red crosses). The areas shown are the 2007 survey area (black outer line) and the core strata (blue lines), with the stratum labels in grey.

3.4.4 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 2022 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.

In 2022, the total number of recruit-sized oysters in core strata infected with Bonamia, detected by heart imprints, was 26.4 million (95% CI 13.7–44.6, Table 16), which was less than in 2021 (34.3 million oysters, 95% CI 16.0–59.0, Michael et al. 2022, referenced in Appendix 6), but more than in 2020 (17.0 million oysters, 95% CI 8.4–29.0, Michael et al. 2021, referenced in Appendix 6). The prevalence of infection in the Bonamia survey area, detected by heart imprints, was 4.7% in 2022, 4.3% in 2021, 3.2% in 2020, and 1.4% in 2019 (Table 16). Of those infections in 2022, 1% were non-fatal, greater than levels in 2019–2021 (Table 18).

The prevalence of Bonamia infection, detected by ddPCR, in the core strata was 6.6.%, higher than levels detected by heart imprints and higher than levels between 2021–2019 using the same method (Table 17).

Table 16: The 2022 estimates of recruit-sized oysters with Bonamia infection (prevalence), estimated by heart imprints, by stratum, 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 (Density CV) of the density estimate, mean population size in millions of infected oysters (Popn infected, shaded 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 2022 recruit-sized oyster population size (Popn recruits, shaded grey, millions of oysters) and prevalence for 2019–2022 (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	Density CV	Popn infected	Lower 95% CI	Upper 95% CI	Area (km²)	Popn recruits	Prev (%) 2022	Prev (%) 2021	Prev (%) 2020	Prev (%) 2019
B1	5	0.05	0.03	0.66	3.8	0.0	9.6	78.2	73.2	5.2	3.4	7.4	0
B3	3	0.06	0.03	0.57	2.5	0.0	5.7	44.7	71.7	3.5	1.5	1.8	0.8
B6	7	0.02	0.01	0.85	0.5	0.0	1.3	30	32.2	1.4	7.4	1.3	2
C1a	3	0.04	0.02	0.55	1.2	0.0	2.7	31.3	30.5	3.9	6	5.8	0
C2	3	0.06	0.03	0.46	1.2	0.1	2.6	21.9	8.3	14.9	3.9	7.2	5.4
C3	3	0.10	0.10	1.00	3.4	0.0	10.7	32.7	44.9	7.5	0	1.4	0.8
C5	5	0.07	0.03	0.48	2.8	0.1	6.0	37.7	31.1	8.9	3.8	3.6	1.2
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	8.6	0.0	0	3.9	0
C7	3	0.12	0.04	0.35	4.3	1.3	8.2	36.1	36.1	11.9	6.9	1.9	3.2
C7a	4	0.07	0.03	0.39	1.7	0.4	3.4	23.6	31.1	5.5	2.3	7.3	2.2
C8	3	0.07	0.07	1.00	1.8	0.0	5.7	26.8	61.1	2.9	2.8	1.1	3.1
C9	3	0.01	0.01	1.00	0.5	0.0	1.6	34.5	56.0	0.9	6.1	3.5	1.9
E2	7	0.04	0.03	0.68	1.9	0.0	4.8	42.8	50.8	3.7	5.3	1.2	0.6
E4	3	0.04	0.04	1.00	1.0	0.0	3.2	28	22.3	4.5	3.5	0	0
Core strata	55	0.05	0.01	0.21	26.4	13.7	44.6	491.8	557.9	4.7	4.3	3.2	1.4
BK	5	0.00	0.00	1.00	0.6	0.0	1.8	578.4	266.6	0.2	0	3.9	0.8
Survey total	60	0.03	0.01	0.21	27.0	14.2	45.3	1 070.2	824.4	3.3	4	3.5	1.2

Table 17: The 2022 estimates of recruit-sized oysters with Bonamia infection (prevalence), estimated from ddPCR, by stratum, 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 (Density CV) of the density estimate, mean population size in millions of oysters (Pop.n infected, shaded 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 2022 recruit-sized oyster population size (Popn recruits, shaded grey, millions of oysters) and prevalence in 2019–2022 (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	Density CV	Popn infected	Lower 95% CI	Upper 95% CI	Area (km²)	Popn recruits	Prev (%) 2022	Prev (%) 2021	Prev (%) 2020	Prev (%) 2019
B1	5	0.10	0.06	0.61	7.5	0.0	18.0	78.2	73.2	10.2	3.4	9.5	0
B3	3	0.10	0.05	0.53	4.3	0.0	9.7	44.7	71.7	6.0	1.5	2.8	5.9
B6	7	0.02	0.01	0.66	0.6	0.0	1.5	30	32.2	1.9	9.5	10.2	4.9
C1a	3	0.04	0.03	0.58	1.4	0.0	3.3	31.3	30.5	4.6	5.6	6.4	0
C2	3	0.09	0.05	0.56	1.9	0.0	4.3	21.9	8.3	22.6	4.6	11.2	9
C3	3	0.10	0.10	1.00	3.4	0.0	10.7	32.7	44.9	7.5	0	1.4	10.4
C5	5	0.10	0.04	0.47	3.6	0.3	7.8	37.7	31.1	11.6	7.2	5.1	3.4
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	8.6	0.0	0	7.2	0
C7	3	0.16	0.05	0.29	5.7	2.3	10.2	36.1	36.1	15.8	10.4	2.9	16
C7a	4	0.07	0.03	0.47	1.6	0.1	3.4	23.6	31.1	5.1	2.9	7.3	6.4
C8	3	0.13	0.13	1.00	3.5	0.0	11.2	26.8	61.1	5.7	6.8	5.3	13.3
C9	3	0.03	0.03	0.91	1.1	0.0	3.4	34.5	56.0	2.0	6.1	3.5	9.7
E2	7	0.03	0.03	1.00	1.3	0.0	4.3	42.8	50.8	2.6	8.8	3.4	2.8
E4	3	0.04	0.04	1.00	1.0	0.0	3.2	28	22.3	4.5	7.1	4	0
Core strata	55	0.08	0.02	0.21	36.9	19.0	62.4	491.8	557.9	6.6	5.7	5.1	6.4
BK	5	0.00	0.00	1.00	2.8	0.0	9.0	578.4	266.6	1.1	0.8	7.8	3.6
Survey total	60	0.04	0.01	0.21	39.7	20.8	66.8	1 070.2	824.4	4.8	5.3	6.2	5.4

Table 18: The 2022 estimates of recruit-sized oysters with non-fatal infections (category 1 and 2), estimated by heart imprints, by stratum, 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 (Density CV) of the density estimate, mean population size in millions of oysters (Pop.n infected, shaded grey), lower and upper 95% confidence intervals (95%CI) in millions of oysters, the area of each stratum (Area (km^2)) in square kilometres, and the 2022 recruit-sized oyster population size (Popn recruits, shaded grey, millions of oysters) and prevalence in 2019–2022 (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	Density CV	Popn infected	Lower 95% CI	Upper 95% CI	Area (km2)	Popn recruits	Prev (%) 2022	Prev (%) 2021	Prev (%) 2020	Prev (%) 2019
B1	5	0.01	0.01	1.00	0.83	0.00	2.67	78.2	73.2	1.1	0	1.9	0
B3	3	0.01	0.01	1.00	0.27	0.00	0.86	44.7	71.7	0.4	1.5	0	0.7
B6	7	0.00	0.00	0.00	0.00	0.00	0.00	30	32.2	0.0	0.4	1.3	0
C1a	3	0.03	0.02	0.83	0.86	0.00	2.44	31.3	30.5	2.8	0	0.6	0
C2	3	0.01	0.00	0.33	0.32	0.11	0.60	21.9	8.3	3.8	1.3	3.2	0
C3	3	0.00	0.00	0.00	0.00	0.00	0.00	32.7	44.9	0.0	0	0.3	0
C5	5	0.04	0.01	0.35	1.47	0.45	2.82	37.7	31.1	4.7	0	0.4	0
C5a	3	0.00	0.00	0.00	0.00	0.00	0.00	23.5	8.6	0.0	0	3.9	0
C7	3	0.01	0.01	1.00	0.30	0.00	0.95	36.1	36.1	0.8	0	0	0
C7a	4	0.04	0.02	0.46	0.95	0.09	2.03	23.6	31.1	3.1	0.5	0.8	0
C8	3	0.00	0.00	0.00	0.00	0.00	0.00	26.8	61.1	0.0	1.4	0	0.6
C9	3	0.00	0.00	0.00	0.00	0.00	0.00	34.5	56.0	0.0	1.5	1.6	0
E2	7	0.01	0.01	1.00	0.53	0.00	1.68	42.8	50.8	1.0	0	1.1	0
E4	3	0.01	0.01	1.00	0.32	0.00	1.01	28	22.3	1.4	3.5	0	0
Core strata	55	0.01	0.00	0.25	5.85	2.68	10.15	491.8	557.9	1.0	0.6	0.9	0.1
BK	5	0.00	0.00	0.00	0.00	0.00	0.00	578.4	266.6	0.0	0	0	0
Survey total	60	0.01	0.00	0.25	5.85	2.68	10.15	1 070.2	824.4	0.7	0.6	0.6	0.1

3.4.5 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 (Michael et al. 2016, referenced in Appendix 6). 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, referenced in Appendix 6) and mainly in central (strata C5 and C9) and eastern (strata C3 and B6) fishery areas, east of a line between Bluff Hill and Saddle Point. The number of stations with non-fatal infections was greatly reduced in 2017 with the occasional station in central, southern, and eastern stations. In 2018, non-fatal infections were detected at relatively few, isolated stations in western, southern, and eastern areas; however, prevalence was higher than in 2017 – 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 (see citations in Appendix 6 for details).

