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Surveys of the Foveaux Strait oyster (*Ostrea chilensis*) fishery (OYU 5) and *Bonamia exitiosa* prevalence, intensity, and disease mortality in February 2021

New Zealand Fisheries Assessment Report 2022/48

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ISSN 1179-5352 (online)
ISBN 978-1-99-105241-4 (online)

September 2022

Te Kāwanatanga o Aotearoa
New Zealand Government

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Please cite this report as:

Michael, K.P.; Bilewitch, J.; Rexin, D.; Forman, J.; Hulston, D.; Moss, G. (2022). Surveys of the Foveaux Strait oyster (*Ostrea chilensis*) fishery (OYU 5) and *Bonamia exitiosa* prevalence, intensity, and disease related oyster mortality in February 2021. *New Zealand Fisheries Assessment Report 2022/48.* 78 p.

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EXECUTIVE SUMMARY

Michael, K.P.[1]; Bilewitch, J.; Rexin, D.; Forman, J.; Hulston, D.; Moss, G. (2022). Surveys of the Foveaux Strait oyster (*Ostrea chilensis*) fishery (OYU 5) and *Bonamia exitiosa* prevalence, intensity, and disease related oyster mortality in February 2021.

***New Zealand Fisheries Assessment Report 2022/48.* 78 p.**

Stock assessments of Foveaux Strait oysters (OYU 5) are undertaken five-yearly. The last stock assessment survey was in 2017. Two-phase, random stratified stock assessment surveys have been undertaken since 1990. From 1999 to 2007, these surveys sampled the same survey area (1054.2 km^2) using the same methods and similar strata to ensure data from these surveys are comparable. An additional stratum (16.0 km^2) was introduced by oyster skippers in 2007 and, since then, the size of survey area has remained at 1070.2 km^2. The 1999 stratum boundaries have also remained similar; however, some of the original strata have been subdivided at various times to better define the areas with relatively high (commercial), moderate, and low oyster densities. Since 2012, 26 survey strata have been sampled for stock assessments. Annual, smaller surveys (Bonamia surveys) that focus sampling effort in the core commercial fishery area are undertaken in the years between stock assessments. The Bonamia survey area represents 14 of the 26 stock assessment survey strata (46% of the area) and 75% and 69% of the recruit-sized oyster population in 2012 and 2017, respectively. To allow Bonamia survey data to be incorporated into stock assessments, the remaining 12 stock assessment strata are combined into a single background, 15th stratum, thereby sampling the whole stock assessment area. Population estimates are presented by stratum, and by three fishery areas: the Bonamia survey area (491.8 km^2), the background stratum (578.4 km^2), and the stock assessment survey area (1070.2 km^2). Only five stations were sampled in the background stratum; estimates for the background stratum and the stock assessment survey area should therefore be viewed with caution. These February surveys provide a ‘weather forecast’ immediately before the start of the six-month oyster season in March. Bonamia surveys update information on the following.

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

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

Foveaux Strait oyster and Bonamia surveys are undertaken in collaboration with the Bluff Oyster Management Company Ltd which provides a vessel, the survey dredge, and crews for the surveys. The February 2021 survey continued this series of collaborative surveys. Dredge sampling was consistent with previous surveys and survey data are comparable with others in the time series. Since 2013, testing for *B. exitiosa* infection used two methods to allow the time series of infection data from heart imprints recorded since 1986 to be adjusted for the higher levels of detection provided by polymerase chain reaction (PCR) methods. A quantitative PCR (qPCR) method was used between 2013 and 2017. An

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improved droplet digital PCR (ddPCR) method with a high level of precision and repeatability, superior levels of sensitivity, detection, and cost-effectiveness was used for the first time in 2018.

All the key indicators for the February 2021 survey are strongly positive for the continued rebuilding of the fishery. 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. 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 was high (1091.2 million oysters, 95% confidence interval (CI) 726.3–1637.0) in 2021. The population size of recruit-sized and pre-recruit oysters started to increase from 2017 and both size groups increased substantially between 2020 and 2021 as small oysters grew into these size groups. Pre-recruits increased 83.5% to 487.0 million oysters (95% CI 320.9–733.6) and recruit-sized oysters increased 51.2% to 801.4 million oysters (95% CI 536.2–1196.7). Commercial-sized oysters also increase between 2020 and 2021 by 28.3% to 405.6 million oysters (95% CI 271.0–606.6). In the absence of significant mortality, continued rapid increases in recruit-sized and commercial-sized oysters driven by the large numbers of small and pre-recruit oysters is highly likely. Oysters growing to recruit size in 2021 may not grow to commercial size for another 1–2 years. Catch rates of commercial-sized oysters for the 2021 season are expected to remain similar to, or slightly higher than the 2020 levels.

Future levels of disease mortality in the fishery are uncertain. *Bonamia exitiosa* mortality has been low since 2018. In 2021, the numbers of gapers and new clocks (the articulated shells of recently dead oysters with the ligament attaching the two valves intact) were low, and similar to base levels for natural mortality, regardless of cause of death. The prevalence of *B. exitiosa* infection by ddPCR was 5.7%, 45.4 million oysters (95% CI 23.9–74.9), higher than the 5.1% observed in 2020, but lower than the 6.4% observed in 2019. The infected population of recruit-sized oysters in the Bonamia survey area estimated using ddPCR is 32.4% higher than that estimated using heart imprints in 2021. Mortality in the Bonamia survey area over the summer of 2020–21 was 4.3% (34.7 million recruit-sized oysters), comprised mostly from post survey mortality of fatally infected oysters. Non-fatal infections of *B. exitiosa* were 0.7% of the recruit-sized oyster population in 2021, and mortality from *B. exitiosa* is expected to be low in 2022. *Bonamia ostreae* was detected in the Foveaux Strait oyster fishery during routine surveillance in February 2021. Retesting of oyster samples collected for *B. exitiosa* in February 2021and extensive sampling for *B. ostreae* in May 2021 did not detect any further infection. The status *B. ostreae* is unknown, and this pathogen 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 from *B. ostreae* could potentially be greater than 90%.

A total allowable commercial catch (TACC) of 15 million oysters represents an exploitation rate of 2.9% of the portion of the stock above 400 oysters per tow (519.3 million oysters), and 1.7% of the recruit sized population in core strata alone. In 2020, the oyster industry landed 8.1 million oysters representing 1.0% 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.

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. An 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. Incorporation of 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 et al., NIWA, unpublished data). 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, non-QMS fish, QMS reported bycatch (Porifera), and QMS reported bycatch (Bryozoa).

The detection of *Bonamia ostreae* at a single site in Foveaux Strait in February 2021 has put greater onus on the top three research priorities agreed in 2019. The 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 (Culloty & Mulcahy 2007). 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. At an individual and 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. To what extent and how quickly *B. ostreae* will affect the oyster fishery is uncertain.

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), referenced in Appendix 6. Since 1999, these surveys have sampled the same survey area (1054 km^2) using the same methods and similar strata to ensure data from these 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. Annual, smaller surveys (Bonamia surveys) that focus sampling effort in the core commercial fishery area are undertaken in the years between stock assessments (Michael et al. 2015b, 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 (15th stratum) thereby sampling the whole stock assessment area. This background stratum receives limited sampling effort.

The introduction of five-yearly stock assessments in 2012 has placed greater onus on the annual Bonamia surveys to monitor changes in the oyster population in commercial fishery areas, as well as the status of Bonamia. These surveys estimate oyster densities and population sizes of four size groups of live oysters, and recruit-sized and pre-recruit new and old clocks (Table 1). Clocks are the articulated shells of recently dead oysters with the ligament attaching the two valves intact. In February surveys, new clocks are assumed to be oysters that have died since summer mortality from Bonamia began, and oysters that died up to three years before this are categorised as old clocks.

Table 1: In 2020, 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 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 and new clocks. A standard Bonamia survey area was established to ensure that surveys are comparable from year to year. This area was determined from fishery independent survey data and fishers' logbook data and thereby represents the core commercial fishery that has been consistent through the fluctuations in relative oyster abundance driven by Bonamia mortality. This survey design and sampling effort predicts a coefficient of variation (CV) for survey estimates of about 11%. The 2014 survey achieved a CV of 11.2% for recruit-sized oysters in the Bonamia survey area, and a CV of 11.7% for the stock assessment survey area from an additional 5 stations in the background stratum (Michael et al. 2015a). Surveys since 2015 have achieved CVs well below the survey target of 20%, 8–12% for recruit-sized oysters in the Bonamia survey area and 9%–17% in the stock assessment survey area (Michael et al. 2020, 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 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 2013 and 2015 (200 million oysters killed between 2012 and 2014), and the continued low replenishment of spat to the oyster population and pre-recruit 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 2020 (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 3.7%) since 2018, as has been the prevalence of fatal infections and non-fatal infections. The low nonfatal infections suggest reduced transmission of Bonamia infection.

This report provides a summary of information from the seventh of the new series of Foveaux Strait oyster surveys in the Bonamia survey area undertaken in February 2021. This survey estimated oyster population size and the status of Bonamia infection and outlines the implications for the future stock status based on the 2017 OYU 5 stock assessment. This survey was undertaken as part of the Fisheries New Zealand research programme OYS2020/01 (Specific objectives 1–4). References for New Zealand Fisheries Assessment Reports for Foveaux Strait oyster and Bonamia surveys 2010–2020 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 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, and 2020. Commercial-sized oyster populations were first estimated in 2019*. The percentage decrease in mean population size between 2012 and 2017 (shaded tan) caused by Bonamia mortality and the percentage increase in mean population size between 2017 and 2020 (shaded green) are also given. Estimates of mean population size from the stock assessment survey area in 2018, 2019, and 2020 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	2012–17	2017–2020
Oyster size: Bonamia survey area							
Commercial	NA	NA	NA	318.7	316.1	NA	99.18*
Recruit	688.1	363.6	494.1	542.5	529.9	-47.2	+145.74
Pre-recruit	297.4	123.1	178.4	216.5	265.3	-58.6	+215.52
Small	451.3	261.9	401.8	595.8	1052.4	-42.0	+401.83
Oyster size: Stock assessment survey area							
Commercial	NA	NA	NA	536.9	504.6	NA	93.98*
Recruit	918.4	527.4	883.3	868.0	879.3	-42.6	+166.7
Pre-recruit	414.3	168.2	225.8	309.8	436.6	-59.4	+259.6
Small	612.2	361.6	552.5	867.8	1356.7	-40.9	+375.2

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 C.V. 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 2021 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 2021 survey were the same as for February Bonamia surveys since 2014 (Figure 1). The 2017 stock assessment survey sampled all the 26 strata (Michael et al. 2019a, referenced in Appendix 6). The inclusion of a single large background stratum (see Figure 1) for Bonamia surveys ensures that the entire stock assessment survey area is sampled, and that data from these annual surveys can be included in future stock assessments for OYU 5.

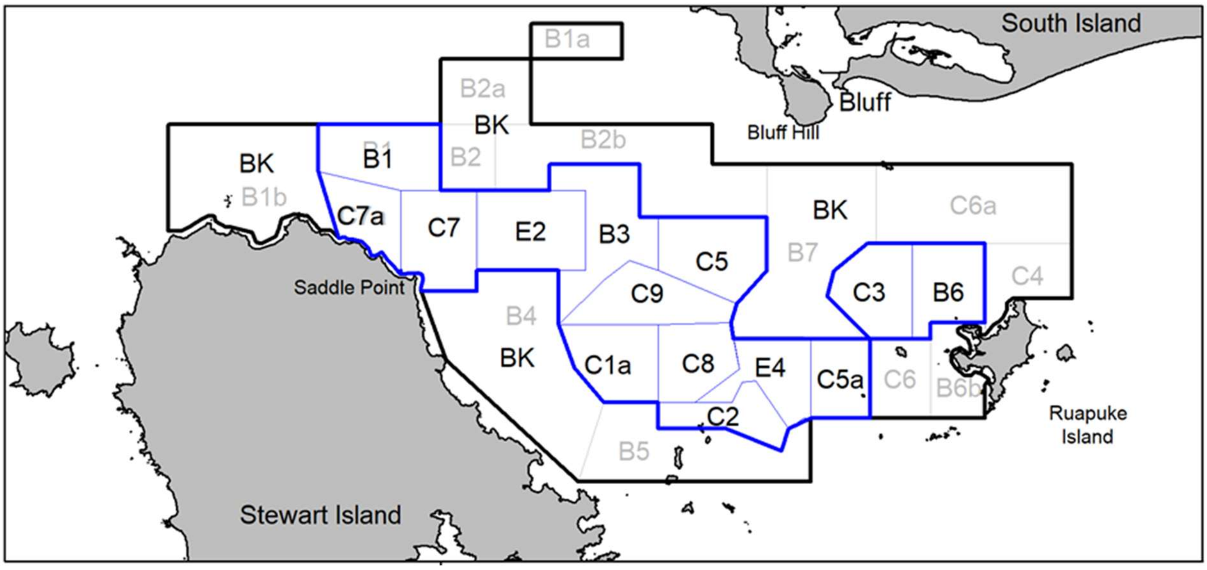

Figure 1: The 2021 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2021 survey strata representing the core commercial fishery area (Bonamia survey area) shown as blue lines. Strata are labelled with grey and black text. The remaining stock assessment survey strata which represent mainly background strata were merged into a single, large background stratum (BK).

Simulations were undertaken in 2014 to determine the optimal stratification and the numbers of stations required to give a survey CV for the recruit-sized population estimate in the range of 8–12% (see Michael et al. 2015a, referenced in Appendix 6). Simulations predicted that 55 stations in the 14 Bonamia survey strata would produce a CV of about 11%. ALLOCATE (Francis 2006) was used to allocate the numbers of stations to strata in 2021 (Table 3). Rand_Stn (Doonan & Rasmussen 2012) was used to generate the location of 50 random first-phase stations and sufficient stations in each stratum to sample 5 second-phase stations in the Bonamia survey strata (hereafter core strata), and 5 stations from the background stratum.

Stations were generated with an exclusion zone of 0.75 nautical miles to spread stations within strata to ensure good spatial coverage and to prevent the overlap of sample tows.

Since 2007, increasing numbers of fixed stations have been sampled annually across the stock assessment survey area to provide a time series of data on changes in oyster density and Bonamia status in localised areas. In 2015, the Fisheries New Zealand (then Ministry for Primary Industries) Shellfish Working Group agreed data from the 12 fixed stations add value to the information obtained from surveys. These 12 fixed stations were sampled in February 2021 (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 during the February 2021 Bonamia survey, and the area of each stratum. A single, large background stratum (BK) represents the merged stock assessment survey strata outside the Bonamia survey area (see Figure 1).

Stratum	First-phase	Second-phase	Fixed	Foul shots	Area (km^2)
B1	5	-	1	-	78.2
B3	3	-	-	-	44.7
B6	3	4	-	-	30.0
BK	5	-	2	1	578.4
C1a	3	-	-	-	31.3
C2	3	-	1	4	21.9
C3	3	-	1	-	32.7
C5	5	-	1	-	37.7
C5a	3	-	1	-	23.5
C7	3	-	-	-	36.1
C7a	3	1	-	3	23.6
C8	3	-	1	-	26.8
C9	3	-	-	-	34.5
E2	7	-	2	-	42.8
E4	3	-	2	-	28.0
Total	55	5	12	8	1 070.2

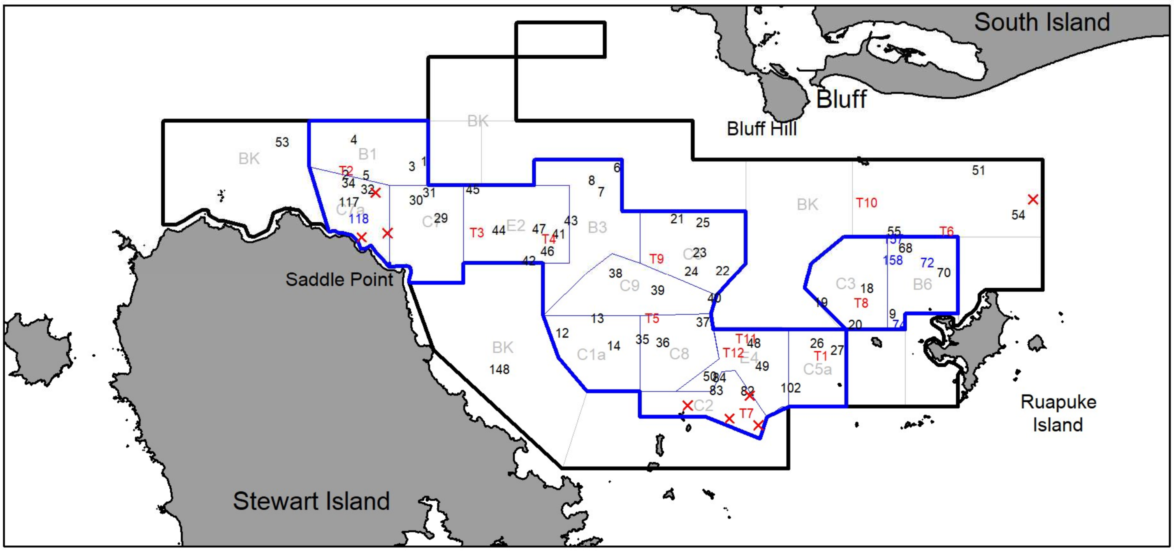

Figure 2: The 2021 survey area with the 2007 survey boundary shown as a heavy, black outer line, the Bonamia survey area as a heavy blue line, and the 2021 Bonamia survey strata shown as fine blue lines. The remaining stock assessment survey strata (fine light grey lines) in the large background stratum were merged into a single stratum (BK). First-phase station numbers are in black text, second-phase in blue text, and fixed stations in red text. Red crosses denote stations that could not be towed because of foul ground (foul shots).