The distributions of non-fatal infections were widespread and patchy in 2020 (Figure 26), 2021 (Figure 27), and 2022 (Figure 28). The densities of non-fatally infected oysters were low in 2020 and in 2021. The numbers of stations with non-fatal infections increased in 2022, especially in the western and central fishery areas (Figure 28); however, non-fatal infection densities remained low. The prevalence of non-fatal infections has not increased above 1% of the recruit-sized population over the last four years (Table 18).

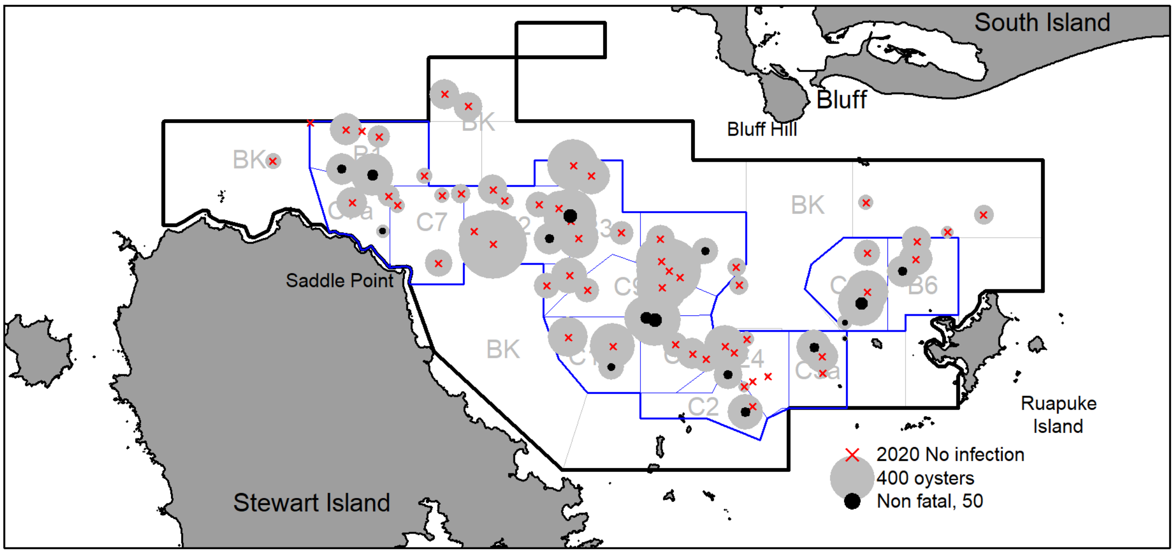

Figure 26: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2020. Stations with no Bonamia infection are shown by red crosses.

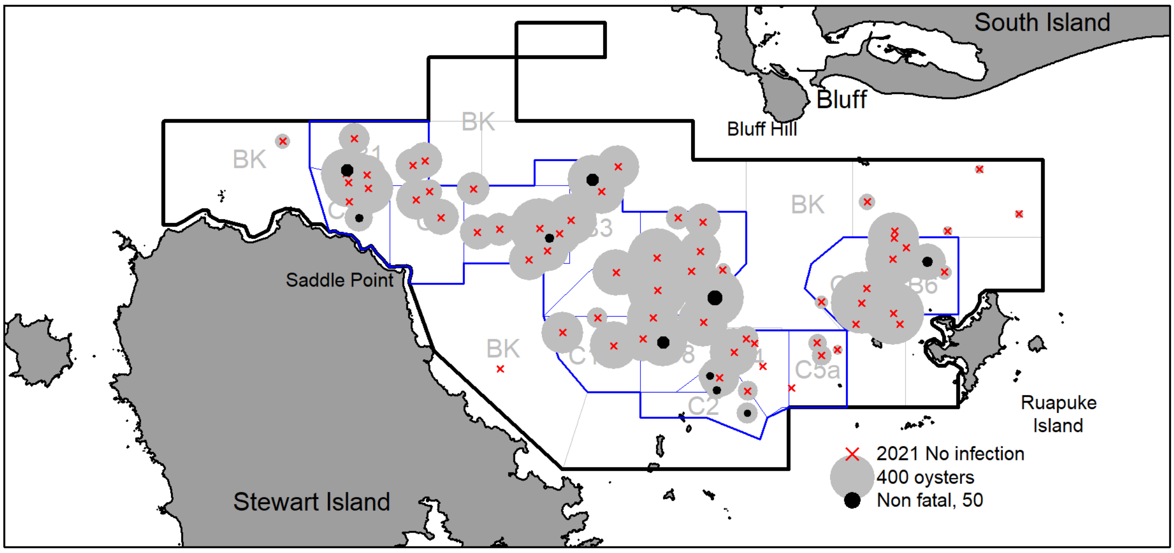

Figure 27: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2021. Stations with no Bonamia infection are shown by red crosses.

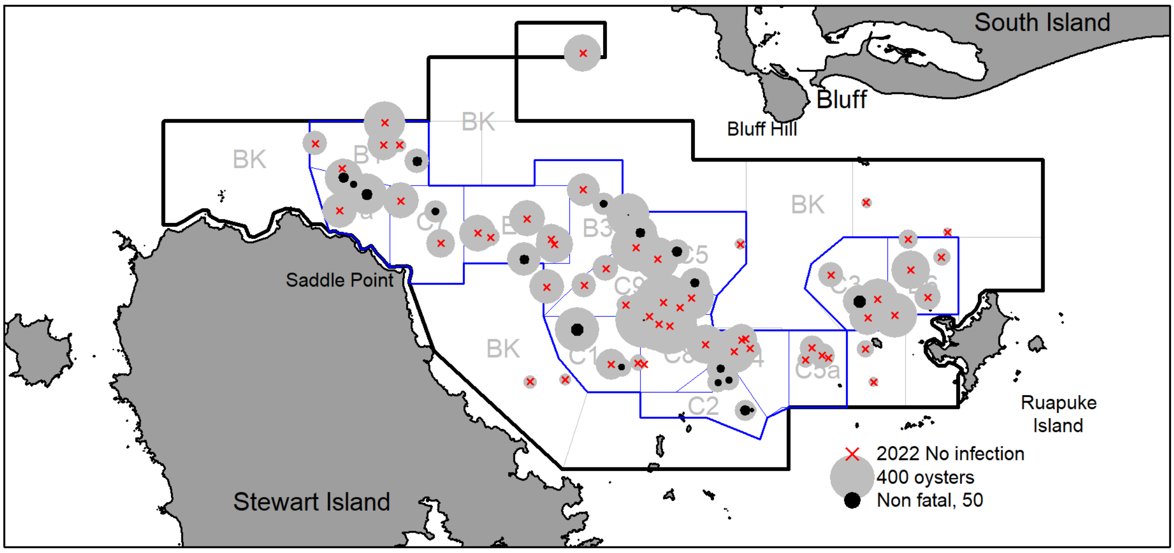

Figure 28: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2022. Stations with no Bonamia infection are shown by red crosses.

3.4.6 Projected short-term mortality from Bonamia infections

Projections of post-survey mortality (within about two months of sampling) from the proportion of oysters with categories 3 and higher (fatal) infections scaled-up to the size of the total recruit-sized oyster population are given in Tables 19–22.