2.1 Catch sampling

Except for minor variations, dredge sampling has followed standard procedures for stock assessment and Bonamia surveys since October 2002: standard dredge sampling methods, standard methods for sorting the catch and recording data (station data forms are shown in Appendix 1), and standard methods for sampling oysters to determine the status of Bonamia. Details of the standard procedures are given by Michael et al. (2015a, 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 2011–2020, except in 2016 when the F.V. *Golden Lea* was used due to the unavailability of the F.V. *Golden Quest*. Stephen Hawke has skippered the survey vessel since 2011, to maintain consistency in the time series. Survey stations were sampled with the standard survey dredge (commercial dredge 3.35 m wide and weighing 430 kg) used since 1993 and rebuilt in 2014 to the same specifications. A traditional friction winch used to deploy the dredge on F.V. *Golden Quest* was replaced with a hydraulic winch system in 2014.

Oyster surveys use straight-line tows to enable the area sampled by the dredge to be calculated. Straight-line tows were made down tide for a distance of 0.2 nautical mile (370 m) at each station. The definitions of start and finish positions and criteria for repeat tows are given in Appendix 1. The area swept by a standard dredge tow is 1240 m^2, the tow length (370 m) multiplied by the dredge width (3.35 m).

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 2021.

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 only those oysters that had died since summer mortality from Bonamia began. Oysters that died before this were categorised as old clocks and these oysters had shells that were fouled or in which the inner valves had lost their lustre. Old clocks can be covered in fouling organisms on both external and internal surfaces, and, because the ligaments of oysters are thought to break down over about a three-year period, old clocks represent oysters that had died between 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 2021, and these were compared with the estimates from strata sampled in 2016–2020 (Michael et al. 2016, 2019a, 2019b, 2020, 2021, 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, and small oysters, and clocks, was calculated by using the combined population sizes in each stratum as:

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

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

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

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

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

The 95% confidence intervals of the mean population size ($\bar{x}$) for each stratum and the Bonamia and stock assessment survey areas are estimated by bootstrapping, i.e., resampling with replacement of a normal distribution for which the variance is based on a CV of the population estimate and the error associated with dredge efficiency.

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.

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 2018 and February 2021 surveys.

2.3 Methods to estimate the annual mortality from Bonamia

Although substantial winter mortality from Bonamia has occurred previously (Hine 1991), most mortality from *B. exitiosa* occurs in the summer. Summer mortality of recruit-sized oysters only is estimated by Bonamia surveys. Summer mortality comprises the aggregate of two different estimates: (1) pre-survey mortality estimated from the population size of recruit-sized new clocks and gapers that had died during the summer; and (2) projections of future (within about two months) disease mortality from the proportion of oysters with categories three and higher (fatal) Bonamia infections (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.

2.4 Methods to estimate the prevalence and intensity of Bonamia infection

Samples of up to 30 randomly selected recruit-sized oysters from each station were flown to the National Institute of Water and Atmospheric Research (NIWA) Wellington for *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 (Appendix 2 and Appendix 3 give an example and details). Data on size, general condition, and whether oysters were incubating larvae were recorded (see Appendix 3). Histological samples were taken from the first five oysters processed for heart imprints at each station.

2.4.1 Estimating prevalence and intensity of *Bonamia exitiosa* infection

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

Prevalence of infection at each station is the proportion of the total sample number that tested positive for Bonamia infection using heart imprints and ddPCR (Bilewitch et al. 2018). The intensity of *B exitiosa* infection was estimated using heart imprints and ddPCR. These estimates are not directly comparable because heart imprints score the numbers of *B. exitiosa* parasites in haemocytes using the methods of Diggles et al. (2003) and ddPCR estimates the numbers of *B. exitiosa* 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).

2.4.2 Review of ddPCR procedures prior to testing and repeat testing

Before the samples from the 2021 survey were analysed, quality control of reagents and methods was undertaken (details in Appendix 3). Each 96-well ddPCR plate tested 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).

2.4.3 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 were also examined to estimate prevalence. Oysters that tested negative for Bonamia using ddPCR analysis was 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) and scaled-up to the size of the recruit-sized oyster population by strata, and for the Bonamia and stock assessment survey areas.

A detailed account of the ddPCR method and testing is given by Bilewitch et al. (2018). This method adapts a previous qPCR assay for the duplex amplification of the Bonamia target (ITS region of the ribosomal genes) plus the *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.

2.4.4 Heart imprints

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

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

from heart imprint samples only

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

2.4.5 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 (Estimates of oyster densities and population size) in section 2.2. These estimates are presented by stratum, for the Bonamia survey area and stock assessment survey area. Method 1 used a correction factor from strata with three or more randomly selected stations only, i.e., target stations were not included. 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 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 are 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 were estimated using standard methods as for population estimates.

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

Under the new management plan for OYU 5, stock assessments are carried out five-yearly, with annual population and Bonamia surveys between assessments. The last assessment was completed in 2017 (Large et al. 2021), updating the stock assessment models with data on recruitment, harvest, catch rates, population size, and mortality (mostly mortality from Bonamia during epizootics). Three projections of future stock status were based on 0%, 10%, and 20% disease mortality.

Projections from the 2009 stock assessment based on a TACC of 15 million oysters, and with no mortality of oysters from Bonamia, predicted an increase in recruit-sized stock abundance of 29% by 2012; however, with a Bonamia mortality of 10%, the population size was expected to increase by only 11% over the same period (Fu & Dunn 2009, Fu 2013). Bonamia mortality was about 10% between 2009 and 2012; and the estimated mortality of recruit-sized oysters between the 2009 survey and the 2012 survey was about 198 million oysters. The population size of recruit-sized oysters increased by 21% between the 2009 and 2012 surveys. If the estimated post-survey mortality in 2012 (81 million oysters) is taken into account, the population size of recruit-sized oysters increased by 13.5%, consistent with the 2009 stock assessment. The 2012 stock assessment based on a TACC of 15 million oysters predicted the population size to remain similar or decline by 23% with 10% and 20% mortality, respectively. The recruit-sized population decreased by 44.5% in 2015 (509.9 million oysters), more than expected because recruitment had been very low since 2010.

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

3. RESULTS

Sea conditions were relatively calm over the February 2021 survey period and better than in February 2020. The survey started the 6th of February 2021 and was completed in the usual time of seven survey days. The weather was good during sampling and tides were mostly neap tides. Observations from the survey suggest little pre-survey mortality (few new clocks), including the eastern fishery areas where mortality has historically been high. The distribution of oysters was widespread. More survey tows recorded counts of 500 or more recruit-sized oysters, 26% of stations in 2021, compared with 10% of stations in 2020. All of the 72 tows recorded at least some recruit-sized oysters. There was good growth in some areas, facilitating an increase in the numbers of recruit-sized oysters. Many of these oysters were legal-sized, but not yet commercial-sized. Large numbers of spat and 1–3-year-old oysters were observed on oysters in many areas in 2021 reflecting continued good recruitment to the population and the survival of juveniles.

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 C3 (station 20) and stratum C5a (stations 26, 27, 102, and T1). Oyster density was most likely underestimated at these stations.

The efficiency of dredge sampling during the 2021 survey was consistent with previous surveys. Dredge tow lengths were almost all about 0.2 nautical miles (370 m) in length. Wind speeds were less than 10 knots, and sea conditions and dredge saturation were similar to previous surveys (Appendix 4, Figures A4.1–A4.3).

3.1 Survey operational detail

NIWA and the BOMC staff began the survey on the 6th of February 2021 and finished on the 14th of February, sampling on seven days during this period. The oyster vessel F.V. *Golden Quest* successfully sampled all 72 stations. 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 10, 11, 15, 16, 17, 33, and 52, and some of their replacements (69, 85, 87, 96, 115, and 116), were replaced by stations 68, 69, 82, 84, 83, 117, and 148, respectively. All five second-phase stations were sampled at their allocated stations.

Target sample size for ddPCR and heart imprints (n = 25 per station) were achieved from 68 of the 72 stations. For samples with fewer than 25 recruit-sized oysters, samples included pre-recruit and small oysters: station 102 (n=6, mostly small), 49 (n=10, mostly small), 148 (n=12, mostly small), and 117 (n=13, mostly small).

3.2 Oyster abundance

3.2.1 Changes in oyster densities between 2018 and 2021

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 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 these size groups in the background stratum (BK) were similar or lower.

Mean catches of commercial-sized oysters generally varied by stratum across the fishery area (Figure 3). Strata west of a line between Saddle Point (Stewart Island) and North Head (Ruapuke Island) (E2, B3, C9, C1a, C5, C8, C3, and B6) had similar or higher catches in 2021 compared with those in 2019 and 2020. Some strata in the eastern fishery area (C5 and C5a) had similar or lower catches in 2021. The background stratum (BK) had lower catches, and this probably reflects the effects of interannual survey variation cause by low sampling effort in BK (Figure 3). Within stratum, catches varied greatly except for strata C5a and BK (Figure 3).

At a stratum level, catches of recruit-sized oysters were generally similar across the fishery area (Figure 4). Strata west of a line between Saddle Point (Stewart Island) and North Head (Ruapuke Island) had similar or higher catches in 2021 compared with those in 2018, 2019, and 2020, as did stratum B6 in the eastern fishery areas. Oyster catches in strata C5, C5a, and BK in the eastern fishery area showed a similar trend to commercial-sized oysters, reflecting sampling variation (Figure 4).

Catches of pre-recruit-sized oysters in 2021 were consistently higher across the whole fishery area than in 2018, 2019, and 2020 (Figure 5). Catches within stratum were variable, reflecting small spatial-scale patchiness. Catches in strata C5, C5a, and BK were similarly low compared with other size groups in 2021 (Figure 5).

Catches of small oysters were high in 2021, similar to those in 2018, 2019, and 2020, except in stratum BK where catches have remained low (Figure 6). The number of small oysters reflects the trend of increasing recruitment to the population indicated by spat monitoring.

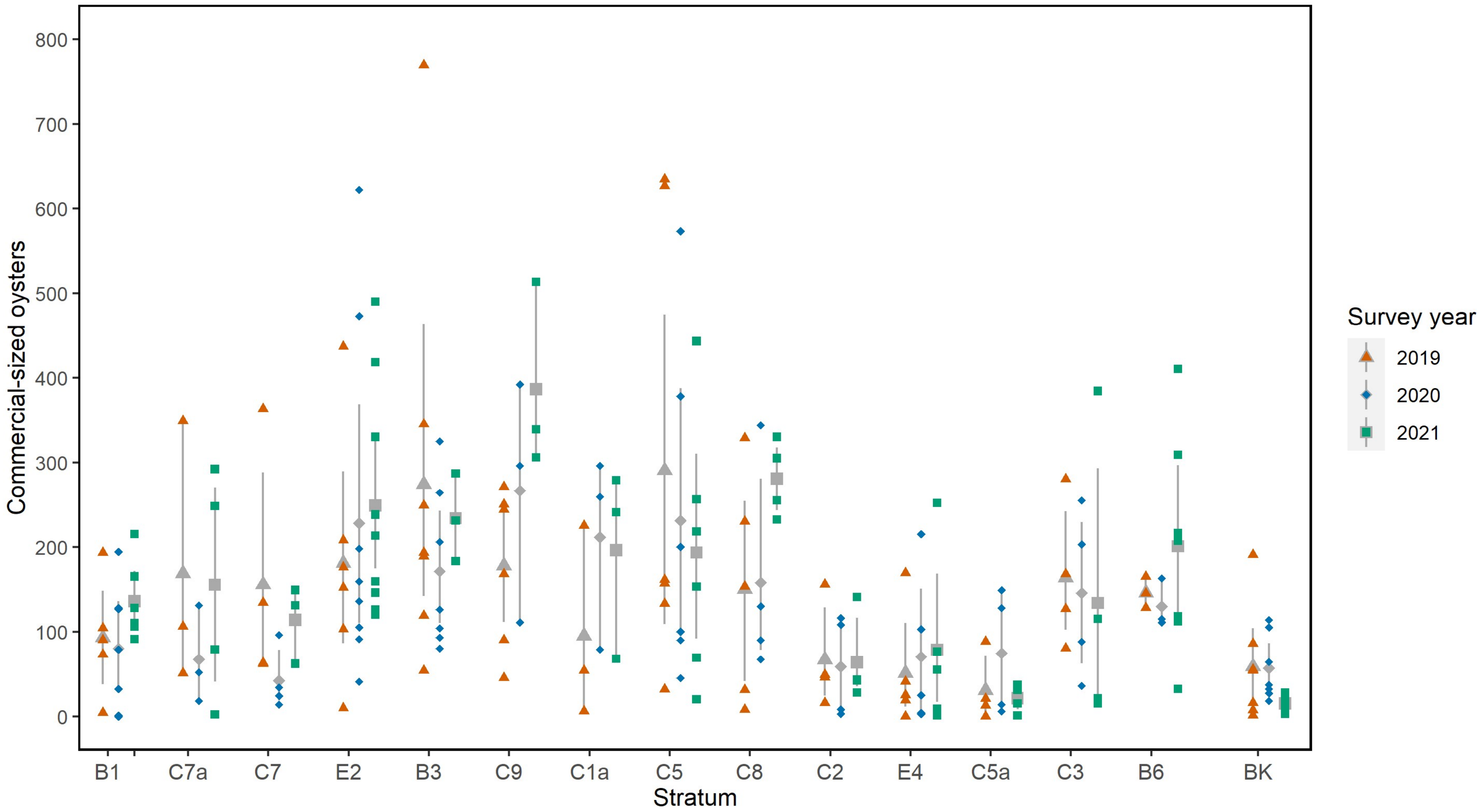

Figure 3: The numbers of commercial-sized oysters (≥ 65 mm in diameter) per tow, means (grey symbols matching shape showing survey year), and 95% confidence intervals (grey lines) by stratum, for surveys during 2019*–2021. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2019 survey are shown as red filled triangles, 2020 as blue filled diamonds, and 2021 as light green filled squares. Bonamia survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthermost right. * Commercial-sized oysters first recorded in 2019.

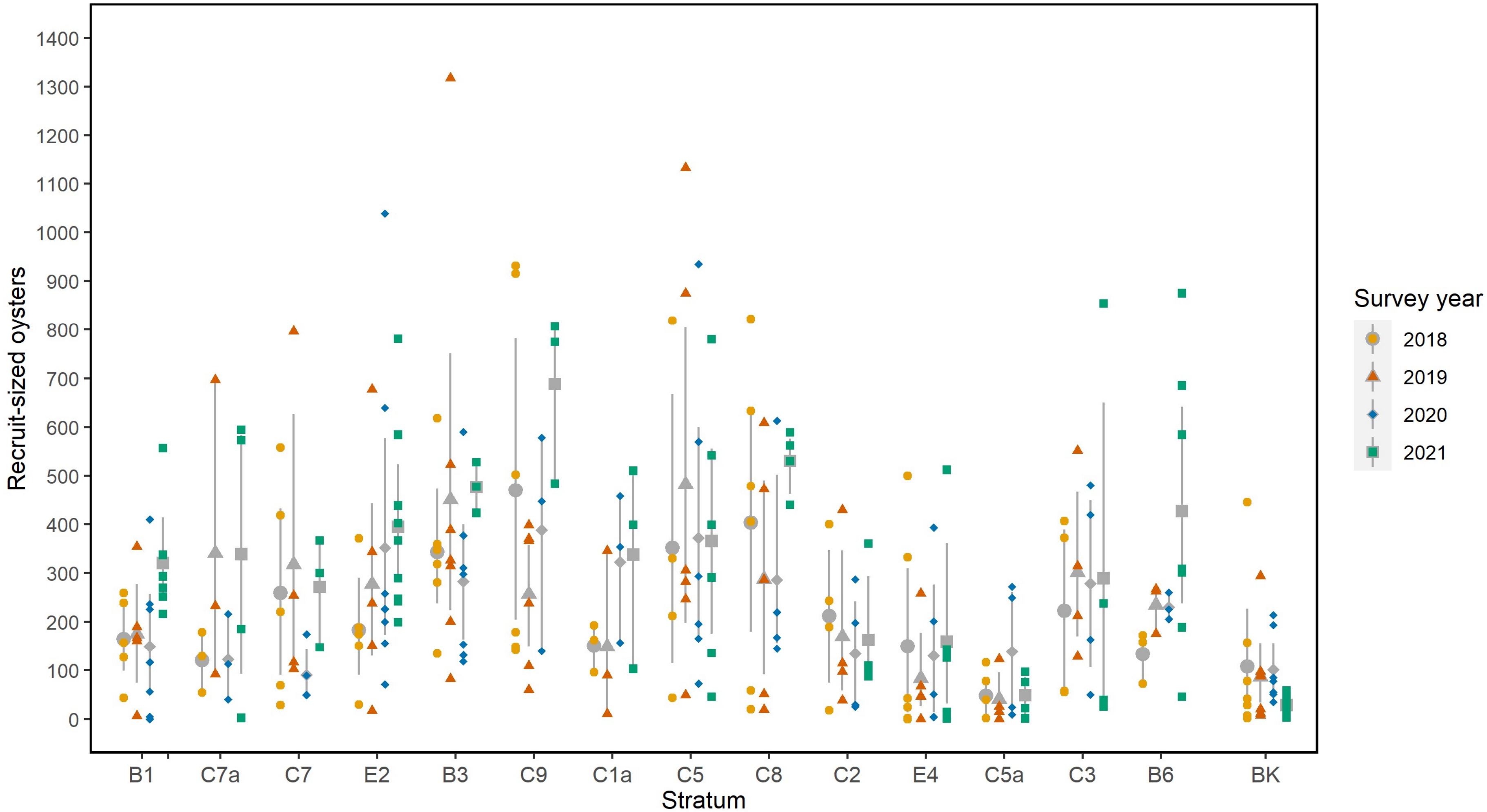

Figure 4: The numbers of recruit-sized oysters (≥ 58 mm in diameter) per tow, means (grey symbols matching shape showing survey year), and 95% confidence intervals (grey lines) by stratum, for surveys during 2018–2021. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2018 survey are shown as tan filled circles, 2019 as red filled triangles, 2020 as blue filled diamonds, and 2021 as light green filled squares. Bonamia survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthermost right.

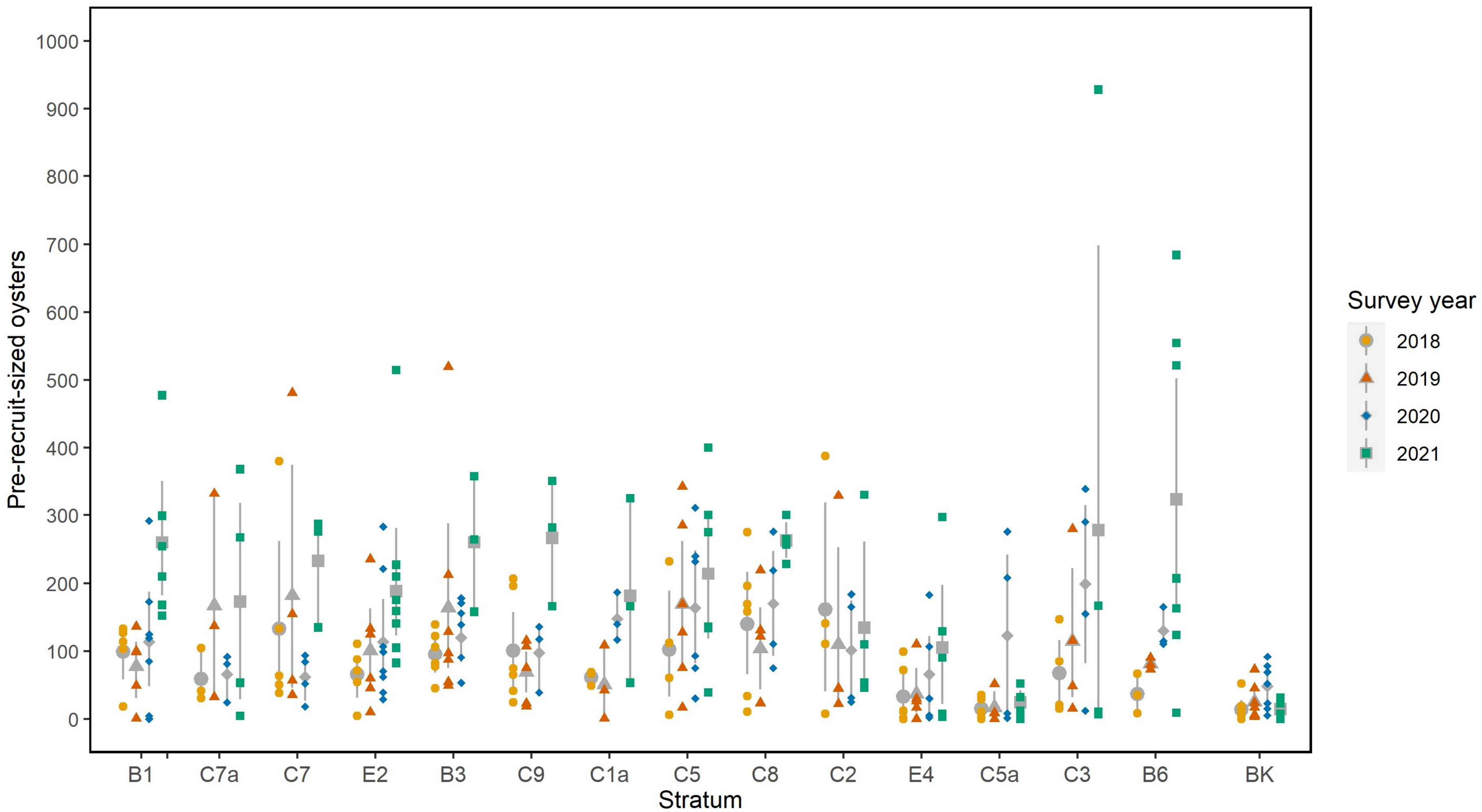

Figure 5: The numbers of pre-recruit- oysters (≥ 50–57 mm in diameter) per tow, means (grey symbols matching shape showing survey year), and 95% confidence intervals (grey lines) by stratum, for surveys during 2018–2021. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2018 survey are shown as tan filled circles, 2019 as red filled triangles, 2020 as blue filled diamonds, and 2021 as light green filled squares. Bonamia survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthermost right.

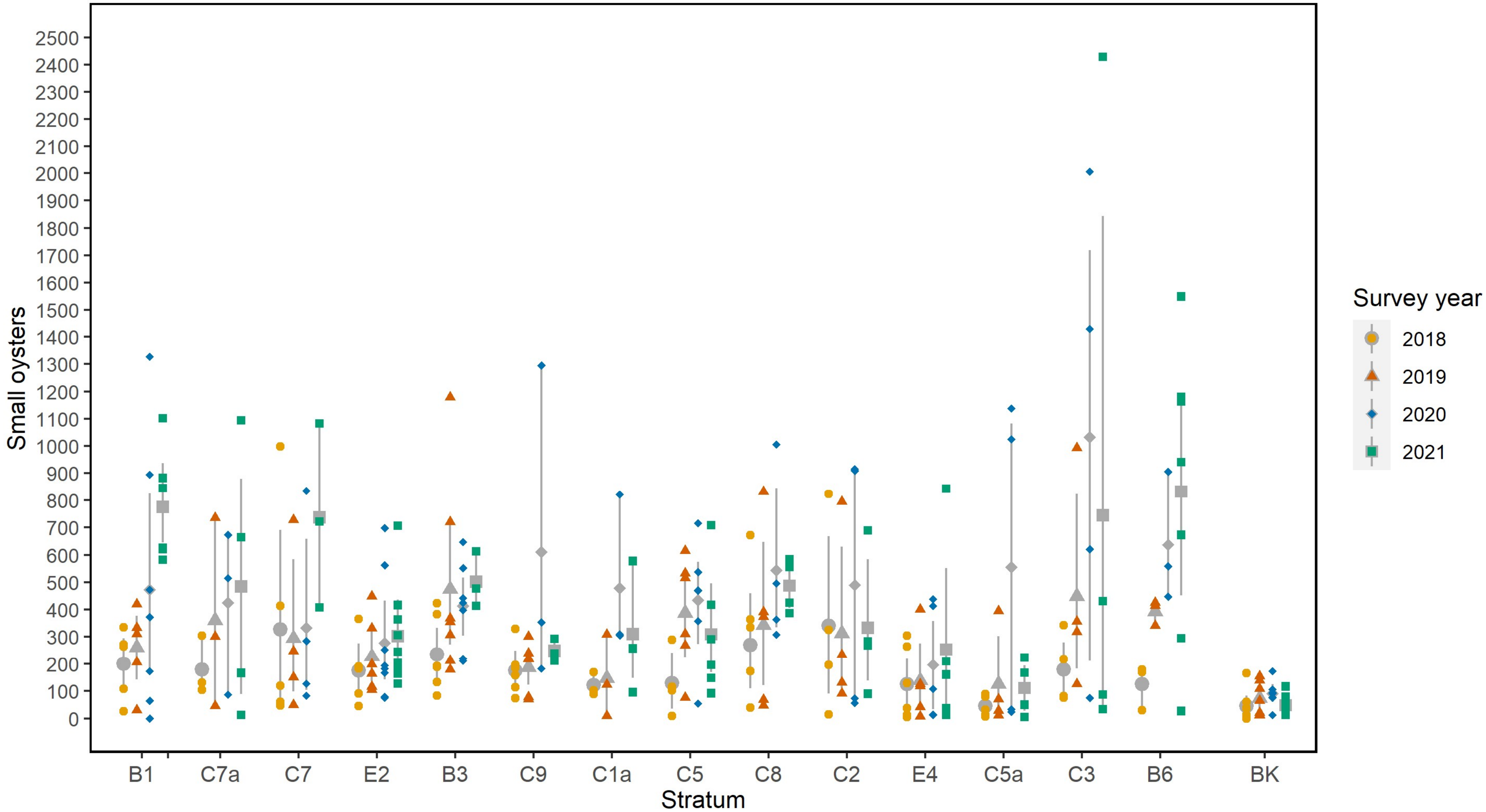

Figure 6: The numbers of small oysters (10–49 mm in diameter) per tow, means (grey symbols matching shape showing survey year), and 95% confidence intervals (grey lines) by stratum, for surveys during 2018–2021. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2018 survey are shown as tan filled circles, 2019 as red filled triangles, 2020 as blue filled diamonds, and 2021 as light green filled squares. 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

Since 2014, annual Bonamia surveys have used fully randomised, two-phase sampling designs aimed at better estimating oyster densities and population sizes of oysters and new clocks, and a standard Bonamia survey area to ensure that surveys are comparable from year to year (Michael et al. 2015a and 2015b, referenced in Appendix 6).

Estimates of absolute population size for commercial-sized, recruit, pre-recruit, and small oysters from the February 2021 survey are given by stratum in Tables 4–7: for the core strata (*n*= 14: B1, B3, B6, C1a, C2, C3, C5, C5a, C7, C7a, C8, C9, E2, and E4), all core strata combined, the background stratum (all background strata combined (BK), *n*= 12: B1a, B1b, B2, B2a, B2b, B4, B5, B6b, B7, C4, C6, and C6a), and the whole 2007 stock assessment survey area (Survey total). The population estimates 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 2016–2021 surveys and for commercial-sized oysters from the 2019–2021 surveys are shown in Table 8 to quantify recent trends in increases or decreases in population size. Mean population sizes and 95% confidence intervals (from s.d.) for these four size groups for 2012 and 2014–2021 in the Bonamia survey area are shown in Figure 7. The survey data for February 2013 are not comparable (sampling does not cover all the Bonamia survey area). New Zealand Fisheries Assessment Reports for Foveaux Strait oyster and Bonamia surveys 2010–2020 that provide estimates of oyster density, population size, and CVs for all size groups are referenced in Table A6.1 (Appendix 6).

Comparisons between the population estimates for the background stratum should be made with caution because there were only 5 stations sampled in total. Bootstrapped estimates of 95% confidence intervals (B.lower and B.upper) were made by resampling a normal distribution where the variance is based on a CV and the error of the estimated dredge efficiency. Bootstrapped estimates are likely to better represent the true range of estimates from this patchily distributed population.

Fishers high-grade their catches (return the smaller oysters that are above minimum legal size) to maximise the numbers of first grade (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 increased by 28.3% between 2020 and 2021 (Table 4), and the population size of oysters above MLS (hereafter recruit-sized oysters) increased by 51.2% over the same time (Table 5). Of the recruit-sized population, 50.6% was of commercial size in the core strata and the same (50.6%) in the stock assessment survey area in 2021 (Tables 4 and 5). The percentages of commercial-sized oysters were lower in 2021 than in 2020 and 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. Mean density in the core strata was 0.82 oysters m^{-2} in 2021, higher than in 2020 and 2019 (0.64 oysters m^{-2} and 0.65 oysters m^{-2}, respectively). In 2021, mean density ranged from 0.08 oysters m^{-2} in stratum C5a to 1.86 oysters m^{-2} in stratum C9 (Table 4). Oyster densities for stratum C5a are likely to be underestimated because this stratum has extensive stands of the stalked ascidian (kāeo, *Pyura pachydermatina*) that substantially reduce oyster catchability.

The density and population size of recruit-sized oysters in core strata both increased by over 50% in 2021 from levels in 2020 and 2019. The mean density in core strata in 2021 (1.63 oysters m^{-2}) was the highest since 2012, which ranged from 0.71 oysters m^{-2} in 2015 to 1.40 oysters m^{-2} in 2012. In 2021, strata with the highest densities were B3, B6, C8, C9, and E2. Usually an increase in recruit-sized oyster density results in an increased catch rate; however, the large numbers of fast growing and thin legal-sized oysters were not of commercial size. The density of recruit-sized oysters in the background stratum is not likely to be well estimated by recent surveys (since 2017) due to the low numbers of stations sampled (*n*=5) over a large area (578.4 km^2). In 2021, mean recruit-sized oyster density in the background stratum was 0.81 oysters m^{-2}. The population size of recruit-sized oysters in core strata is the highest it has been since 2012. 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 (see Table 8). The CV for the estimate of recruit-sized population in core strata was 6%, lower than it has been between 2012 and

2020 (8.0% to 13.0%). The CV for the estimate of recruit-sized population in the background stratum was 33% in 2021, and between 13% in 2020 and 59.0% in 2018, reflecting the low sampling effort in the large stratum area.

Pre-recruit mean oyster density in all core strata combined increased 83.3% from 0.54 oysters m^{-2} in 2020 to 0.99 oysters m^{-2} in 2021 (Table 6) and increased 396.0% since 2016 (see Table 8). Mean densities in several strata (B1, B3, B6, C2, C7, C7a, and C9) increased substantially, while only three strata showed decreases (C3, C5a, and E4). Mean pre-recruit oyster density decreased from 0.60 oysters m^{-2} in 2012 to 0.25 oysters m^{-2} in 2017, in a fluctuating trend, and then increased to 0.99 oysters m^{-2} in 2021 (Table 8). 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 6). Pre-recruits decreased from 297.4 million oysters in 2012 to 89.2 million oysters in 2015, and then increased to 2021 to 487.0 million oysters. The population sizes in the background stratum decreased by 79.4% from 171.3 million oysters in 2020 to 35.3 million oysters in 2021 (Table 6). The CV for the estimate of recruit-sized population in the background stratum was 45% in 2021, reflecting the low sampling effort.

The mean densities and population sizes of small oysters for all the core strata combined remained similarly high between 2020 and 2021. Mean density in 2020 was 2.14 oysters m^{-2} and 2.22 oysters m^{-2} in 2021. Population size increased by 3.7% from 1052.4 million oysters in 2020 to 1091.2 million oysters in 2021 (Table 7). The population size of small oysters declined by 43% from 451.3 million oysters in 2012 to 256.1 million oysters in 2016 and has since increased substantially by 326.1% in 2021 (Table 8). Similar numbers of strata slightly either increased or decreased in mean densities in 2021. In order of rank, strata B1, C7, B6, and B3 had the highest population sizes. The CV for the estimate of population size for small oysters in the background stratum was 33% in 2021 and the population size decreased from 304.3 million oysters in 2020 to 111.0 million oysters in 2021 (Table 7).

In 1995 and 1997, Cranfield et al. (1999) estimated Current Annual Yield (CAY) from a "commercial population" that reflected the patchy distribution of oyster density. Cranfield et al. (1999) defined the commercial population as the recruited population in the stock assessment survey area above a density of 400 oysters per tow (equivalent to about 6–8 sacks per hour during commercial dredging). This threshold was based on a historical, economic catch rate, and, when the catch rate dropped below 6 sacks per hour, fishers would move to new fishery areas. Although this method is no longer used for stock assessments, estimates of commercial population size allow some comparison with previous years and the Shellfish Working Group requested that these estimates be included in this report. Table 9 shows estimates of commercial population size, using the catch of recruit-sized oysters at each station minus 400 oysters, for the 2021 core strata (*n*= 14), all core strata combined, all background strata combined (*n*= 12), and for the whole 2007 stock assessment survey area sampled. Nine core strata only (B1, B3, B6, C1a, C5, C7a, C8, C9, and E2) supported commercial densities in 2021 (Table 9). Mean densities in these strata ranged from 0.50 oysters m^{-2} to 3.33 oysters m^{-2}, and mean density for all core strata combined was 1.06 oysters m^{-2}. The proportion of the recruited population above the commercial threshold in core strata increased by 116.7% from 239.6 million oysters in 2020 to 519.3 million oysters in 2021 (Table 9). 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 mean commercial density in the core strata was 0.17 oysters m^{-2} in 2017 and has increased continually through to 2021. There were no commercial densities of oysters in the background stratum either in 2020 or in 2021 (Table 9).

In 2021, three strata had commercial densities of commercial-sized oysters (B6, C9, and E2) ranging from 0.29 oysters m^{-2} to 0.81 oysters m^{-2}, and a mean population size of 28.3 million commercial-sized oysters.