Projected short-term mortality using Method 1 (see Section 2.5.5), post-survey mortality of oysters in 2022 reduced the recruit-sized oyster population in core strata by 20.7 million oysters, 3.7% of the recruit-sized population, from 557.9 million oysters at the time of the survey (February 2022) to 537 million oysters by early March 2022 (the beginning of the new oyster season) (Table 19). Percentage post-survey mortality was similar or higher in 2022 than in 2018–2021 (3.2%, 1.3%, 2.2%, and 3.6%, respectively). In 2022, post-survey mortality of recruit-sized oysters by stratum ranged from no mortality to 11.0%; two strata had projected mortalities over 10% (Table 19).

The estimate of post-survey mortality in core strata from fatally infected oysters scaled to the size of the catch (Method 2) using heart imprints was similar to that estimated using averaged correction factors (Method 1), 20.5 million oysters (3.7%, Table 20). The estimate of post-survey mortality in the core strata using ddPCR relative infection ratios (see Figure A3.3, Appendix 3) and Method 2 was 20.4 million oysters (3.7%), the same as that from heart imprints (Table 21).

The speed at which low level category 1 and 2 infections progress to category 3+ infections or the variability amongst individual oysters is not known. Where the prevalence of category 1 and 2 infections was high and infections 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). 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%, see references in Appendix 6). In 2022, summer mortality was 4.9% (Method 1) and 5.0% (Method 2) (Table 22). Summer mortality was higher in core strata in 2022 than in 2018–21 (Table 22). However, summer mortality in the Bonamia survey area was still relatively low (Figure 29).

Table 19: Absolute population estimates for recruit-sized oysters after projected mortality from Bonamia based on category 3 and higher infections (correction factor method) by stratum in the core strata, background stratum (BK), and for the stock assessment survey area (Survey total) sampled in February 2022. Columns give the area of each stratum (Area (km^2)), 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 (Density s.d.) of the density estimate, coefficient of variation (Density CV) of the oyster density, mean population size at the time of survey (Pop.n1, filled light grey) in millions of oysters, mean post survey mortality population size (Pop.n2, filled medium grey) in millions of oysters, lower and upper 95% confidence intervals (95% CI) for the post-mortality estimate, losses of oysters (Losses, millions), and the percentage mortality (% Mortality, shaded dark grey).

Core Strata	Area (km^2)	No. stns	Correction factor	Mean density	Density s.d.	Density CV	Pop.n1	Pop.n2	Lower 95% CI	Upper 95% CI	Losses	% Mortality
B1	78.2	5	0.961	0.90	0.29	0.32	73.2	70.3	24.0	131.0	2.9	3.9
B3	44.7	4	0.966	1.42	0.43	0.30	71.7	69.3	24.6	115.1	8.3	3.4
B6	30.0	5	0.986	1.06	0.36	0.34	32.2	31.7	9.7	59.7	0.5	1.4
C1a	31.3	4	0.990	0.96	0.44	0.46	30.5	30.2	2.6	64.5	0.3	1.0
C2	21.9	3	0.899	0.34	0.12	0.36	8.3	7.4	2.1	14.4	0.8	10.1
C3	32.7	3	0.925	1.27	0.32	0.25	44.9	41.5	19.2	71.4	3.4	7.5
C5	37.7	4	0.959	0.79	0.25	0.32	31.1	29.8	10.3	55.5	1.3	4.1
C5a	23.5	3	1.000	0.37	0.17	0.47	8.6	8.6	0.8	18.5	0.0	0.0
C7	36.1	3	0.890	0.89	0.22	0.25	36.1	32.2	14.9	56.0	4.0	11.0
C7a	23.6	4	0.976	1.28	0.30	0.23	31.1	30.3	14.6	51.9	0.8	2.4
C8	26.8	4	0.968	1.97	0.75	0.38	61.1	59.1	12.9	104.5	8.3	3.2
C9	34.5	6	0.991	1.61	0.50	0.31	56.0	55.5	20.2	102.8	0.5	0.9
E2	42.8	4	0.975	1.16	0.25	0.21	50.8	49.5	25.5	82.9	1.3	2.5
E4	28.0	3	0.971	0.77	0.21	0.27	22.3	21.7	9.6	38.2	0.7	2.9
Core strata	491.8	55	0.961	1.07	0.10	0.09	557.9	537.2	342.4	799.2	20.7	3.7
BK	578.4	5	0.997	0.46	0.27	0.59	266.6	265.9	0.0	635.9	0.7	0.3
Survey total	1 070.2	62	0.964	0.75	0.15	0.20	824.4	803.1	420.9	1 331.6	21.3	2.6

Table 20: Scaled up estimates of the population size of recruit-sized oysters with fatal infections (category 3–5) estimated by heart imprints by stratum in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey area) sampled in February 2022. Columns give the area of each stratum (Area (km^2)) in square kilometres, the number of stations sampled (No. stns), the mean oyster density of oysters expected to die per square metre (Mean density), the standard deviation of the mean density (Density s.d.), the coefficient of variation (Density CV) of the density estimate, mean population size at the time of survey (Popn recruits, filled light grey) in millions of oysters, mean population size of the millions of oysters estimated to die (Losses, millions, shaded medium grey), lower and upper 95% confidence intervals (95% CI) in millions of oysters, and the percentage mortality in 2019–2022 (Mort (%), shaded dark grey).

Stratum	Area (km^2)	No. stns	Mean density	Density s.d.	Density CV	Popn recruits	Losses	Lower 95% CI	Upper 95% CI	Mort (%) 2022	Mort (%) 2021	Mort (%) 2020	Mort (%) 2019
B1	78.2	5	0.04	0.03	0.82	73.2	2.9	0.0	8.4	4.0	3.4	5.4	0
B3	44.7	3	0.05	0.03	0.69	71.7	2.2	0.0	5.6	3.1	0	1.8	0.8
B6	30.0	7	0.02	0.01	0.85	32.2	0.5	0.0	1.3	1.4	7	0	2
C1a	31.3	3	0.01	0.01	1.00	30.5	0.3	0.0	1.1	1.1	6	5.2	0
C2	21.9	3	0.04	0.02	0.50	8.3	0.9	0.0	1.9	10.7	2.6	4	5.4
C3	32.7	3	0.10	0.10	1.00	44.9	3.4	0.0	10.7	7.5	0	1.1	0.8
C5	37.7	5	0.03	0.03	0.74	31.1	1.3	0.0	3.4	4.1	3.8	3.2	1.2
C5a	23.5	3	0.00	0.00	0.00	8.6	0.0	0.0	0.0	0.0	0	0	0
C7	36.1	3	0.11	0.04	0.36	36.1	4.0	1.1	7.7	11.1	6.9	1.9	3.2
C7a	23.6	4	0.03	0.02	0.60	31.1	0.8	0.0	1.8	2.5	1.8	6.5	2.2
C8	26.8	3	0.07	0.07	1.00	61.1	1.8	0.0	5.7	2.9	1.4	1.1	3.1
C9	34.5	3	0.01	0.01	1.00	56.0	0.5	0.0	1.6	0.9	4.6	2	1.9
E2	42.8	7	0.03	0.03	1.00	50.8	1.3	0.0	4.3	2.6	5.3	0.1	0.6
E4	28.0	3	0.02	0.02	1.00	22.3	0.6	0.0	2.1	2.8	0	0	0
Core strata	491.8	55	0.04	0.01	0.26	557.9	20.5	9.0	36.5	3.7	3.6	2.3	1.4
BK	578.4	5	0.00	0.00	1.00	266.6	0.6	0.0	1.8	0.2	0	3.9	0.8
Survey total	1 070.2	60	0.02	0.01	0.26	824.4	21.1	9.6	37.3	2.6	3.4	2.9	1.2

Table 21: Scaled up estimates of the population size of recruit-sized oysters with fatal infections (category 3–5) estimated by ddPCR by stratum in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in February 2022. Columns give the area of each stratum (Area (km²)) in square kilometres, the number of stations sampled (No. stns), the mean oyster density of oysters expected to die per square metre (Mean density), the standard deviation of the mean density (Density s.d.), the coefficient of variation (Density CV) of the density estimate, mean population size at the time of survey (Popn recruits, filled light grey) in millions of oysters, mean population size of the millions of oysters estimated to die (Losses, millions, shaded medium grey), lower and upper 95% confidence intervals (95% CI) in millions of oysters, and the percentage mortality in 2022 and 2021 by ddPCR (Mort (%) 2022 (ddPCR) and Mort (%) 2021 (ddPCR), shaded dark grey) and by heart imprints (Mort (%) 2022 (Heart imp.), shaded light blue grey).

Stratum	Area (km²)	No. stns	Mean density	Density s.d.	Density CV	Popn recruits	Losses	Lower 95% CI	Upper 95% CI	Mort (%) 2022 (ddPCR)	Mort (%) 2021 (ddPCR)	Mort (%) 2022 (Heart imp.)
B1	78.2	5	0.04	0.03	0.73	73.2	3.3	0.0	8.8	4.5	3.4	4.0
B3	44.7	3	0.06	0.04	0.59	71.7	2.8	0.0	6.6	3.9	1.5	3.1
B6	30	7	0.02	0.01	0.66	32.2	0.6	0.0	1.5	1.9	7	1.4
C1a	31.3	3	0.02	0.02	1.00	30.5	0.7	0.0	2.1	2.2	6	1.1
C2	21.9	3	0.05	0.03	0.56	8.3	1.2	0.0	2.8	14.5	2.6	10.7
C3	32.7	3	0.08	0.08	1.00	44.9	2.5	0.0	8.0	5.6	0	7.5
C5	37.7	5	0.06	0.04	0.62	31.1	2.4	0.0	5.8	7.6	3.8	4.1
C5a	23.5	3	0.00	0.00	0.00	8.6	0.0	0.0	0.0	0.0	0	0.0
C7	36.1	3	0.10	0.05	0.53	36.1	3.5	0.0	8.0	9.8	6.9	11.1
C7a	23.6	4	0.05	0.04	0.70	31.1	1.2	0.0	3.1	3.9	1.8	2.5
C8	26.8	3	0.00	0.00	0.00	61.1	0.0	0.0	0.0	0.0	1.4	2.9
C9	34.5	3	0.01	0.01	1.00	56.0	0.5	0.0	1.6	0.9	6.1	0.9
E2	42.8	7	0.03	0.03	1.00	50.8	1.3	0.0	4.3	2.6	5.3	2.6
E4	28	3	0.01	0.01	1.00	22.3	0.3	0.0	1.0	1.4	3.5	2.8
Core strata	491.8	55	0.04	0.01	0.24	557.9	20.4	9.7	34.9	3.7	4.1	3.7
BK	578.4	5	0.00	0.00	1.00	266.6	1.1	0.0	3.6	0.4	0	0.2
Survey total	1070.2	60	0.02	0.00	0.24	824.4	21.6	10.3	37.3	2.6	3.8	2.6

Table 22: Summer mortality for 2018–2022 in the stock assessment survey area and for 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				
Pre-survey mortality	2018	2019	2020	2021	2022	2018	2019	2020	2021	2022
Recruit-sized new clocks (NC, millions)	3.4	9.2	8.5	5.5	10.3	2.9	4.1	3.5	5.5	7.4
Post-survey mortality										
Correction factor (millions of oysters)	23.1	9.4	24.9	29.2	21.3	15.7	6.8	12	29.2	20.7
Scaled catch (millions of oysters)	10.7	9.4	25.5	29.2	21.1	7.3	6.8	12	29.2	20.5
Combined summer mortality										
Correction factor +NC (millions of oysters)	26.5	18.6	33.4	34.7	31.6	18.6	10.9	15.5	34.7	28.1
Scaled catch +NC (millions of oysters)	14.1	18.6	34	34.7	31.4	10.2	10.9	15.5	34.7	27.9
Population before summer mortality										
Recruit-sized oysters +NC (millions of oysters)	886.7	868	887.8	873.6	834.7	497	542.5	533.4	806.9	565.3
Percent summer mortality										
Correction factor +NC (%)	3	2.1	3.8	4	3.8	3.7	2	2.9	4.3	5.0
Scaled catch +NC (%)	1.6	2.1	3.8	4	3.8	2.1	2	2.9	4.3	4.9

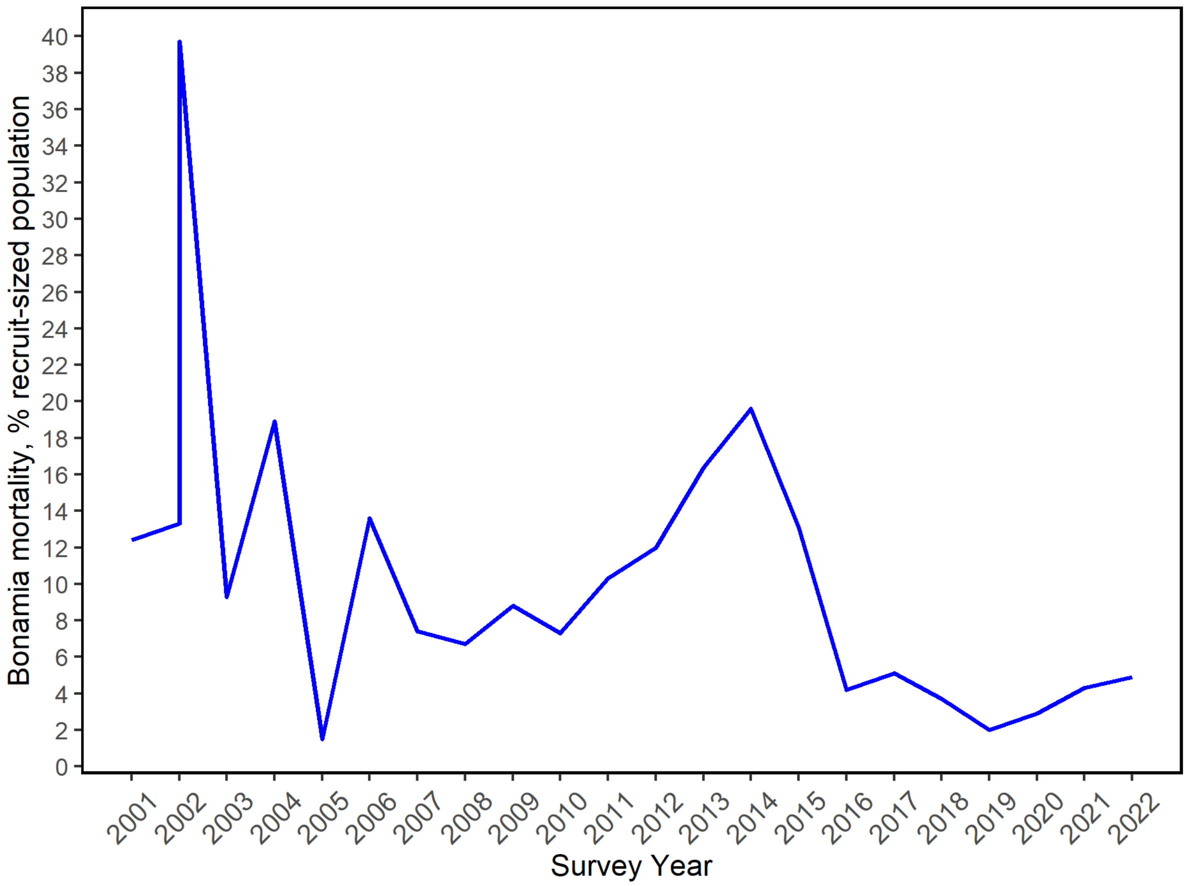

Figure 29: Percentage mortality of the recruit-sized oyster population in the Bonamia survey area between 2001 and 2022.

3.5 The status of the OYU 5 fishery in 2022 and future trends

The 2017 stock assessment for OYU 5 suggested that an annual commercial harvest of up to 30 million oysters was not likely to have a significant effect on the future (1–5 years) status of the stock (Figure 30) (Large et al. 2021). Disease mortality and recruitment to the fishery were the main drivers of future stock size in the OYU 5 fishery. Since 1985, OYU 5 has shown cyclic trends in oyster abundance.

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 30).

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 a continued increase in future recruit-sized stock abundance. The recruit-sized population increased around 36% between 2017 and 2018, as did pre-recruit oysters (around 45%) and small oysters (around 53%). Significant recruitment to the oyster population was recorded by spat monitoring, catch sampling, and 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 oysters (around 21.4%) and small oysters (around 48.3%). Between 2019 and 2020 both commercial- sized and recruit-sized oysters remained similar, and pre-recruit oysters increased by 22.5% and small oysters by 76.7% (Table 8). The February 2021 survey showed increases of 28.3%, 51.2%, 83.6%, and 3.7% for commercial-sized, recruit-sized, pre-recruit and small oysters. The 24.0%–30.5% decline in population sizes for commercial-sized, recruit-sized, and pre-recruit oysters in 2022 is unexplained (Table 8). The high population size of small oysters and moderate numbers of pre-recruits should support continued rebuilding of the stock.