Table 4: **Absolute population estimates for commercial-sized (≥ 65 mm in diameter) oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2021 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 (CV) of the density estimate, the mean population size in millions of oysters 2021 (Pop.n), upper and lower 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 2020 mean population estimates (2020 Pop.n) and the 2021 mean population size represented as a percentage of the 2020 mean population size by stratum (% of 2020). The percentage change from the 2020 estimate is shaded green for increases in population size in 2021 and tan for decreases.**

Stratum	Number stations	Mean density	Density s.d.	CV	2021 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2020 Pop.n	% of 2020
B1	5	0.64	0.10	0.16	50.2	29.7	79.9	78.2	27.6	+81.7
B3	3	1.16	0.14	0.12	51.6	32.4	78.9	44.7	36.9	+40.0
B6	7	0.98	0.24	0.25	29.5	13.7	50.8	30.0	18.6	+58.7
C1a	3	0.96	0.31	0.33	29.9	10.2	56.1	31.3	32.3	-7.4
C2	3	0.35	0.18	0.51	7.6	0.2	16.8	21.9	4.5	+68.6
C3	3	0.25	0.16	0.65	8.2	0.0	20.4	32.7	17.5	-52.8
C5	5	0.70	0.22	0.31	26.3	9.4	48.5	37.7	36.8	-28.4
C5a	3	0.08	0.04	0.55	1.8	0.0	4.1	23.5	6.4	-72.2
C7	3	0.55	0.13	0.23	19.8	9.8	33.8	36.1	7.4	+166.4
C7a	4	0.78	0.35	0.45	18.4	2.1	38.3	23.6	7.8	+136.6
C8	3	1.32	0.14	0.10	35.4	22.5	54.0	26.8	12.5	+184.0
C9	3	1.86	0.30	0.16	64.2	37.3	102.9	34.5	44.7	+43.5
E2	7	1.39	0.27	0.19	59.7	32.2	98.1	42.8	53.1	+12.4
E4	3	0.11	0.08	0.77	3.0	0.0	8.3	28.0	10.1	-70.7
Core total	55	0.82	0.05	0.07	405.6	271.0	606.6	491.8	316.1	+28.3
BK	5	0.06	0.01	0.23	33.6	16.4	57.4	578.4	188.4	-82.1
Survey total	60	0.41	0.03	0.06	439.3	289.9	643.3	1070.2	504.6	-12.9

Table 5: Absolute population estimates for recruit-sized (≥ 58 mm in diameter) oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2021 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 (CV) of the density estimate, the mean population size in millions of oysters 2021 (Pop.n), upper and lower 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 2020 mean population estimates (2020 Pop.n) and the 2021 mean population size represented as a percentage of the 2020 mean population size by stratum (% of 2020). The percentage change from the 2020 estimate is shaded green for increases in population size in 2021 and tan for decreases.

Stratum	Number stations	Mean density	Density s.d.	CV	2021 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2020 Pop.n	% of 2020
B1	5	1.56	0.26	0.17	122.1	71.3	195.2	78.2	52.6	+132.2
B3	3	2.36	0.14	0.06	105.3	70.5	154.9	44.7	61.0	+72.7
B6	7	2.09	0.56	0.27	62.8	27.2	110.1	30.0	33.1	+90.0
C1a	3	1.64	0.58	0.35	51.5	14.9	99.4	31.3	49.4	+4.1
C2	3	0.89	0.44	0.50	19.5	0.7	42.8	21.9	8.9	+119.4
C3	3	0.50	0.35	0.69	16.5	0.0	42.2	32.7	33.8	-51.2
C5	5	1.38	0.43	0.31	52.0	18.1	96.6	37.7	60.7	-14.3
C5a	3	0.16	0.11	0.67	3.7	0.0	9.4	23.5	11.5	-67.6
C7	3	1.31	0.31	0.24	47.3	22.9	81.0	36.1	16.1	+194.3
C7a	4	1.70	0.75	0.44	40.2	5.0	83.1	23.6	14.3	+182.0
C8	3	2.48	0.18	0.07	66.4	43.9	99.0	26.8	23.1	+187.0
C9	3	3.33	0.51	0.15	114.8	67.5	182.9	34.5	65.2	+76.1
E2	7	2.17	0.39	0.18	92.8	52.3	150.3	42.8	82.0	+13.2
E4	3	0.23	0.19	0.84	6.5	0.0	18.7	28.0	18.4	-64.9
Core total	55	1.63	0.10	0.06	801.4	536.2	1196.7	491.8	529.9	+51.2
BK	5	0.12	0.04	0.33	66.7	22.0	125.9	578.4	349.4	-80.9
Survey total	60	0.81	0.05	0.06	868.1	572.8	1272.8	1070.2	879.3	-1.3

Table 6: Absolute population estimates for pre-recruit (50–57 mm in diameter) oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2021 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 (CV) of the density estimate, the mean population size in millions of oysters 2021 (Pop.n), upper and lower 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 2020 mean population estimates (2020 Pop.n) and the 2021 mean population size represented as a percentage of the 2020 mean population size by stratum (% of 2020). The percentage change from the 2020 estimate is shaded green for increases in population size in 2021 and tan for decreases.

Stratum	Number stations	Mean density	Density s.d.	CV	2021 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2020 Pop.n	% of 2020
B1	5	1.30	0.25	0.19	101.6	56.3	166.2	78.2	42.9	+136.8
B3	3	1.29	0.29	0.22	57.6	29.4	96.3	44.7	25.8	+123.0
B6	7	1.59	0.48	0.31	47.6	17.5	86.7	30.0	18.7	+154.7
C1a	3	0.88	0.38	0.43	27.6	4.1	57.3	31.3	22.7	+21.9
C2	3	0.81	0.42	0.52	17.7	0.1	39.6	21.9	8.5	+109.1
C3	3	0.31	0.27	0.86	10.1	0.0	29.3	32.7	24.3	-58.7
C5	5	0.86	0.24	0.27	32.5	13.4	58.1	37.7	27.3	+19.2
C5a	3	0.07	0.04	0.63	1.7	0.0	4.1	23.5	8.2	-79.7
C7	3	1.12	0.24	0.21	40.5	21.1	67.5	36.1	10.9	+272.7
C7a	4	0.88	0.45	0.51	20.7	0.0	45.5	23.6	7.6	+172.3
C8	3	1.21	0.06	0.05	32.5	21.8	47.8	26.8	17.6	+84.9
C9	3	1.29	0.27	0.21	44.5	22.9	74.8	34.5	16.4	+171.3
E2	7	1.08	0.28	0.26	46.0	20.5	80.9	42.8	25.8	+78.7
E4	3	0.23	0.20	0.89	6.4	0.0	19.1	28.0	8.7	-26.8
Core total	55	0.99	0.08	0.08	487.0	320.9	733.6	491.8	265.3	+83.6
BK	5	0.06	0.03	0.45	35.3	3.8	74.8	578.4	171.3	-79.4
Survey total	60	0.49	0.04	0.08	522.3	338.8	773.9	1070.2	436.6	+19.6

Table 7: Absolute population estimates for small oyster (1049 mm in diameter) oysters in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2021 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 (CV) of the density estimate, the mean population size in millions of oysters 2021 (Pop.n), upper and lower 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 2020 mean population estimates (2020 Pop.n) and the 2021 mean population size represented as a percentage of the 2020 mean population size by stratum (% of 2020). The percentage change from the 2020 estimate is shaded green for increases in population size in 2021 and tan for decreases.

Stratum	Number stations	Mean density	Density s.d.	CV	2021 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2020 Pop.n	% of 2020
B1	5	3.80	0.37	0.10	297.3	192.7	448.5	78.2	180.7	+64.5
B3	3	2.48	0.30	0.12	111.0	69.8	169.3	44.7	88.7	-25.1
B6	7	4.08	1.00	0.25	122.3	56.9	210.5	30.0	91.2	+34.1
C1a	3	1.51	0.68	0.45	47.2	5.2	99.8	31.3	73.3	-35.6
C2	3	1.74	0.87	0.50	38.1	1.2	84.0	21.9	37.0	+3.0
C3	3	0.92	0.63	0.68	30.1	0.0	76.7	32.7	113.2	-73.4
C5	5	1.52	0.55	0.36	57.4	15.5	112.1	37.7	77.8	-26.2
C5a	3	0.36	0.23	0.65	8.4	0.0	20.9	23.5	45.2	-81.4
C7	3	3.55	0.93	0.26	128.2	56.8	225.8	36.1	59.0	+117.2
C7a	4	2.46	1.28	0.52	57.9	0.0	128.9	23.6	49.3	+17.6
C8	3	2.47	0.31	0.13	66.2	40.6	102.7	26.8	50.7	+30.7
C9	3	1.20	0.13	0.11	41.3	26.2	63.1	34.5	102.8	-59.8
E2	7	1.72	0.36	0.21	73.8	38.3	123.4	42.8	62.3	+18.5
E4	3	0.43	0.30	0.71	12.0	0.0	31.5	28.0	21.3	-43.8
Core total	55	2.22	0.16	0.07	1091.2	726.3	1637.0	491.8	1052.4	+3.7
BK	5	0.19	0.06	0.33	111.0	37.2	208.6	578.4	304.3	-63.5
Survey total	60	1.12	0.08	0.07	1202.1	788.8	1768.5	1070.2	1356.7	-11.4

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 2016–2021 surveys and for commercial-sized oysters from the 2019–2021 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 upper and lower 95% confidence intervals (95%CI) in millions of oysters that reflect the variability in the catches, and the percentage change in population size. Increases in population size are shaded green and decreases tan.

Bonamia survey area

2012	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	
Recruit	1.40	0.09	688.1	449.2	1046.7	
Pre-recruit	0.60	0.10	297.4	192.6	454.4	
Small	0.92	0.16	451.3	261.5	731.7	
2016	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2012–2016
Recruit	0.78	0.09	385.2	246.9	593.8	-44.0
Pre-recruit	0.25	0.03	120.5	186.7	491.8	-59.5
Small	0.52	0.07	256.1	155.0	407.3	-43.3
2017	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2016–2017
Recruit	0.74	0.11	363.6	233.9	559.1	-5.6
Pre-recruit	0.25	0.12	123.1	77.5	191.7	+2.2
Small	0.53	0.10	261.9	168.8	401.6	+2.3
2018	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2017–2018
Recruit	1.00	0.11	494.1	315.0	764.9	+35.9
Pre-recruit	0.36	0.11	178.4	113.5	276.5	+44.9
Small	0.82	0.13	401.8	249.2	631.2	+53.4
2019	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2018–2019
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	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 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	1052.4	644.4	1665.9	+76.6
2021	Mean density	CV	Pop.n	B.lower 95%CI	B.upper 95%CI	% change 2020–2021
Commercial	0.82	0.07	405.6	271.0	606.6	+28.3
Recruit	1.63	0.06	801.4	536.2	1196.7	+51.2
Pre-recruit	0.99	0.08	487.0	320.9	733.6	+83.6
Small	2.22	0.07	1091.2	726.3	1637.0	+3.7

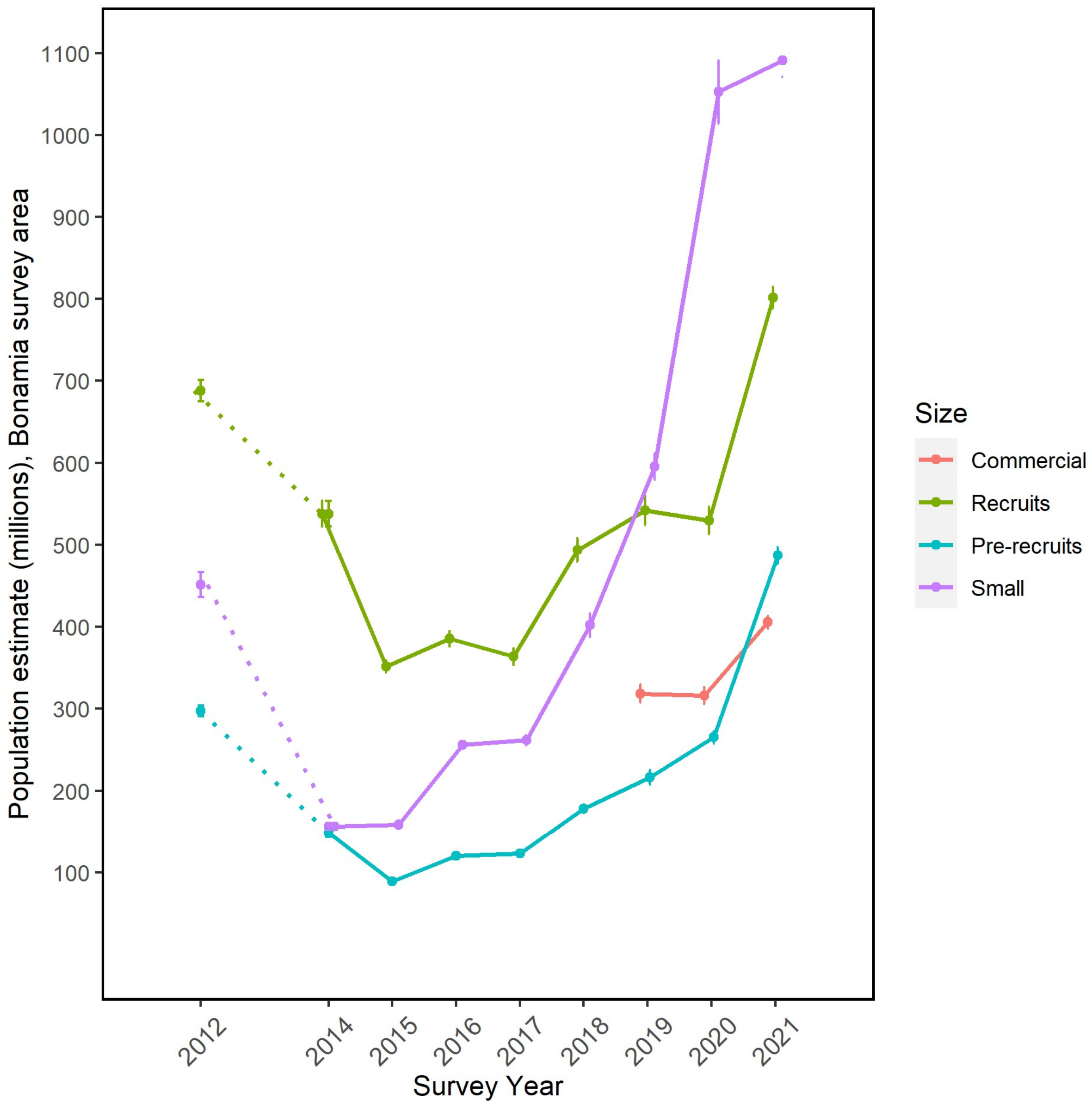

Figure 7: Mean population sizes, 95% confidence intervals from survey s.d. (solid lines) for commercial-sized, recruit-sized, pre-recruit, and small oysters in the Bonamia survey area between 2012 and 2021. The survey data for February 2013 are not comparable (sampling does not cover all the Bonamia 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) 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 2021 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 (CV) of the density estimate, the mean population size in millions of oysters 2021 (Pop.n), upper and lower 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 2020 mean population estimates (2020 Pop.n) and the 2021 mean population size represented as a percentage of the 2020 mean population size by stratum (% of 2020). The percentage change from the 2020 estimate is shaded green for increases in population size in 2021 and tan for decreases.

Stratum	Number stations	Mean density	Density s.d.	CV	2021 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2020 Pop.n	% of 2020
B1	5	0.50	0.50	1.00	39.4	0.0	126.5	78.2	25.9	+52.3
B3	3	2.36	0.14	0.06	105.3	70.5	154.9	44.7	18.1	+483.3
B6	7	1.51	0.73	0.48	45.4	2.1	97.6	30	0.0	-
C1a	3	0.82	0.82	1.00	25.7	0.0	82.4	31.3	23.4	+9.9
C2	3	0.00	0.00	0.00	0.0	0.0	0.0	21.9	0.0	-
C3	3	0.00	0.00	0.00	0.0	0.0	0.0	32.7	22.3	-100.0
C5	5	0.53	0.53	1.00	20.0	0.0	63.4	37.7	34.1	-41.2
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	3	0.00	0.00	0.00	0.0	0.0	0.0	36.1	0.0	-
C7a	4	1.48	0.86	0.58	34.9	0.0	81.6	23.6	0.0	-
C8	3	2.48	0.18	0.07	66.4	44.0	99.0	26.8	0.0	-
C9	3	3.33	0.51	0.15	114.8	68.1	180.7	34.5	57.4	+99.9
E2	7	1.58	0.61	0.39	67.4	15.7	134.3	42.8	58.5	+15.3
E4	3	0.00	0.00	0.00	0.0	0.0	0.0	28	0.0	-
Core total	55	1.06	0.14	0.13	519.3	321.9	801.4	491.8	239.6	+116.7
BK	5	0.00	0.00	0.00	0.0	0.0	0.0	578.4	0.0	-
Survey total	60	0.49	0.06	0.13	519.3	321.9	801.4	1070.2	239.6	+116.7

3.2.3 Changes in the distribution of live oysters

The February 2021 survey sampled 60 first- and second-phase random stations generated with a 0.75 nautical mile exclusion zone that spread sampling effort and 12 fixed stations. All 72 stations were used to describe oyster distribution. Sampling effort was focused in core strata with background strata receiving only 5 stations for 51.4% of the survey area. The sampling was therefore insufficient to provide a consistent or complete coverage of the fishery area in 2021, and hence the survey is not likely to have estimated the distributions of oyster density well for live commercial-sized, recruit, pre-recruit, and small oysters outside the core strata (delimited by a blue line in Figures 8–12). These distributions of oysters are compared with the last Bonamia survey in 2020 which also sampled 72 stations in total, mostly in the commercial fishery area.

The distributions of oyster densities of all sizes are widespread, covering most of the fishery area with the highest densities in core fishery strata, 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 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 more widespread in 2021 than in 2020, especially in strata C3 and B6 in the eastern fishery area (Figure 10). The increasing patches of relatively high recruit-sized oyster densities are 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 are patchily distributed. The numbers and sizes of relatively high-density patches have increased and were more widespread in 2021 than in 2020, especially in strata C3 and B6 in the eastern fishery area (Figure 11), similar to the distribution of recruits (see Figure 10). Densities are still relatively low in some central fishery areas.