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 *B. exitiosa* 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. Between 2018 and 2021, substantial increases have occurred in recruit-sized, pre-recruit, and small oysters (see Figure 7 and Table 8). In 2021, the population size of recruit-sized oysters in the Bonamia survey area was 2.2 times that in 2017, declining to 1.5 times that in 2017 in 2022 (see Table 8).

Bonamia infection and subsequent mortality from Bonamia has been low since 2016 (see Figure 29). Prevalence of Bonamia infection was relatively low in 2022, 4.7% of recruit-sized oysters in the Bonamia survey area by heart imprints and was similar to the 4.3% in 2021. Prevalence by ddPCR was 6.7% in 2022 and 5.7% in 2021.

The future status of the fishery is best represented by a midpoint between series "a" and "b" in Figure 30, which assumes no Bonamia mortality and 10% mortality. Mortality estimated by Methods 1 and 2 ranged from 4.9% to 7.1%, below the 10% mortality of series "b". Moreover, non-fatal infections are low (1.0 % of the recruit-sized population), suggesting low *B. exitiosa* mortality in 2023.

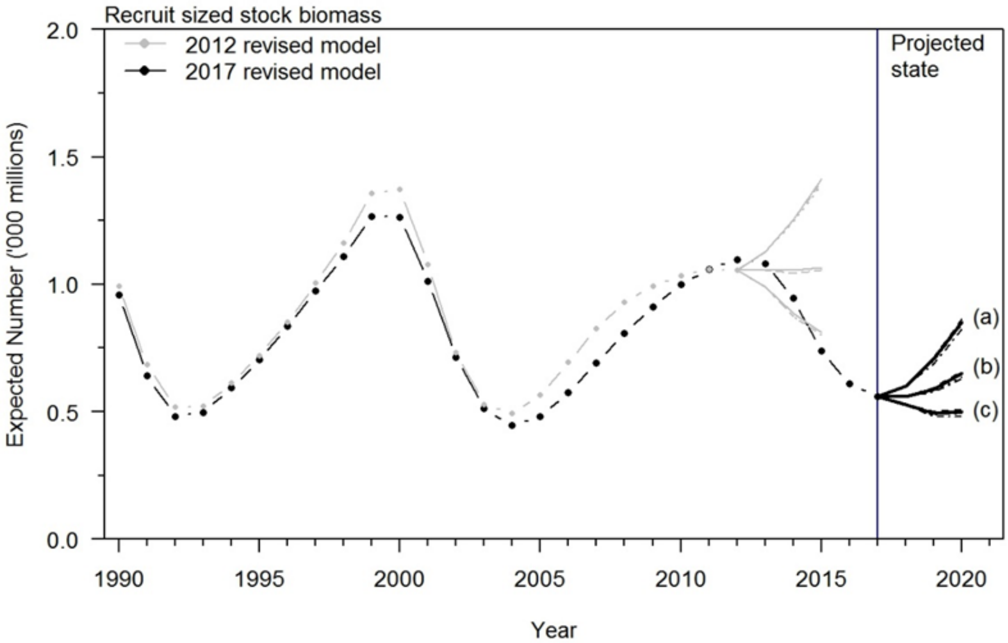

Figure 30: Model estimates of recent recruit-sized stock abundance and projected recruit-sized stock abundance with catches of 7.5 (solid line), 15 (dash dot), and 30 million oysters (dash line) under assumptions of (a) no disease mortality, (b) disease mortality of 0.10 y^{-1}, and (c) disease mortality 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. 2021).

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 six-month oyster season begins on the 1st of March. 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 to fishers and for management. 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 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. In 2022, the survey attained coefficients of variation (CVs) of 9–10% (Table 8) for the four size groups of oysters sampled in the Bonamia survey area.

The objectives of Bonamia surveys have changed over time (see Michael et al. 2016, referenced in Appendix 6). A new time series of Bonamia and oyster surveys, incorporating a fully randomised, two-phase sampling design and a standardised Bonamia survey area, was established in 2014 to make these surveys comparable from year to year. The February 2022 survey is the ninth 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 The 2022 survey results

4.1.1 Sampling efficiency

Sampling of oysters in the February 2022 survey used the same vessel, skipper, and crew, standard sampling methods, and survey dredge as for previous Foveaux Strait surveys 2009–21, and because of this consistency in gear and methods, it should better reflect changes in the oyster fishery. Sampling conditions during the February 2022 survey were generally good and not expected to have affected dredge efficiency. Oyster densities in C5a and E2 were probably underestimated because of the low dredge efficiency caused by extensive stands of kāeo (*Pyura pachydermatina*). These low estimates of density have flow-on effects to the estimates of oyster densities and *B. exitiosa* prevalence, intensity, and oyster mortality at the levels of stratum, all core strata combined, and the stock area. Dredge contents of survey tows were generally fuller in 2022 than in 2021 (see Figures A4.5 & A4.6, Appendix 4), with dredge catches in 2022 containing more gravel. These observations suggest a change to the seabed between surveys and lower oyster catchability in 2022. Oyster skippers also observed more gravel in dredge catches and lower catch rates at the beginning of the 2022 oyster season, possibly indicating fishery-scale changes to the seabed by storms. Overall, the catchability of oysters may have been lower during the 2022 oyster survey than for previous surveys.

4.1.2 Survey design performance

Stock assessment and Bonamia surveys estimate oyster densities and mean population sizes by stratum, for the Bonamia survey and stock areas. The five random survey stations in the large background stratum (BK) have a large influence on stock size and contribute greatly to the inter-survey variation in BK. Therefore, interpretation of the Bonamia survey results focuses on core strata rather than the whole stock area. The CV obtained from population estimate of recruit-sized oysters was lower (9%) than the CV (11%) predicted by the survey design using ALLOCATE (Francis 2006) and suggests that the stratification of the survey area is appropriate.

4.1.3 Distribution of oysters

The design of the surveys does not describe the spatial structure of the stock well, especially the distribution and spatial extent 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 400 oysters per tow (1.0 oyster m^{-2}).

There was an unexplained decrease in “oyster bed” densities between 2021 and 2022. Fewer core strata (7) had oyster densities above 400 per tow in 2022 (the same as in 2020) than in 2021 (10), and more strata than in 2012–2019 (2–6 strata). The mean density in strata with recruit-sized oysters greater than 400 per tow was lower in 2022 (1.05 oysters m^{-2}) than in 2021 (1.62 oysters m^{-2}). In 2022, the population size in core strata above 400 oysters per tow represented 30.8% of the recruit-sized population, lower than in 2021 (64.8%). Recruit-sized oyster densities greater than 400 oysters per tow were highest in strata B3, C8, and C9.

4.1.4 The status of Bonamia infection and summer mortality of oysters

The timing of Bonamia surveys coincides with a period of peak seasonal mortality from Bonamia and the shedding of infective particles. In 2022, 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 uses a ddPCR method for the detection of Bonamia infection that also provides for quantification of infection. Overall, this 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 since 2018 most likely improved the detection of low-level infection, increasing the estimates of prevalence in the Foveaux Strait population.

4.2 Fishery trends

4.2.1 Historical trends

Fishers target high density patches of commercial-sized oysters. In 2017, 66% of the catch was 70 mm in length or larger (recruit size is 58 mm in length or larger). Between 2012 and 2017, Bonamia mortality 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 2.3 S/H (95% CI 2.2–2.4) in 2019. Increasing oyster densities from 2017 (see Table 8) were reflected in an increase in catch rates to 3.0 S/H (95% CI 2.9–3.1) in 2021.

4.2.2 Unexplained decrease in oyster population sizes

In 2022, the population sizes of commercial and recruit-sized oysters decreased by 30.5% and 30.4%, respectively, and pre-recruits by 24.0% (Table 8). Commercial catch rates are usually the highest at the beginning of the oyster season. In 2022, mean catch rate in March was 2.6 S/H, lower than for the entire 2021 season, at 3.0 S/H. The difference of 243.5 million recruit-sized oysters between 2021 and 2022 is difficult to explain with the available data. The decrease of 243.5 million recruit-sized oysters between 2021 and 2022 cannot be accounted for by the 8 million oysters landed by the commercial fishery in 2021 and the estimated 45 million oysters that died between surveys. The February 2022 survey data may not be directly comparable with data in the OYU 5 survey time series because of these decreases in population sizes.

There is a possibility that inter-survey variation, i.e., whether random stations on average land on high or low density areas in a patchily distributed population in any given year, may have contributed to the substantial decrease in the estimates of population size. However, sampling methods, sampling effort, and survey designs were consistent with previous surveys. The lower catches of commercial recruit-sized and pre-recruit oysters in 2022 (Figure 31) suggest reduced catchability of oysters, i.e., a reduction in dredge efficiency, most likely caused by changes to seabed structure by storms as mentioned previously. The unusually high volumes of gravel in most dredge tows combined with seawater temperature anomalies since mid-January 2022 suggest that different weather systems may have mobilised bottom sediments throughout Foveaux Strait.

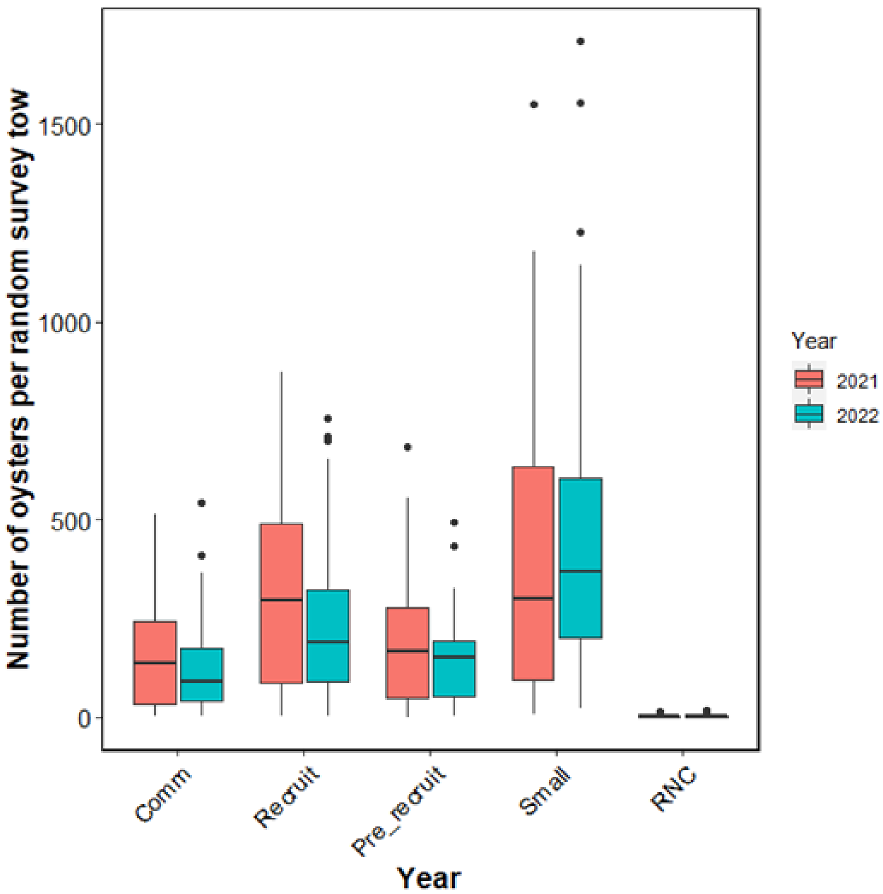

Figure 31: Boxplots of the number of oysters caught in each dredge tow for commercial (comm), recruit-sized (Recruit), pre-recruit (Pre_recruit), small oysters (Small), and recruit-sized new clocks (RNC) from random survey tows in 2021 (tan colour) and 2022 (teal colour). Medians shown as solid lines, boxes represent 50th percentiles, whiskers 95th percentiles, and outliers as filled black circles.

4.3 Outlook for the 2022 oyster season

The February 2022 survey data suggest either an unexplained decline in oysters larger than recruit-sized or reduced catchability. The lower than expected catch rates at the beginning of the 2022 oyster season, reports of more gravel in catches (oyster skippers meeting, May 2002), and subsequent reports of increasing catch rates in areas revisited by fishers later in the season suggest a catchability issue that is improving, rather than a decrease in oyster densities. The trend in the recruited stock will be reassessed by the February 2023 stock assessment survey.

Some indicators from the 2022 survey are strongly positive for continued rebuilding of the fishery in the medium-term. The spat monitoring programme showed recruitment to the oyster population, which has been consistently high since the summer of 2014–15, was especially high over the summer of 2020–21 and recruitment remains strong in 2021–22. These strong cohorts of settlers can be tracked through the oyster population by annual surveys. The population size of small oysters in the Bonamia survey area has been increasing since 2015 and has remained high (1116.6 million oysters, 95% CI 723.1–1709.9) in 2022. The population size of pre-recruit oysters was 370.3 million oysters (95% CI 241.5–563.8) in 2022, which is still above estimates from 2020. The population size of recruit-sized oysters, 557.9 million oysters (95% CI 66.6–842.9), is similar to that in 2020. The population size of commercial-sized oysters (282.1 million oysters; 95% CI 184.5–428.3) is not greatly different to that in 2020. In the absence of significant mortality, continued rapid increases in pre-recruit sized oysters is likely. Depending on annual growth rates, which are known to vary considerably, increases to commercial-sized and recruit-sized oysters may be slow. Commercial catch rates for the 2022 season are expected to remain similar to or be slightly lower than the 2021 levels.

Future levels of disease mortality in the fishery are uncertain. *B. exitiosa* mortality has been low since 2018. In 2022, non-fatal infections of *B. exitiosa* were 1.0% of the recruit-sized oyster population and mortality from *B. exitiosa* is expected to be low in 2023. The status and effects of other co-infections such as *B. ostreae*, the Apicomplexan APX, and *Alcicornis longicornutus* are unknown. *Bonamia ostreae* has the potential to cause catastrophic mortality in this naïve oyster population. The rapidly increasing oyster density could potentially fuel high oyster mortality, resulting in high propagule pressure and effective and extensive transmission through the oyster population. Oyster mortality could potentially be greater than 90%.

A TACC of 15 million oysters represents an exploitation rate of 8.7% of the portion of the stock above 400 oysters per tow in core strata (46% of the stock area) and 2.7% of the recruit-sized population in core strata. The Bluff Oyster Management Company runs a voluntary quota shelving programme with which almost all vessels comply. In 2021, the oyster industry landed 50.8% of the TACC, 7.6 million oysters representing 0.9% of the recruit-sized population in the Bonamia survey area in 2021. However, catch rates and the economics of fishing are determined by the numbers of localised high-density patches ("oyster beds") and not the size of the recruited population.

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APPENDIX 1: SURVEY STATION FORM

FOVEAUX STRAIT OYSTER SURVEY, STATION DATA RECORD

Vessel name

Recorder ....

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

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

Finish position: Latitude S | Longitude E

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

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

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

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

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

Sediment type

Circle the main type (one only)

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

Comments:

1 Nautical mile = 1.853 km

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

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

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

APPENDIX 2: BONAMIA SAMPLING FORM, VERSION 2022

***Bonamia* Testing: Laboratory Sampling Record**

B.ex		*B.ost*	Y	Other	

Project Code	Survey	Station	Area code	Tag
	19	|		

Date collected (dd/mm/yyyy)			Time (24hr)		Date Processed (dd/mm/yyyy)		
|	|	2022	|	|	|	|	2022

Comments

Plate Layout

Oyster no.	Length (mm)			Height (mm)			Size	Gaper	Larvae colour	Gonad section	Heart imprint score
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											

Recorder:	Checker:

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 were 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 on 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); 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 S. 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 (Maas et al. 2013) (early-stage) larvae, grey (late-stage) larvae, yellow (almost ready to settle) larvae, or with no larvae present (coded NA).

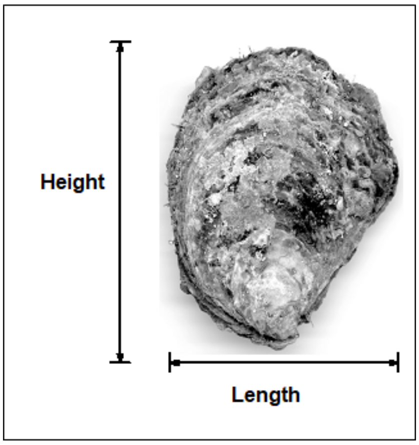

Figure A3.1: An oyster showing length (anterior-posterior axis) and height (dorsal-ventral axis) dimensions.

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 2022 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 2022, 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. Four 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 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 2022, heart imprints were examined by a single experienced reader. A review of scoring protocols was undertaken before screening samples.

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

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

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.