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

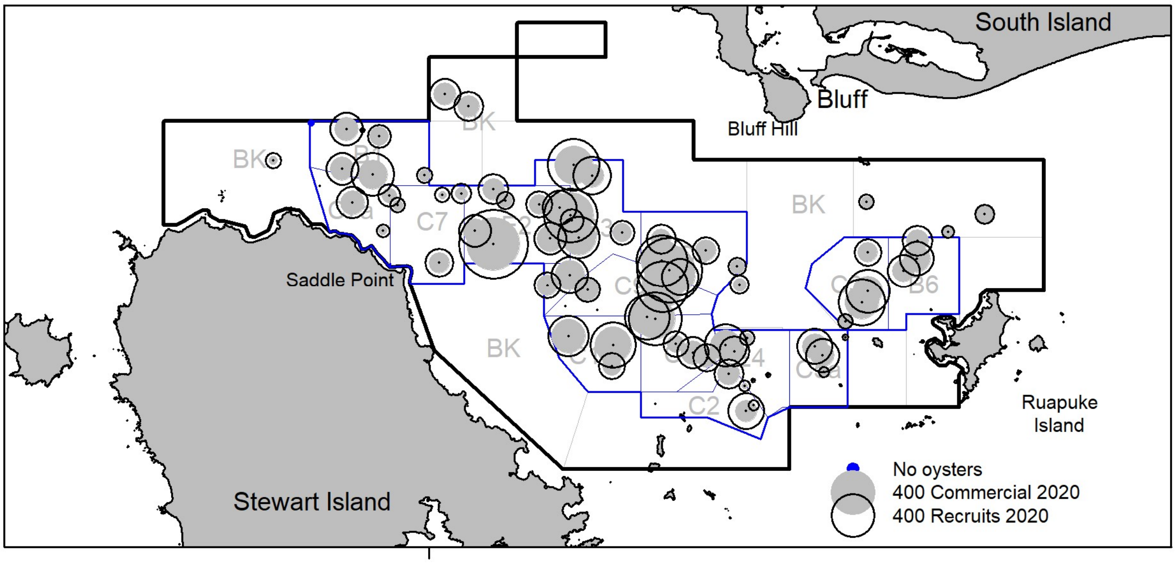

Figure 8: Density (numbers of oysters per standard tow representing an area swept of 1240 m²) of commercial-sized (filled grey circles) and recruit-sized (black circles) oysters sampled during the February 2020 survey. Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

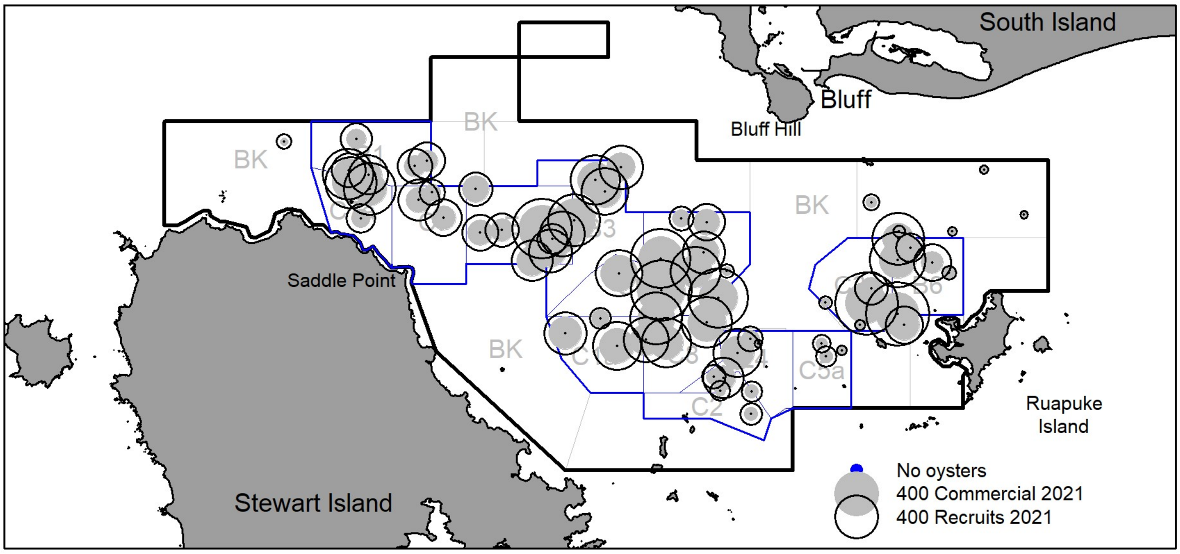

Figure 9: Density (numbers of oysters per standard tow representing an area swept of 1240 m^2) of commercial-sized (filled grey circles) and recruit-sized (black circles) oysters sampled during the February 2021 survey. Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

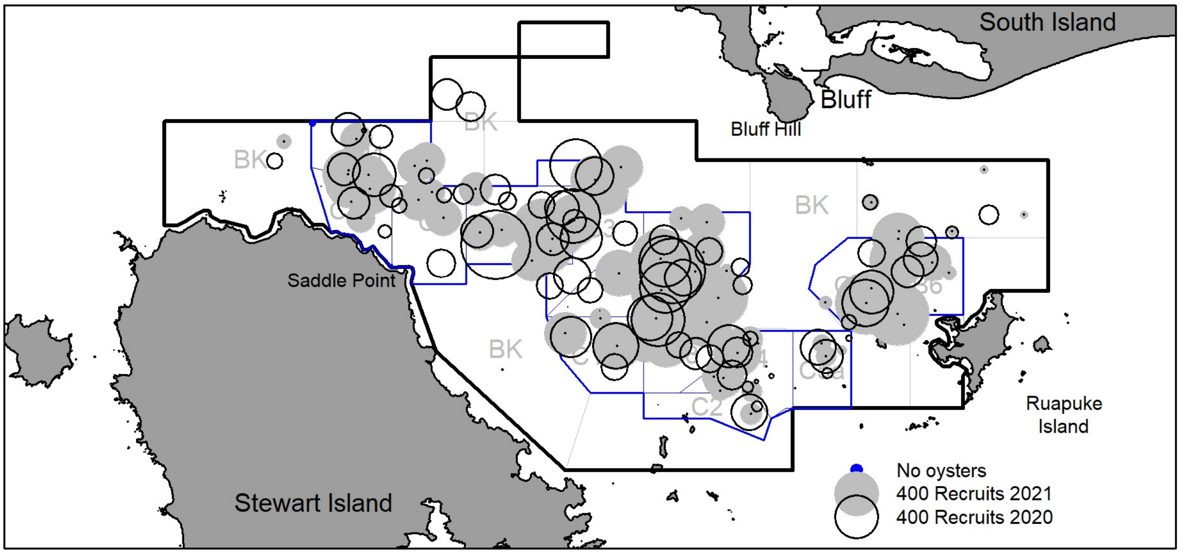

Figure 10: Density (numbers of oysters per standard tow representing an area swept of 1240 m^2) of recruit-sized oysters sampled during the February surveys in 2021 (filled grey circles) and in 2020 (black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

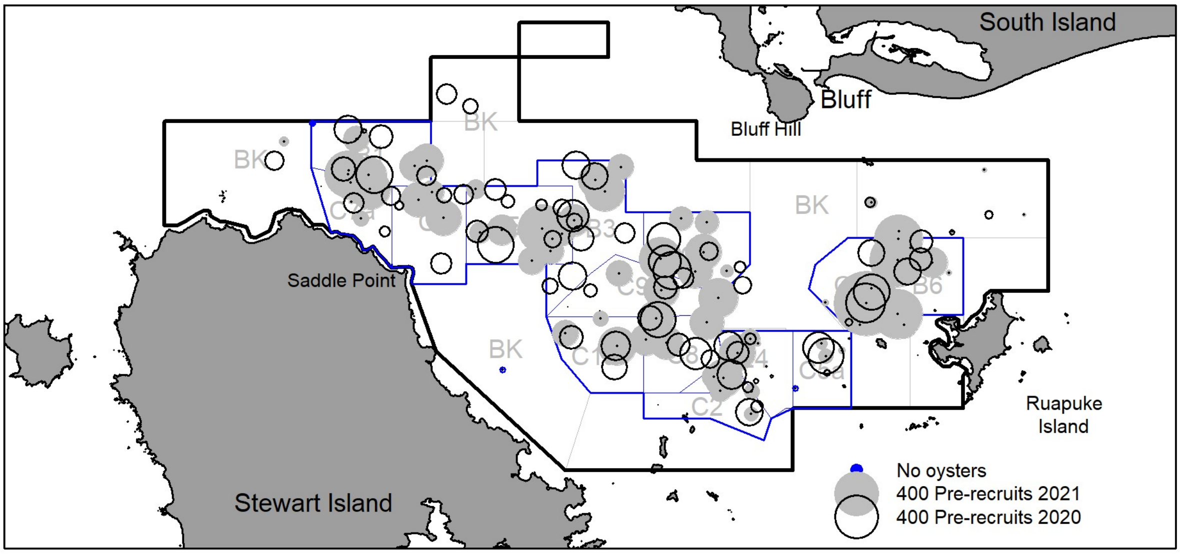

Figure 11: Density (numbers of oysters per standard tow representing an area swept of 1240 m^2) of pre-recruit oysters sampled during the February surveys in 2021 (filled grey circles) and in 2020 (black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

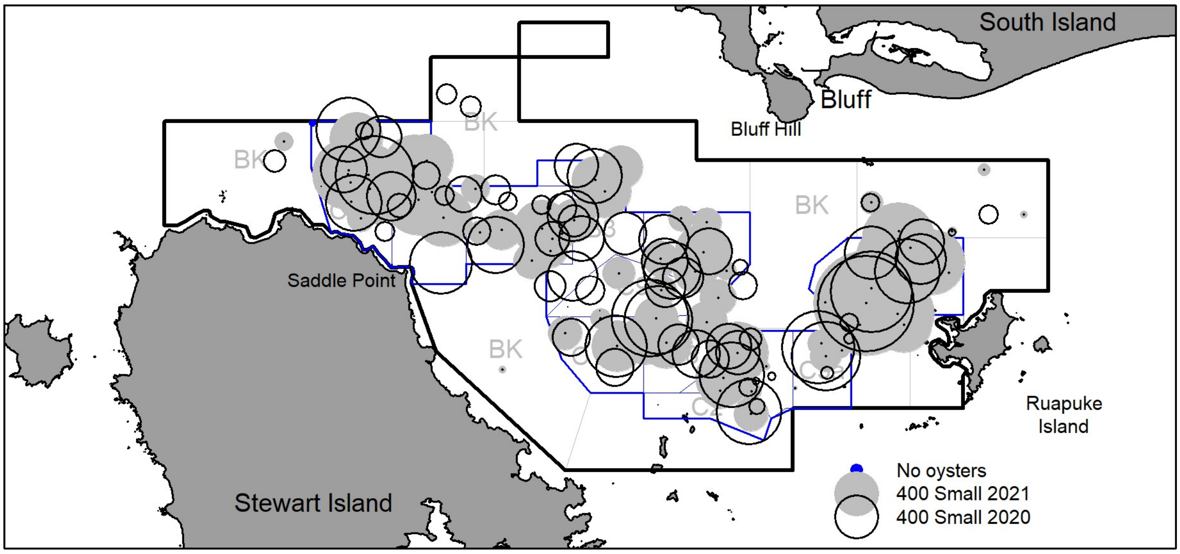

Figure 12: Density (numbers of oysters per standard tow representing an area swept of 1240 m^2) of small oysters sampled during the February surveys in 2021 (filled grey circles) and in 2020 (black circles). Blue filled circles denote no oysters caught. The Bonamia survey area is shown by the blue lines.

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*, a 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 shows large fluctuations in a broadly cyclic trend between 1993 and 2021 (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-size oyster population was increasing. The numbers of spat per recruit increased substantially between 2016 and 2020 (Figure 13). In 2021, the numbers of spat have remained similarly high; however, the numbers of recruits have increased markedly, resulting in a downward 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).

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. The total numbers of spat per collector sampled over the summers of 2005–06 to 2020–21 are shown in Figure 14. Data are 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).

3.4 Status of Bonamia infection and mortality

3.4.1 Estimates of oyster mortality before and during the February 2021 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 and pre-recruit-sized oysters between 2018 and 2021, are given in Table 10. There were few gapers observed during the February 2021 survey. Over all stations sampled, those with recruit-sized gapers represented 34% (21 stations) in 2021, 22% (16 stations) in 2020, and 10.9% in 2019. Pre-recruit gapers accounted for 13% (8 stations) in 2021, 12.5% (9 stations) in 2020, and a single station (1.4%) in 2019 (see Appendix 6 for a list of survey reports).

Counts of recruit-sized new clocks ranged from 1 to 16, and for pre-recruits 1 to 17; however, the percentages of new clocks were low, a maximum of 0.04% and 0.08% for recruit and pre-recruit size groups, respectively. These low percentages suggest that pre-survey mortality has remained low.

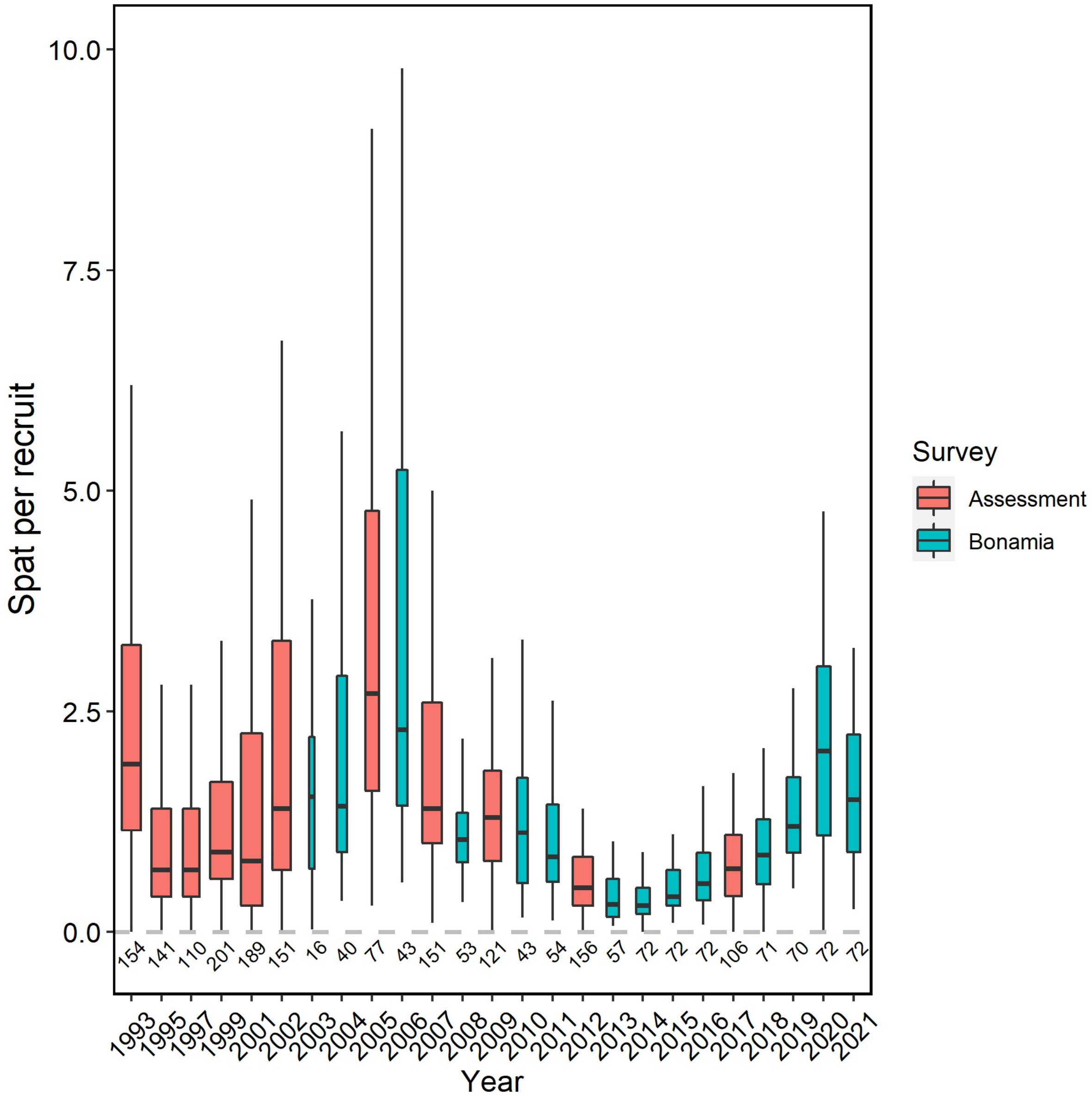

Figure 13: The numbers of small oysters per recruited oyster sampled between 1993 and 2021 stock assessment surveys (Assessment), and Bonamia surveys (Bonamia). Medians are shown as solid lines, boxes represent 50th percentiles (25–75%), and whiskers 90th percentiles (5–95%). Outliers smaller than 5% 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).