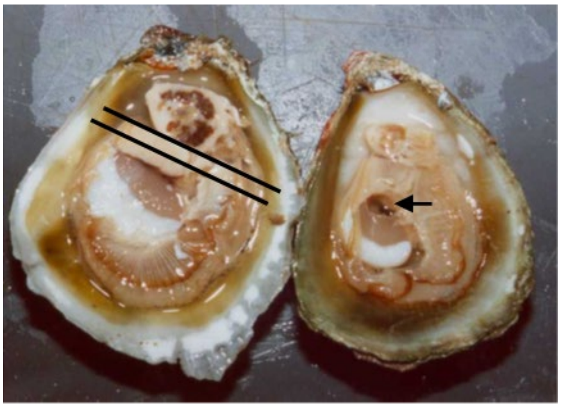

Figure A3.2: Lines on left oyster show location of 5 mm thick standard section taken for histology. The arrow on the oyster on the right shows the heart, a black organ adjacent to the adductor muscle.

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 Diggles 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 where heart imprints were not able to detect infection (score 0) (Figure A3.3). 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).

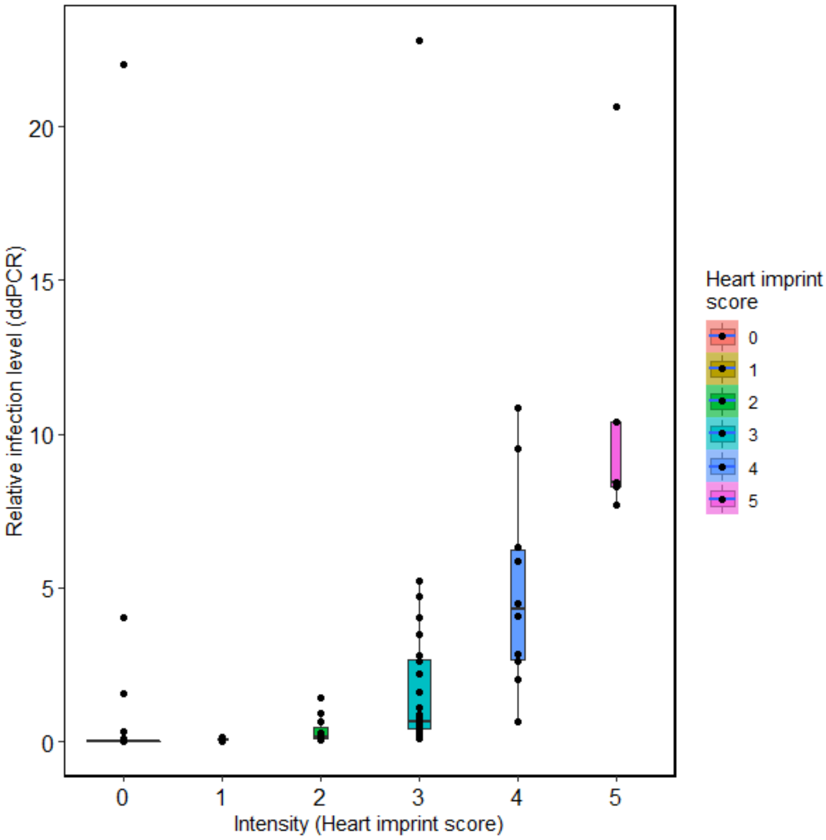

Figure A3.3: Boxplots of the intensity of *Bonamia exitiosa* from ddPCR calculated as the ratio of the concentration of Bonamia targets to the concentration of β-actin targets in each sample plotted by the categorical intensity of infection from heart imprints using the methods of Diggles et al. (2003). Medians shown as solid lines, boxes represent 50th percentiles, whiskers are 95th percentiles, and outliers as filled black circles.

APPENDIX 4: FEBRUARY 2022 SURVEY CONDITIONS AND COMPARABILITY

Dredge tow lengths in February 2022 were almost all 0.2 nautical miles (371 m, 5th percentile 0.19 and 95th percentile 0.21) in length (Figure A4.1), similar to 2021 (Figure A4.2). All oyster and clock densities were standardised to a 0.2 nautical mile standard tow length for analysis. Most of the survey stations in 2022 were sampled in wind conditions less than 10 knots (Figure A4.3), and generally in better sea conditions than in 2021 (Figure A4.4). The median wind force was 1 on the Beaufort scale (1−2 knots), with 5th and 95th percentiles of Beaufort scale 0 (calm) and 4 (11−15 knots), respectively. Maximum wind speed during sampling was about 15 knots. Operational limits for dredge sampling of 20 knots and Figure A4.3 show the February 2022 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 5% to 80% with a median fullness of 40%, similar to 2020, but lower than in 2014–2019 (50%). 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 may have had an effect on sampling effectiveness in the 2022 survey (Figure A4.5) as dredges were generally fuller than in 2021 (Figure A4.6). 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 2022 survey data. Future surveys could identify stations with this pattern in the distribution of catch; however, it is difficult to incorporate these data into estimates of population size.

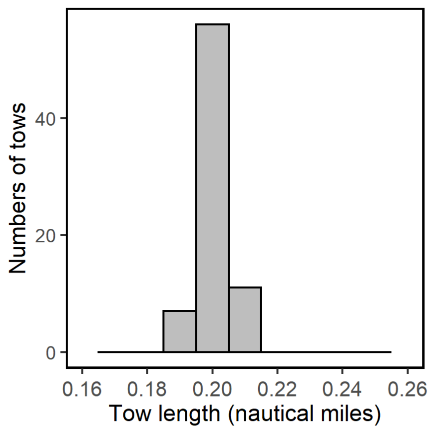

Figure A4.1: Distribution of dredge tow lengths from the February 2022 survey. The standard tow length was 0.2 nautical mile (371 m).

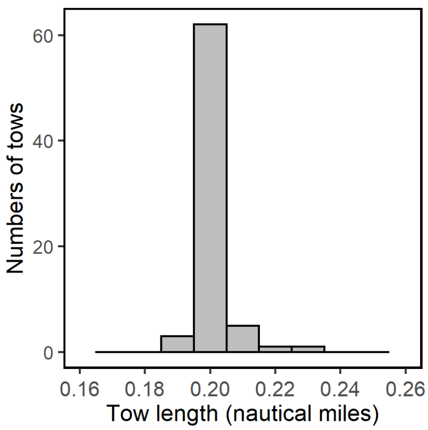

Figure A4.2: Distribution of dredge tow lengths from the February 2021 survey. The standard tow length was 0.2 nautical mile (371 m).

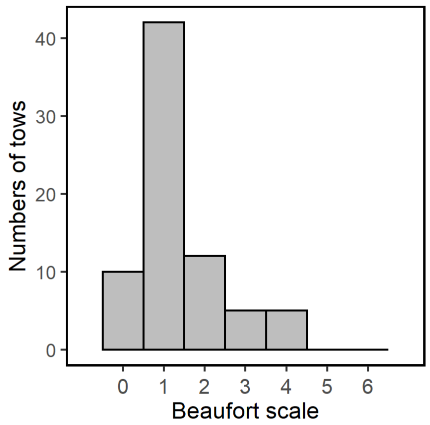

Figure A4.3: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2022. Beaufort scale: 0, < 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

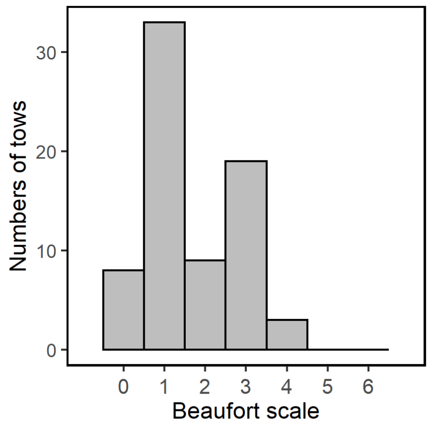

Figure A4.4: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2021. Beaufort scale: 0, < 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

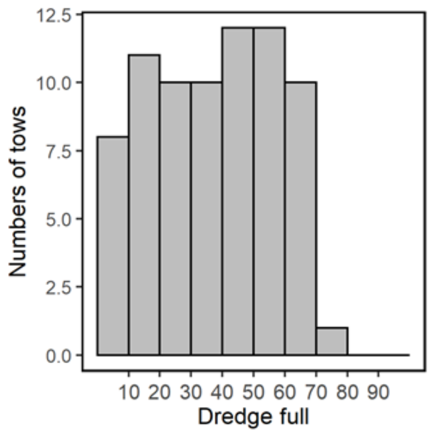

Figure A4.5: Distribution of dredge fullness (%) recorded for survey tows in February 2022. No tows were landed with a dredge fullness of greater than 80%. Unpublished video data suggest that dredge saturation may occur below 80% full.