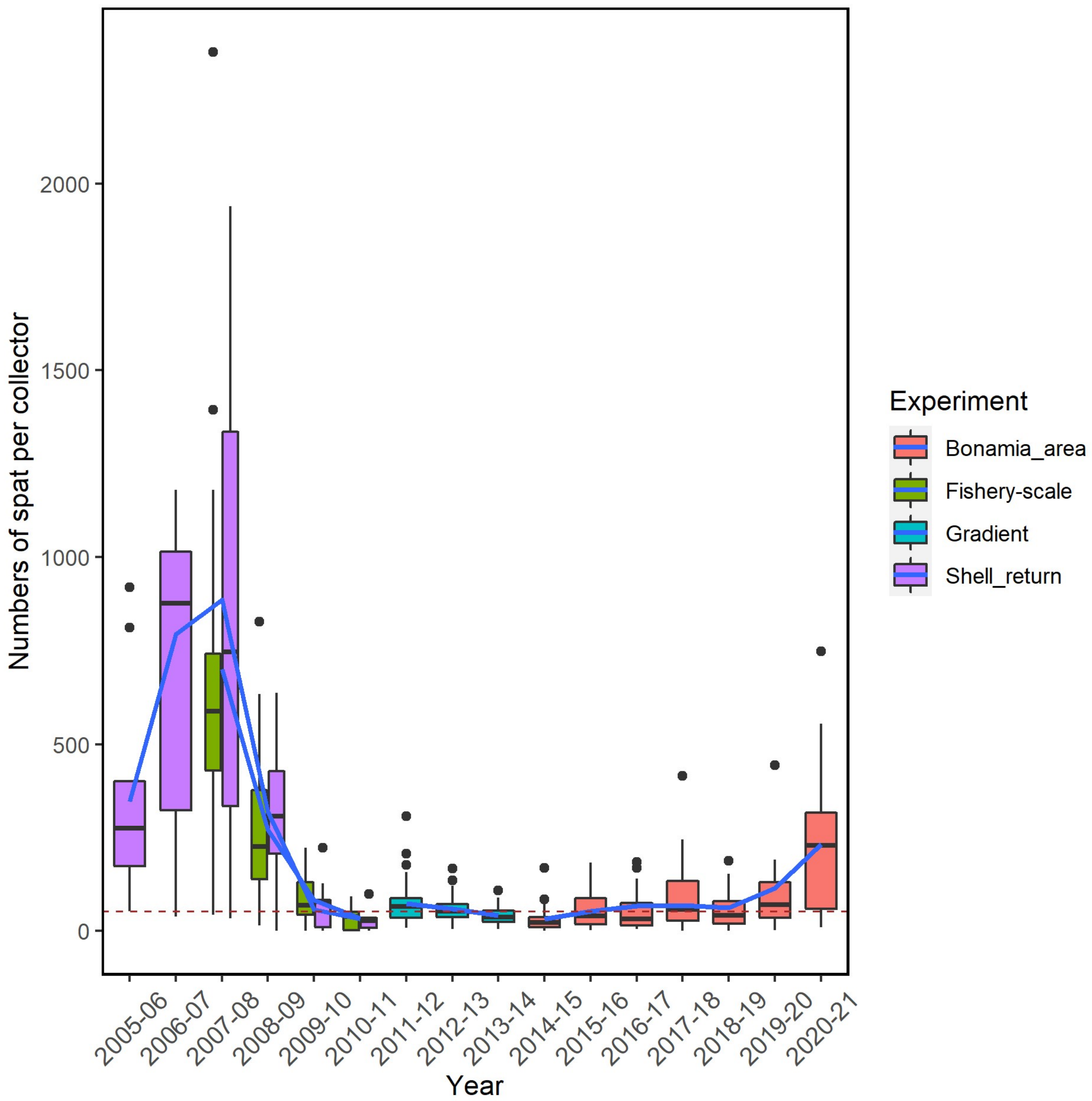

Figure 14: The total numbers of spat per collector sampled over the summers of 2005–06 to 2020–21. Spat settlement shows the success of spawning and indicates the levels of replenishment to the oyster population. Data represent four different experiments and different areas: the shell return site (south of Bluff Hill) and 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 in 2014–15.

Table 10: The number of stations with more than 50 live recruit and pre-recruit-sized oysters combined for surveys 2018–2021 (No. stations), and descriptive statistics for the percentages of new clocks and gapers combined for recruit and pre-recruit size groups. Percentages are new clocks and gapers of new clocks, gapers, and live oysters combined. The number of stations with no new clocks and gapers (No. zero stations) and percentage by year and size (% zero stations).

Percentage new clocks and gapers

	Recruit-sized				Pre-recruits			
Year	2018	2019	2020	2021	2018	2019	2020	2021
No. stations	55	57	62	57	40	40	53	54
Median	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.09	0.04	0.04	0.03	0.03	0.05	0.10	0.02
5th percentile	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
95th percentile	0.04	0.03	0.03	0.02	0.03	0.02	0.04	0.02
No. zero stations	23	11	19	9	33	21	34	24
% zero stations	41.8	19.3	30.6	15.8	82.5	52.5	64.2	44.4

The numbers of recruit-sized new clocks sampled in survey tows between 2018 and 2021 are compared in Figure 15. Recruit-sized new clock densities have generally remained low and consistent within strata; however, in 2021, there have been noticeably higher counts in strata B6, C2, C9, and E2 (Figure 15).

The distribution of pre-survey mortality of recruit-sized oysters has remained low, widespread, and locally variable between 2019 and 2021 (Figures 16 and 17), and new clock densities were higher at stations where recruit-sized oyster densities were higher, i.e., in strata designated as commercial (C2, C2, C3, C5, C9, and E2).

The population size of recruit-sized new clocks in core strata was 57.6% higher in 2021 (5.5 million new clocks) than in 2020 (3.5 million) (Table 11), and similar to the increase in live, recruit sized oysters (see Table 8). All but four 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 was 288.7% higher in 2021 (2.0 million new clocks) than in 2020 (0.5 million, Table 12). Pre-recruit oysters increased substantially over the same period. Pre-recruit new clocks increased in half the core strata and the other half remained similar (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 2021 (0.7%), as it was between 2016 and 2020, ranging between 0.4% and 1.4% (Table 13). It was higher in 2012, 2014, and 2015, ranging between 3.2% and 6.8%. Pre-survey mortality of recruit-sized oysters in the 2007 stock assessment survey area was similar to that in core strata, 0.6% in 2021, ranging between 0.4% and 1.5% between 2016 and 2020, and between 3.2% and 7.6% in 2012, 2014, and 2015 (Table 13).

Pre-survey mortality of pre-recruit oysters in core strata remained low in 2021 (0.4%) and similar to that between 2016 and 2020 (0.2% and 0.7%, Table 13). Pre-survey mortality of pre-recruit oysters was higher in 2012, 2014, and 2015 ranging between 2.4% and 2.9%. In the stock assessment survey area, pre-survey mortality of pre-recruit oysters was 0.5% in 2021, similar to that between 2016 and 2020 (0% and 0.8%, Table 13) and lower than pre-survey mortality in 2012, 2014, and 2015 that ranged between 2.3% and 3.6% (Table 13).

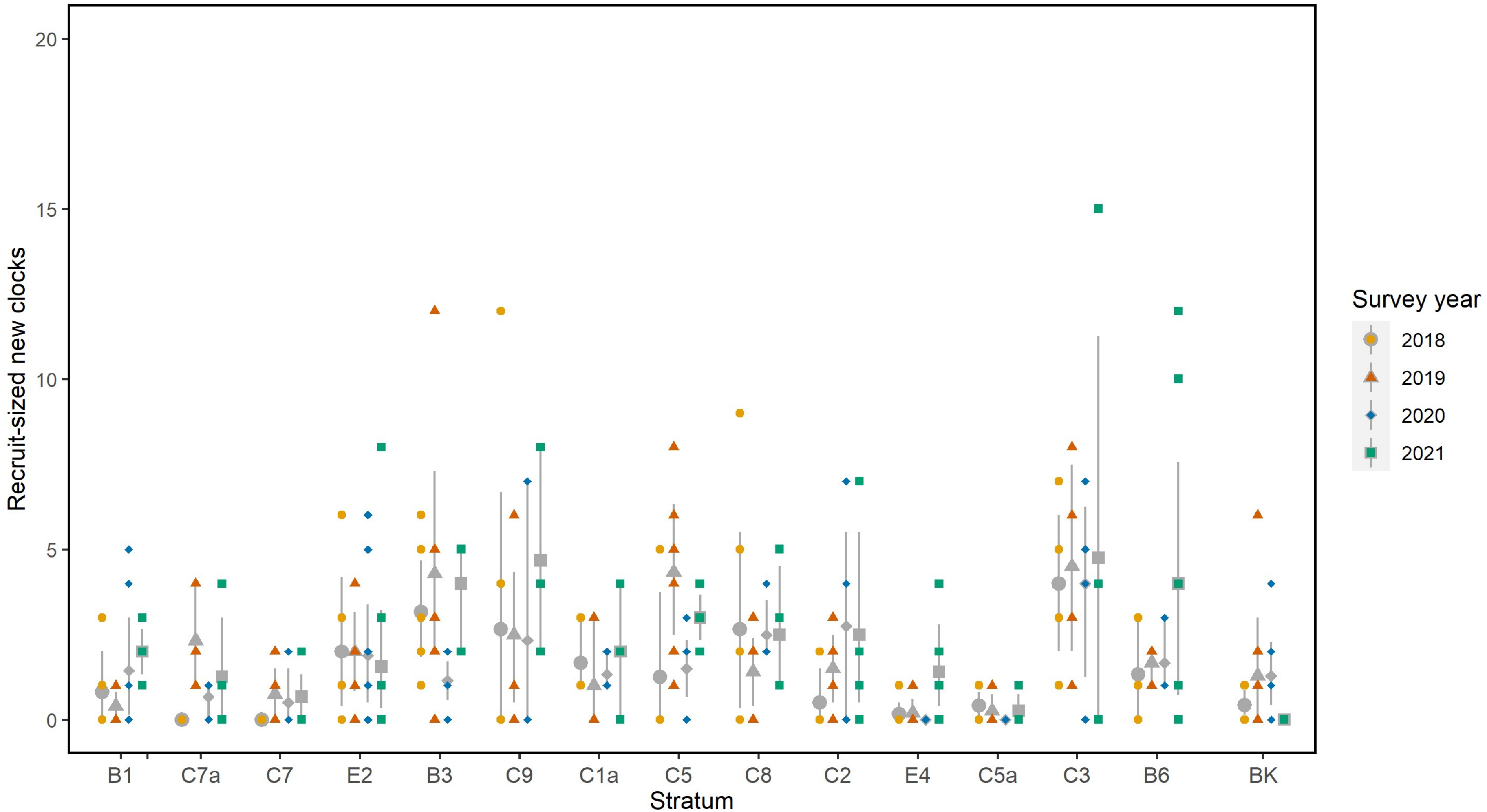

Figure 15: The numbers of recruit-sized new clocks (≥ 58 mm in diameter) per tow, means (grey symbols matching shape showing survey year) and 95% confidence intervals (grey lines) by stratum, for surveys 2018–21. Tow numbers are adjusted to a standard tow length of 0.2 nautical miles. Numbers from the 2018 survey are shown as tan filled circles, 2019 as red filled triangles, 2020 as blue filled diamonds, and 2021 as light green filled squares. Bonamia survey strata are arranged west to east with northern strata at similar longitudes shown first and the background stratum (BK) furthermost right.

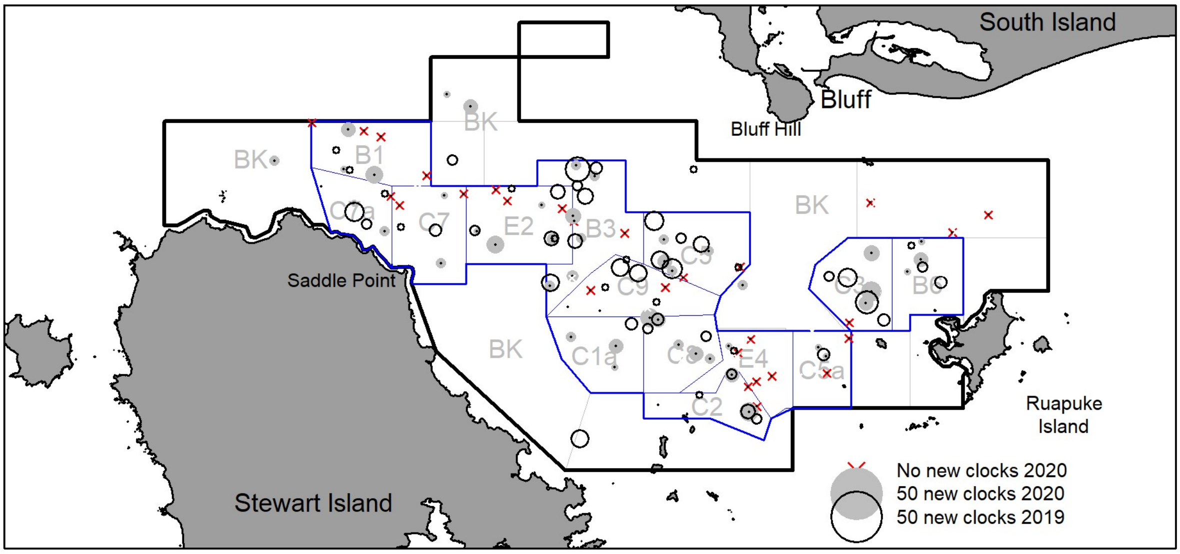

Figure 16: The distribution of recruit-sized new clocks and gaper densities combined in 2020 and 2019 (2020, filled grey circles and 2019, black circles) showing the pre-survey mortality in February 2020 and 2019. Stations with no recruit-sized new clocks and gapers are shown as red crosses.

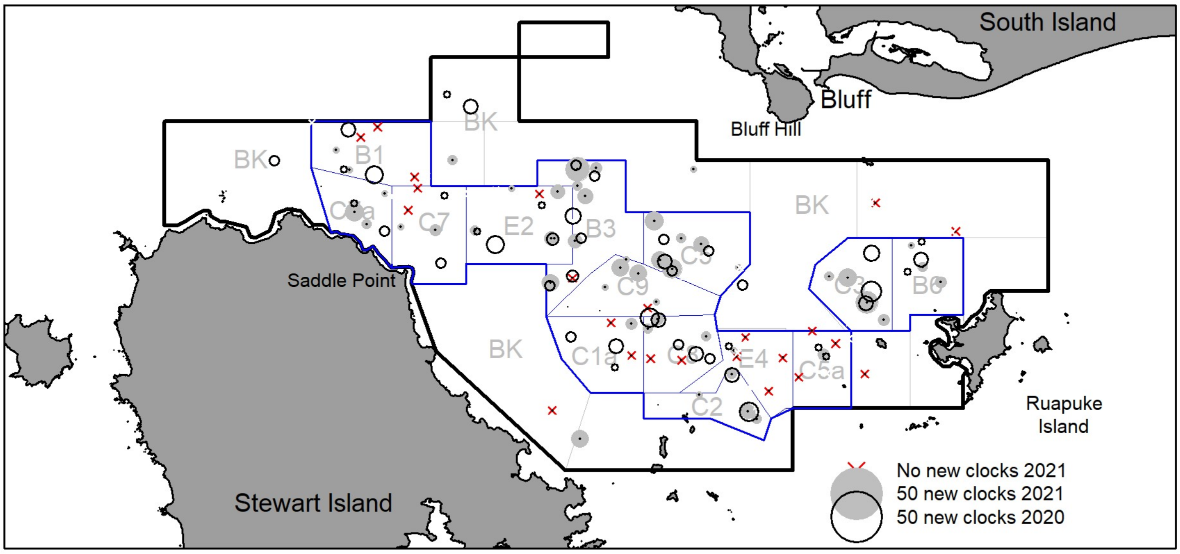

Figure 17: The distribution of recruit-sized new clocks and gaper densities combined in 2021 and 2020 (2021, filled grey circles and 2020, black circles) showing the pre-survey mortality in February 2021 and 2020. Stations with no recruit-sized new clocks and gapers are shown as red crosses.

Table 11: Absolute population estimates for recruit-sized (≥ 58 mm in diameter) new clocks in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2021 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 (CV) of the density estimate, the mean population size in millions of oysters 2021 (Pop.n), upper and lower 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 2020 mean population estimates (2020 Pop.n) and the 2021 mean population size represented as a percentage of the 2020 mean population size by stratum (% of 2020). The percentage change from the 2020 estimate is shaded green for decrease in population size in 2021 and tan for increases.

Stratum	Number stations	Mean density	Density s.d.	CV	2021 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2020 Pop.n	% of 2020
B1	5	0.01	0.00	0.22	0.7	0.4	1.2	78.2	0.6	+27.2
B3	3	0.02	0.00	0.25	0.9	0.4	1.5	44.7	0.2	+257.1
B6	7	0.02	0.01	0.47	0.6	0.0	1.3	30.0	0.2	+146.9
C1a	3	0.01	0.01	0.57	0.3	0.0	0.7	31.3	0.2	+51.3
C2	3	0.01	0.01	0.82	0.3	0.0	0.8	21.9	0.1	+102.6
C3	3	0.01	0.01	1.00	0.2	0.0	0.7	32.7	0.6	-65.7
C5	5	0.02	0.00	0.12	0.6	0.4	0.9	37.7	0.2	+169.9
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	3	<0.01	<0.01	1.00	0.1	0.0	0.4	36.1	0.1	+27.9
C7a	4	0.01	0.00	0.77	0.2	0.0	0.4	23.6	0.1	+99.9
C8	3	0.01	0.00	0.41	0.2	0.0	0.4	26.8	0.3	-16.3
C9	3	0.02	0.01	0.37	0.8	0.2	1.5	34.5	0.4	+96.7
E2	7	0.01	0.01	0.67	0.4	0.0	0.9	42.8	0.4	-10.1
E4	3	0.01	0.01	0.57	0.3	0.0	0.7	28.0	0.0	-
Core total	55	0.01	0.00	0.13	5.5	3.4	8.6	491.8	3.5	+57.6
BK	5	0.00	0.00	0.00	0.0	0.0	0.0	578.4	5.0	-100.0
Survey total	60	0.01		0.13	5.5	3.4	8.6	1070.2	8.5	-35.4

Table 12: Absolute population estimates for pre-recruit (50–57 mm in diameter) new clocks in the core strata (Stratum), background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total) sampled in 2021 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 (CV) of the density estimate, the mean population size in millions of oysters 2021 (Pop.n), upper and lower 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 2020 mean population estimates (2020 Pop.n) and the 2021 mean population size represented as a percentage of the 2020 mean population size by stratum (% of 2020). The percentage change from the 2020 estimate is shaded green for decrease in population size in 2021 and tan for increases.

Stratum	Number stations	Mean density	Density s.d.	CV	2021 Pop.n	B.lower 95%CI	B.upper 95%CI	Area (km^2)	2020 Pop.n	% of 2020
B1	5	<0.01	<0.01	0.75	0.3	0.0	0.8	78.2	0.3	+17.0
B3	3	<0.01	<0.01	0.58	0.2	0.0	0.5	44.7	0.0	+609.7
B6	7	<0.01	<0.01	0.73	0.1	0.0	0.4	30.0	0.1	+52.6
C1a	3	<0.01	<0.01	0.50	0.1	0.0	0.2	31.3	0.1	+98.9
C2	3	0.01	<0.01	0.66	0.1	0.0	0.4	21.9	0.0	-
C3	3	<0.01	<0.01	1.00	0.1	0.0	0.2	32.7	0.0	-
C5	5	0.01	<0.01	0.41	0.2	0.0	0.5	37.7	0.0	+510.5
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	0.0	-
C7	3	<0.01	<0.01	0.58	0.2	0.0	0.4	36.1	0.0	+284.1
C7a	4	<0.01	<0.01	1.00	0.0	0.0	0.1	23.6	0.0	-
C8	3	0.01	<0.01	0.50	0.2	0.0	0.4	26.8	0.0	-
C9	3	<0.01	<0.01	1.00	0.2	0.0	0.5	34.5	0.0	-
E2	7	<0.01	<0.01	0.53	0.1	0.0	0.3	42.8	0.0	-
E4	3	<0.01	<0.01	0.58	0.1	0.0	0.3	28.0	0.0	-
Core total	55	<0.01	<0.01	0.20	2.0	1.1	3.3	491.8	0.5	+288.7
BK	5	<0.01	<0.01	1.00	0.6	0.0	1.8	578.4	4.5	-87.5
Survey total	60	<0.01	<0.01	0.27	2.6	1.1	4.6	1070.2	5.0	-49.0

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, 2014–2021 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

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

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 and pre-recruit sized oysters were collected from all but ten stations in 2021; this included 62 stations of recruits only and 2 stations of recruits and pre-recruits. In all, 1747 heart imprint slides were sampled and archived. This sample comprised 1669 recruit-sized oysters (95.5% of oysters sampled), 30 pre-recruits, and 48 small oysters. In previous years, a similarly high proportion of recruit-sized oysters were sampled. Only a subsample of these samples was screened (*n*= 304). Stations with fewer than 15 recruit and pre-recruit sized oysters (station 117, *n*= 6; station 49, *n*= 4; station 148, *n*= 3; and station 102, *n*= 1) were not used in the analysis of infection.

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

3.4.2.2 ddPCR detection of Bonamia in oyster heart tissues

A summary of ddPCR samples tested and the corresponding heart imprint slides examined in 2021 is shown in Table 14. Of the 1747 slides taken from random stations with more than 15 recruit and pre-recruit sized oysters in 2021, a subset of 304 heart imprint slides were examined for Bonamia infection.

The remaining 1443 slides were from oysters screened using ddPCR and were not infected. In 2021, 97.3% of oysters had no detectable infection using histology, similar to 2020 and 2019 (97.0% in both years), but higher (less infection) than in previous years (see Table A6.1 in Appendix 6 for references). The ddPCR, as expected, showed high sensitivity in the detection of low-level infections. Unscaled prevalence of Bonamia by ddPCR was 75% higher (4.8%) than for heart imprints (2.7%).

Table 14: The numbers of oyster heart tissue samples screened for Bonamia using ddPCR and heart imprints in 2021. The total numbers of samples tested (Sample (N)), the numbers of samples that tested negative (ddPCR-) and 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	1 747	1 663	84
Heart imprints			
Slides read (N)	304		
Heart imprint -ve	256		
Heart imprint +ve	48		

3.4.2.3 Prevalence and intensity of infection from 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.

Heart imprints underestimate the true prevalence of Bonamia infection and are lower than ddPCR estimates (Table 15). The mean prevalence from heart imprints in 2021 (3.0%) was lower than in 2020 (3.2%) and higher than in 2019 (1.9%), but lower than in older surveys (2009–2017, 4.3–15.3%, referenced in Appendix 6). The ddPCR analysis of heart tissues is more sensitive than heart imprints. Mean prevalence from ddPCR in 2021 (4.8%) was lower than in 2020 (5.7%) and in 2019 (7.4%); 60% higher than from heart imprints in 2021. Differences in the prevalence of infection between ddPCR and heart imprints are in part determined by the intensity of infection.

Table 15: Comparisons of infection levels (prevalence (Prev %) and intensity (Inten)) between 2019, 2020, and 2021 in survey tows. Number of samples for each method (N), mean and median prevalence and intensity estimated by heart imprints (Hist.), and prevalence from ddPCR, and standard deviation (s.d.) and 5% and 95% percentiles (5% and 95%) are reported. Data are from random stations sampled for Bonamia with more than 15 recruit and pre-recruit oysters in the sample.

	2019			2020			2021		
	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)	Hist. Prev (%)	Hist. Inten	ddPCR Prev (%)
N	40	22	55	55	27	55	53	23	53
Mean	1.9	3.4	7.4	3.2	3.0	5.7	3.0	3.4	4.8
Median	0	3.3	0	0	3.0	4.0	0	3.4	4.0
s.d.	3.0	1.0	9.7	3.9	1.0	5.7	4.2	1.0	5.7
5%	0	2.0	0	0	1.0	0	0	1.6	0.0
95%	8.0	5.0	20.0	12.0	5.0	17.2	12.0	5.0	16.0

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 2.7% of oysters with detectable infections in 2021, mean light (category 1 and 2) infections was 0.7% (0.1–5% in 2010–2020), and 2.0% had category 3 and 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 2021 and was the lowest since 2007 (Figure 18). During periods of relatively high prevalence (2012–2015), qPCR showed higher prevalence than heart imprints, but levels were similar at relatively low prevalence. Prevalence from ddPCR increased from 2018 to 2019 and then decreased in 2020 and remained similar in 2021; and was higher than for heart imprints reflecting the increased sensitivity of PCR methods.

Boxplots (Figure 19) show the mean intensity of infection for each tow, each year for 2007 to 2021. The proportion of infected oysters with non-fatal infections (less than category 3, Diggles et al. 2003) in 2021 was similar to that in 2020. The proportion of infected oysters with fatal (category 3 and higher) infections in 2020 and in 2021 decreased markedly from 2017–2019. The median intensity of infection in 2021 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 may reflect this variation.

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 2019 and 2021 is widespread, but spatially patchy over multiple scales (Figures 20–22). Sites with Bonamia infection were interspersed with sites with no Bonamia infection across the fishery area in 2019, 2020, and 2021(shown as red crosses, Figures 20–22). In all years, ddPCR detected higher numbers of infected oysters than were detected by heart imprints, and ddPCR detected infection at some sites, presumably low intensity infections, where there was no infection detected by heart imprints.

Infection was widespread, it was very patchy in 2019 (Figure 20) and infected stations were interspersed with stations with no detectable infection. In 2019, there was greater variation in the prevalence estimated by heart imprints and ddPCR, possibly because of an increase in low level infections not easily detected by heart imprints (Figure 20). Prevalence followed a similar pattern in 2020 (Figure 21); however, prevalence was lower and there was less variation between heart imprints and ddPCR at some stations. The distribution of infection remained similar in 2021; however, the prevalence estimated from both ddPCR and heart imprints increased at infected sites, suggesting an increase in both prevalence and intensity of infection at localised sites (Figure 22).

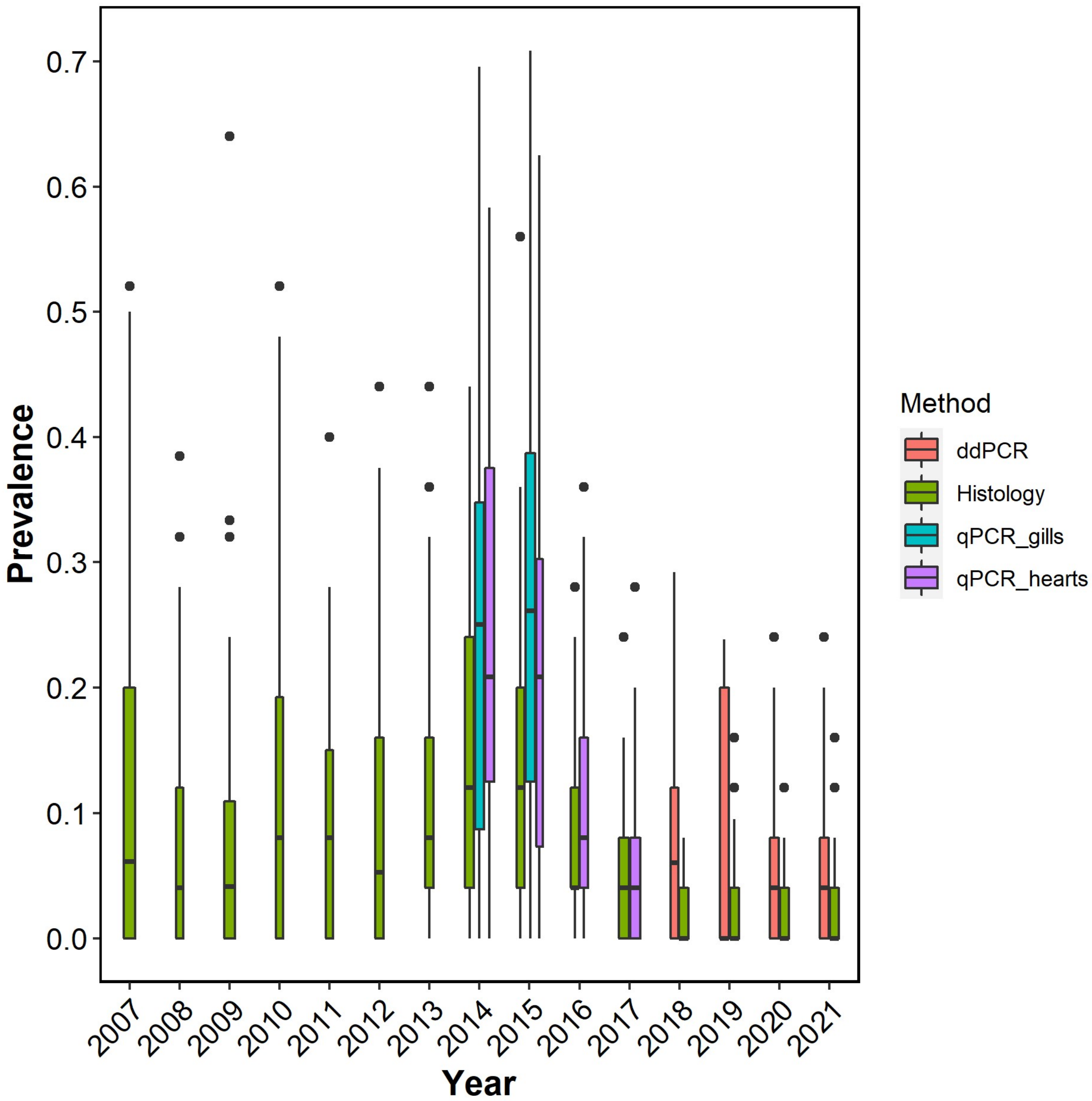

Figure 18: Boxplots of the median prevalence of Bonamia infection 2007–2021. The median prevalence of infection at all stations determined from histology (heart imprints) 2007–2021, and for qPCR heart tissues (qPCR_hearts) 2014-2017, and gill tissues (qPCR_gills) in 2014 and 2015. Heart tissues were only tested with ddPCR since 2018. Medians shown as solid lines, boxes represent 50th percentiles , whiskers 95th percentiles, and outliers as filled black circles.

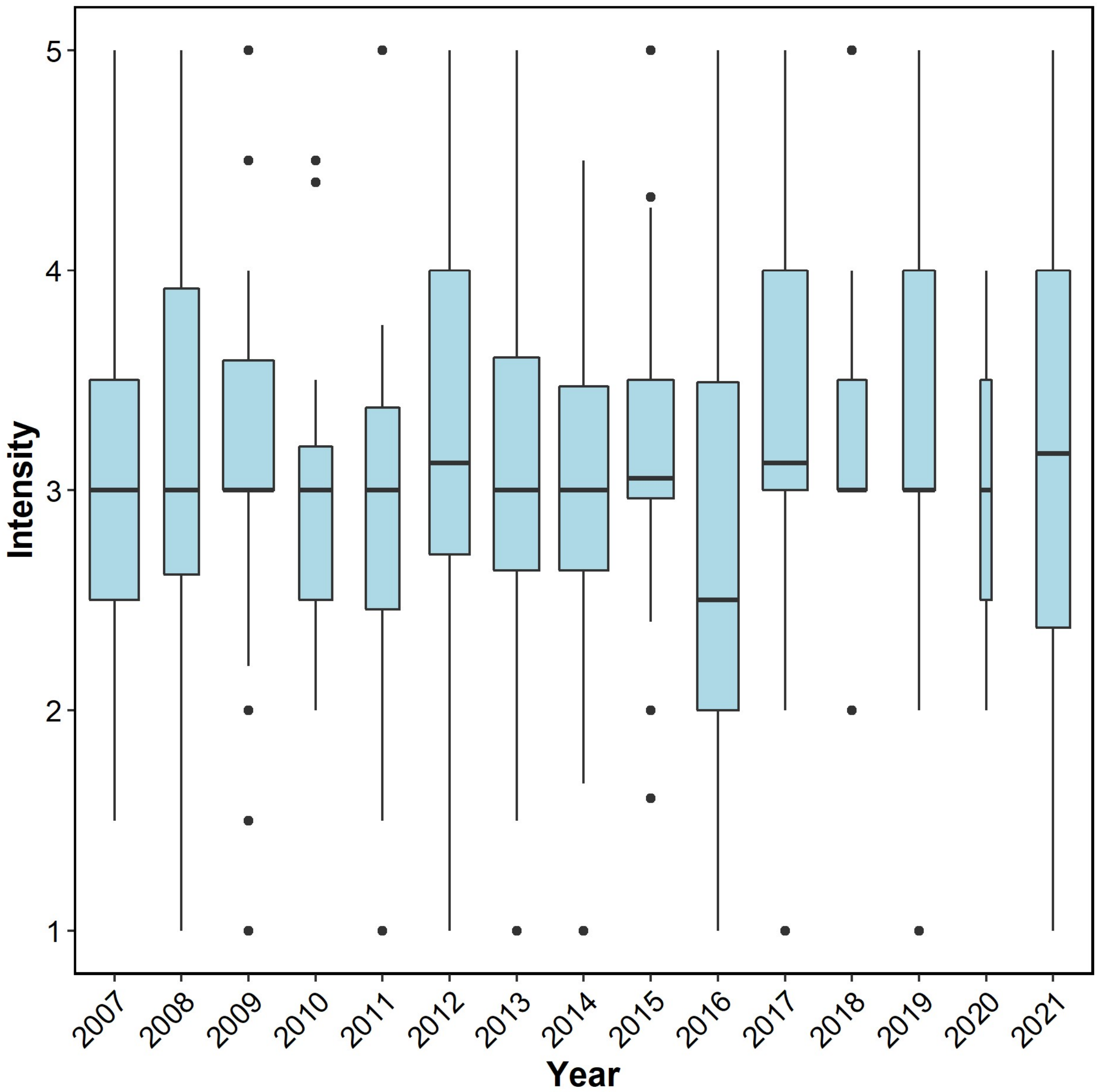

Figure 19: Boxplots of the mean intensity of Bonamia infection by tow, 2007–2021. The mean intensity of infection at all stations was determined from histology. Medians are shown as solid lines, boxes represent 50th percentiles, whiskers 95th percentiles, and outliers are shown as filled black circles. The width of boxes scaled by the number of stations with Bonamia infection.