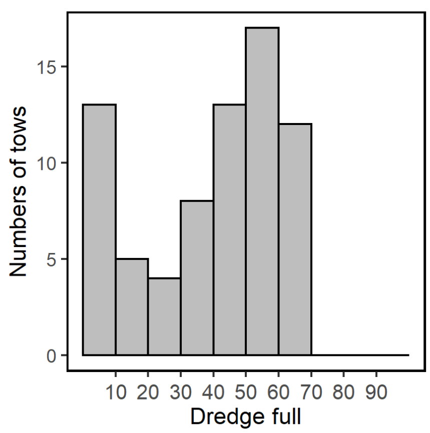

Figure A4.6: Distribution of dredge fullness (%) recorded for survey tows in February 2021. No tows were landed with a dredge fullness of greater than 80%. Unpublished video data suggest that dredge saturation may occur below 80% full.

APPENDIX 5: 2022 SURVEY CATCH AND INFECTION DETAILS

Table A5.1: Details by stratum (Str) and station (Stn) of recruit-sized oysters (Recruits) and densities m^{-2} (Density); 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. The percentage prevalence of Bonamia infection detected by heart imprints (%Prev), by ddPCR (%ddPCR), and the difference in detection between the two methods (Diff) from the February 2022 survey. (Continued on next page)

Str	Stn	Recruit	Density	Total	Un.inf	NF.inf	Fat.inf	%Prev	% ddPCR	Diff
B1	1	395	3.26	25	363	0	32	8	16	8
B1	2	143	1.18	25	126	11	6	12	16	4
B1	3	140	1.16	25	140	0	0	0	0	0
B1	4	37	0.31	25	37	0	0	0	4	4
B1	5	239	1.98	25	239	0	0	0	4	4
B3	6	277	2.29	25	266	0	11	4	16	12
B3	7	559	4.62	25	559	0	0	0	0	0
B3	8	121	1.00	25	116	5	0	4	4	0
B6	9	452	3.74	25	452	0	0	0	0	0
B6	10	88	0.73	25	88	0	0	0	0	0
B6	11	333	2.75	25	333	0	0	0	0	0
B6	13	165	1.36	23	151	0	14	8	9	1
BK	14	40	0.33	25	40	0	0	0	0	0
BK	15	311	2.57	25	311	0	0	0	0	0
BK	16	31	0.26	25	30	0	1	3	16	13
BK	18	63	0.52	25	63	0	0	0	0	0
C1a	19	220	1.82	25	211	0	9	4	8	4
C1a	20	74	0.61	25	71	3	0	4	0	-4
C1a	21	58	0.48	25	58	0	0	0	0	0
C1a	22	445	3.68	24	426	19	0	4	4	0
C2	23	109	0.90	25	92	4	13	16	32	16
C2	25	21	0.17	25	20	1	0	5	4	-1
C3	26	293	2.42	25	293	0	0	0	0	0
C3	27	150	1.24	24	150	0	0	0	0	0
C3	28	376	3.11	25	316	0	60	16	16	0
C5	29	210	1.74	25	202	8	0	4	4	0
C5	30	27	0.22	25	27	0	0	0	0	0
C5	31	267	2.21	25	235	11	21	12	12	0
C5	32	151	1.25	25	133	12	6	12	24	12
C5a	33	54	0.45	25	54	0	0	0	0	0
C5a	34	27	0.22	24	27	0	0	0	0	0
C5a	35	146	1.21	25	146	0	0	0	0	0
C7	36	119	0.98	25	90	5	24	24	28	4
C7	37	298	2.46	25	262	0	36	12	16	4

Table A5.1: Continued.

Str	Stn	Recruit	Density	Total	Un.inf	NF.inf	Fat.inf	%Prev	% ddPCR	Diff
C7	38	198	1.64	25	190	0	8	4	8	4
C7a	39	322	2.66	24	309	13	0	4	4	0
C7a	40	96	0.79	25	92	4	0	4	0	-4
C7a	41	275	2.27	25	264	0	11	4	4	0
C7a	42	388	3.21	25	357	16	16	8	8	0
C8	43	345	2.85	24	345	0	0	0	0	0
C8	44	710	5.87	25	710	0	0	0	16	16
C8	45	2	0.02	25	2	0	0	0	0	0
C9	47	77	0.64	25	77	0	0	0	4	4
E2	49	291	2.40	23	266	0	25	9	9	0
E2	50	253	2.09	25	243	10	0	4	0	-4
E2	51	92	0.76	24	92	0	0	0	0	0
E2	52	318	2.63	25	318	0	0	0	0	0
E4	53	82	0.68	25	82	0	0	0	0	0
E4	54	228	1.88	25	228	0	0	0	0	0
E4	55	181	1.50	25	159	7	14	12	12	0
B3	61	250	2.07	25	220	0	30	12	12	0
B6	69	62	0.51	25	60	0	2	3	12	9
BK	71	22	0.18	25	22	0	0	0	0	0
C2	78	104	0.86	25	87	4	12	16	16	0
C8	106	652	5.39	24	598	0	54	8	0	-8
C9	114	756	6.25	25	756	0	0	0	0	0
C9	115	153	1.26	25	153	0	0	0	0	0
C9	127	460	3.80	25	442	0	18	4	8	4
C9	128	411	3.40	25	411	0	0	0	0	0
C9	129	143	1.18	25	143	0	0	0	0	0
C5a	T1	150	1.24	25	150	0	0	0	0	0
B1	T2	85	0.70	25	82	0	3	4	8	4
E2	T3	310	2.56	25	298	0	12	4	4	0
E2	T4	200	1.65	25	200	0	0	0	4	4
C8	T5	539	4.45	24	539	0	0	0	0	0
BK	T6	17	0.14	25	17	0	0	0	8	8
C2	T7	105	0.87	25	80	13	13	24	28	4
C3	T8	235	1.94	25	197	19	19	16	12	-4
C5	T9	424	3.50	25	407	0	17	4	4	0
BK	T10	25	0.21	25	25	0	0	0	0	0
E4	T11	102	0.84	25	102	0	0	0	8	8
E4	T12	459	3.79	25	459	0	0	0	0	0

APPENDIX 6: REFERENCES FOR SURVEYS 2010–2021

Table A6.1: Fisheries Assessment Reports for Foveaux Strait oyster and Bonamia surveys 2010–2020. 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 (*Bonamia exitiosa*) in Foveaux Strait oysters (*Ostrea chilensis*), changes in the distributions and densities of recruit, pre-recruit, and small oysters in February 2010, and projections of disease mortality. *New Zealand Fisheries Assessment Report 2011/5*. 51 p.
2011	Michael, K.P.; Forman, J.; Hulston, D.; Fu, D. (2012). The status of infection by bonamia (*Bonamia exitiosa*) in Foveaux Strait oysters (*Ostrea chilensis*) 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. *New Zealand Fisheries Assessment Report 2012/37*. 57 p.
2012	Michael, K.P.; Fu, D.; Forman, J.; Hulston, D. (2013). The Foveaux Strait oyster (*Ostrea chilensis*, OYU 5) stock assessment survey and status of bonamia infection and mortality, February 2012. *New Zealand Fisheries Assessment Report 2013/09*. 64 p.
2013	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.
2014	Michael, K.P.; Forman, J.; Hulston, D.; Maas, E.; Fu, D. (2015a). 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.
2015	Michael, K.P.; Forman, J.; Hulston, D. (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 2015. *New Zealand Fisheries Assessment Report 2015/73*. 86 p.
2016	Michael, K.P.; Forman, J.; Hulston, D.; Sutherland, J. (2016). 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 2016. *New Zealand Fisheries Assessment Report 2016/67*. 95 p.
2017	Michael, K.P.; Forman, J.; Hulston, D.; Bilewitch, J.; Moss, G. (2019a). Foveaux Strait oyster and Bonamia surveys, February 2017. *New Zealand Fisheries Assessment Report 2019/46*. 83 p.
2018	Michael, K.P.; Bilewitch, J.; Forman, J.; Hulston, D.; Sutherland, J.; Moss, G.; Large, K. (2019b). 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.
2019	Michael, K.P., Bilewitch, J., Forman, J., Hulston, D., Moss, G. (2020) 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.* 79 p.
2020	Michael, K.P.; Bilewitch, J.; Forman, J.; Hulston, D.; Moss, G. (2021). Surveys of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU 5) and *Bonamia exitiosa* prevalence, intensity, and disease related oyster mortality in February 2020. *New Zealand Fisheries Assessment Report 2021/06*. 71 p.
2021	Michael, K.P.; Bilewitch, J.; Rexin, D.; Forman, J.; Hulston, D.; Moss, G. (2022). Surveys of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU 5) and *Bonamia exitiosa* prevalence, intensity, and disease related oyster mortality in February 2021. *New Zealand Fisheries Assessment Report 2022/48*. 78 p.