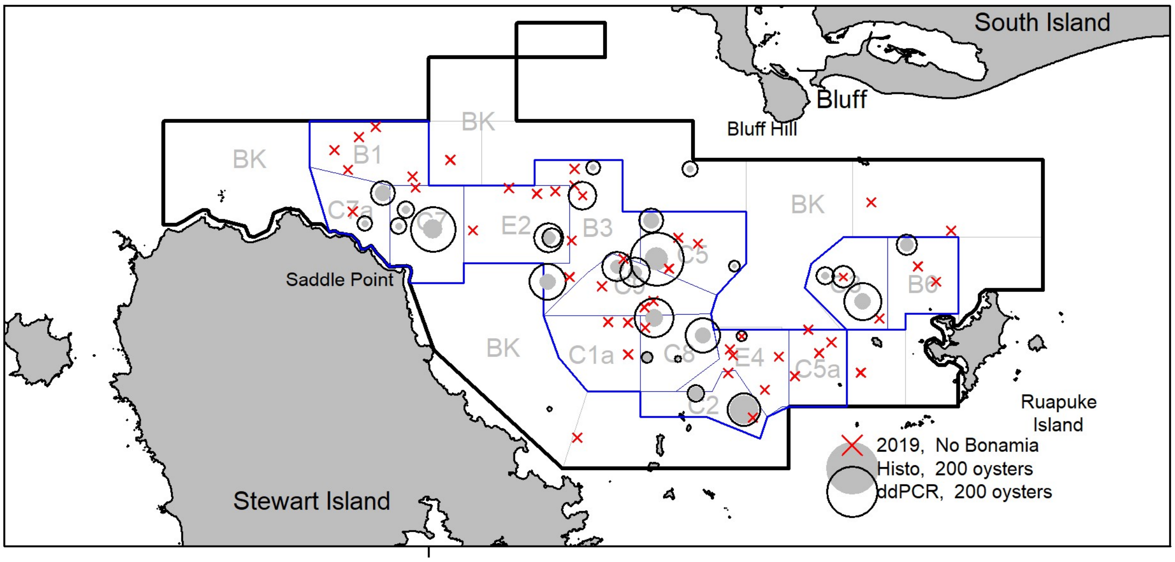

Figure 20: The distributions of Bonamia infection in February 2019 estimated from heart imprints and ddPCR analysis of heart tissues only: numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and ddPCR heart tissues (ddPCR, black circles) and stations with no Bonamia (red crosses). Black circles with red crosses only, 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.

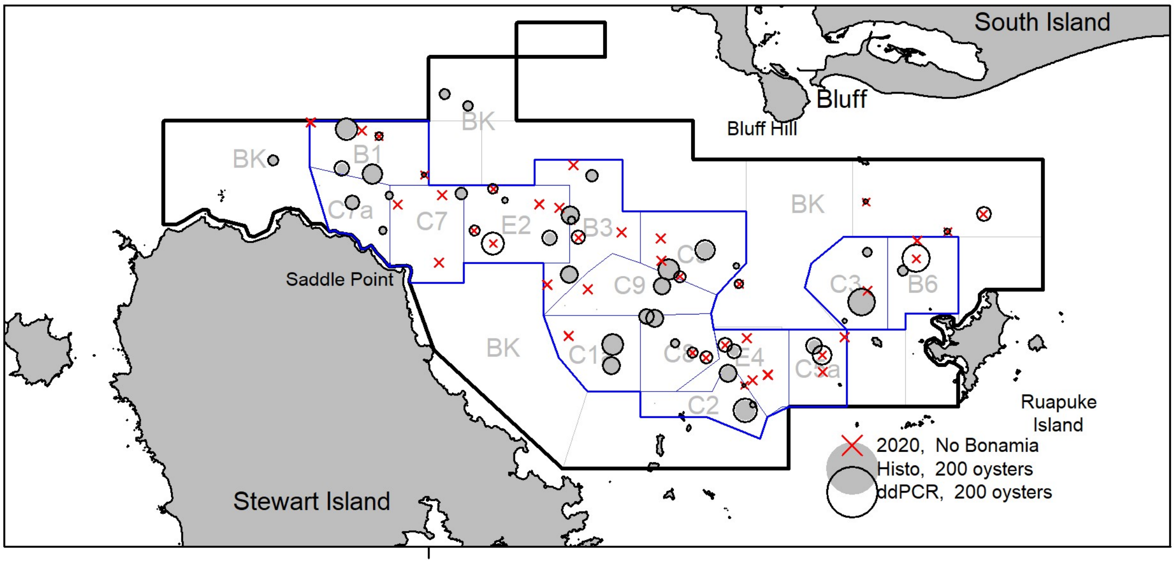

Figure 21: The distributions of Bonamia infection in February 2020 estimated from heart imprints and ddPCR analysis of heart tissues only: numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and ddPCR heart tissues (ddPCR, black circles) and stations with no Bonamia (red crosses). Black circles with red crosses only, 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.

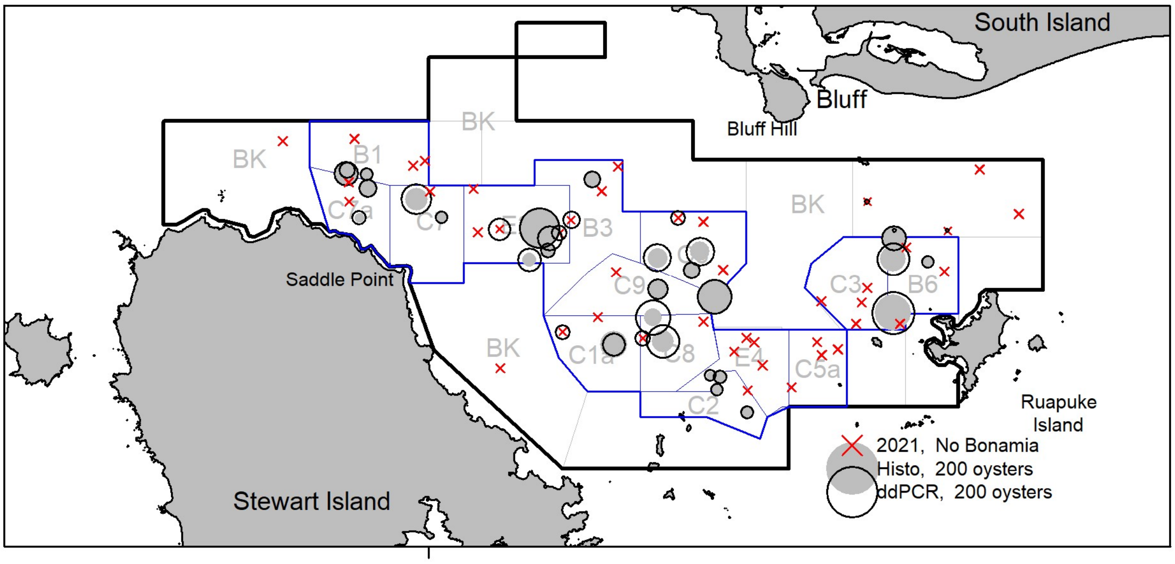

Figure 22: The distributions of Bonamia infection in February 2021 estimated from heart imprints and ddPCR analysis of heart tissues only: numbers of oysters with Bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles) and ddPCR heart tissues (ddPCR, black circles) and stations with no Bonamia (red crosses). Black circles with red crosses only, 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 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 (Figure 23). Non-fatal infection remained relatively low in 2019 (Figure 23). In 2020 fatal infection remained widespread and patchy, with densities of fatal infections similar to in 2019 (Figure 24). In 2021, fatal infection became more widespread and increased slightly compared to previous years (Figure 25).

Patterns in the distribution of prevalence and intensity of infection between 2012 and 2021 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.

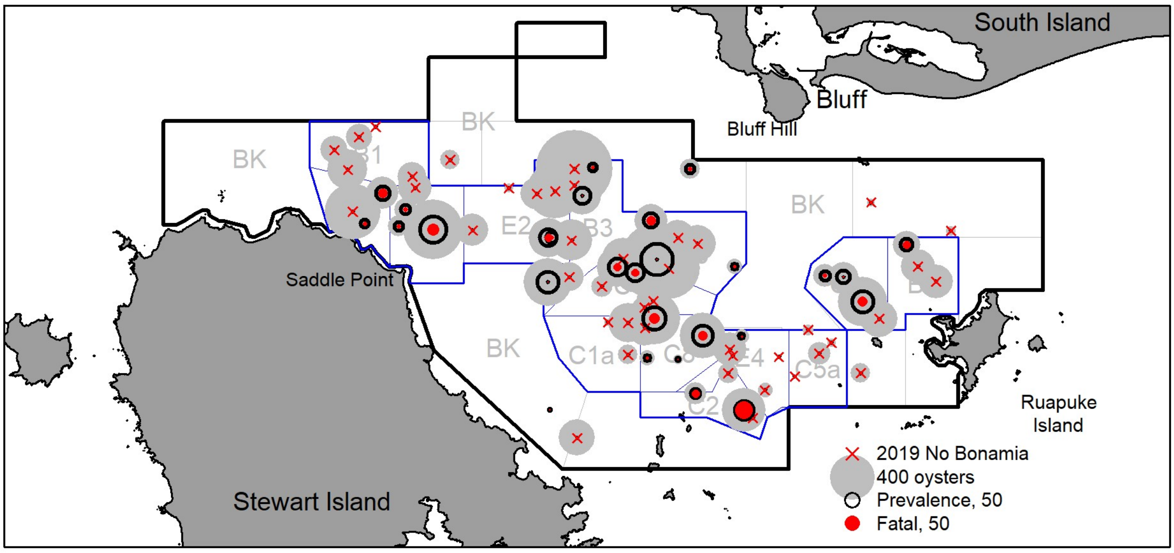

Figure 23: The distributions of recruit-sized oysters and Bonamia infection in February 2019: numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, black circles), fatal infections (intensity categories 3–5 combined, filled red circles), 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.

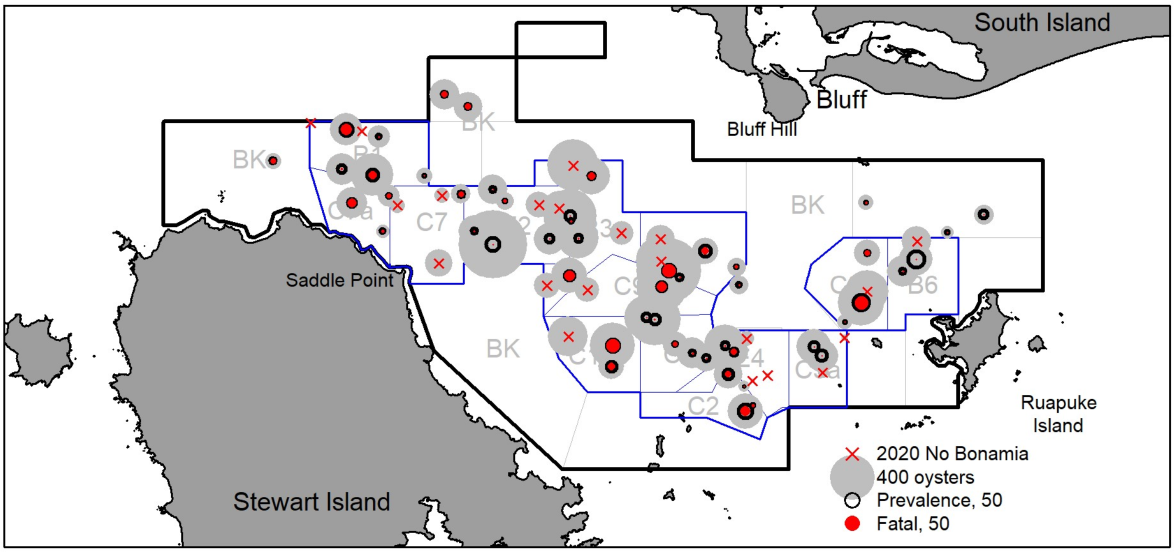

Figure 24: The distributions of recruit-sized oysters and Bonamia infection in February 2020: numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, black circles), fatal infections (intensity categories 3–5 combined, filled red circles), 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.

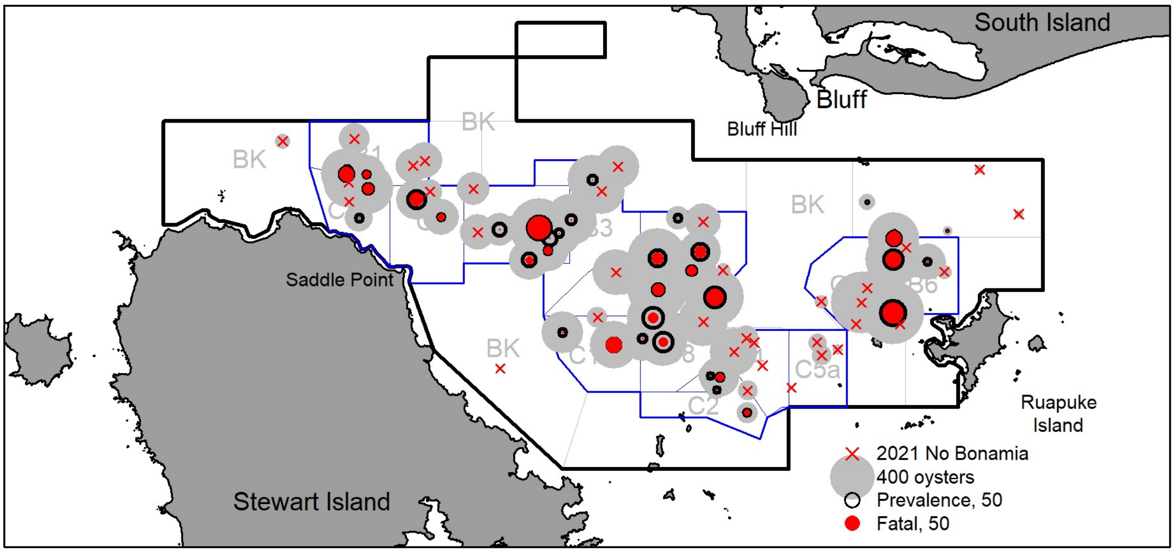

Figure 25: The distributions of recruit-sized oysters and Bonamia infection in February 2021: numbers of oysters (filled grey circles), numbers of oysters with Bonamia infection (intensity categories 1–5 combined, black circles), fatal infections (intensity categories 3–5 combined, filled red circles), 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 2021 are shown in Table 16, by ddPCR in Table 17, and non-fatal infections (intensity categories 1 and 2) from heart imprints in Table 18.

The total numbers of recruit-sized oysters infected with Bonamia in 2021 from heart imprints was 34.3 million oysters (95% confidence interval (CI) 16.0–59.0, Table 16), double that in 2020 (17.0 million oysters, 95% CI 8.4–29.0). The prevalence of infection in the Bonamia survey area was 4.3% in 2021, 3.2% in 2020, and 1.4%in 2019. Of those infections in 2021, only 0.6% were non-fatal, fewer than in 2020 (0.9%) but more than in 2019 (0.1%) (see Table 18).

The prevalence by ddPCR was 5.7%, 45.4 million oysters (95% CI 23.9–74.9, Table 17), higher than 5.1% in 2020 (27.2 million oysters, 95% CI 14.9–44.9).

Table 16: The 2021 estimates of recruit-sized oysters with Bonamia infection (prevalence), estimated by heart imprints, scaled to population size in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total). Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of infected oysters (Popn infected, shaded grey), upper and lower 95% confidence intervals (95% CI) in millions of oysters, the area of each stratum (Area (km^2)) in square kilometres, by stratum, and the 2020 recruit-sized oyster population size (Popn recruits, shaded grey, millions of oysters) and prevalence for 2021, 2020, and 2019 (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	CV	Popn infected	Lower 95% CI	Upper 95% CI	Area (km^2)	Popn recruits	Prev (%) 2021	Prev (%) 2020	Prev (%) 2019
B1	5	0.05	0.04	0.74	4.2	0.0	11.2	78.2	122.1	3.4	7.4	0
B3	3	0.03	0.03	1.00	1.5	0.0	4.8	44.7	105.3	1.5	1.8	0.8
B6	7	0.15	0.07	0.48	4.6	0.2	10.0	30.0	62.8	7.4	1.3	2
C1a	3	0.10	0.10	1.00	3.1	0.0	9.9	31.3	51.5	6.0	5.8	0
C2	3	0.03	0.02	0.58	0.8	0.0	1.8	21.9	19.5	3.9	7.2	5.4
C3	3	0.00	0.00	0.00	0.0	0.0	0.0	32.7	16.5	0.0	1.4	0.8
C5	5	0.05	0.03	0.63	2.0	0.0	4.8	37.7	52.0	3.8	3.6	1.2
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	3.7	0.0	3.9	0
C7	3	0.09	0.06	0.70	3.2	0.0	8.4	36.1	47.3	6.9	1.9	3.2
C7a	4	0.04	0.03	0.74	0.9	0.0	2.5	23.6	40.2	2.3	7.3	2.2
C8	3	0.07	0.07	1.00	1.8	0.0	5.9	26.8	66.4	2.8	1.1	3.1
C9	3	0.20	0.14	0.66	7.0	0.0	17.7	34.5	114.8	6.1	3.5	1.9
E2	7	0.11	0.09	0.79	4.9	0.0	13.5	42.8	92.8	5.3	1.2	0.6
E4	3	0.01	0.01	1.00	0.2	0.0	0.7	28.0	6.5	3.5	0	0
Core strata	55	0.07	0.02	0.25	34.3	16.0	59.0	491.8	801.4	4.3	3.2	1.4
BK	5	0.00	0.00	0.00	0.0	0.0	0.0	578.4	66.7	0.0	3.9	0.8
Survey total	60	0.03	0.01	0.25	34.3	16.0	59.0	1070.2	868.1	4.0	3.5	1.2

Table 17: The 2021 estimates of recruit-sized oysters with Bonamia infection (prevalence), estimated from ddPCR, scaled to population size in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total). Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n infected, shaded grey), upper and lower 95% confidence intervals (95% CI) in millions of oysters, the area of each stratum (Area (km^2)) in square kilometres, by stratum, and the 2020 recruit-sized oyster population size (Popn recruits, shaded grey, millions of oysters) and prevalence in 2021, 2020, and 2019 (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	CV	Popn infected	Lower 95% CI	Upper 95% CI	Area (km^2)	Popn recruits	Prev (%) 2021	Prev (%) 2020	Prev (%) 2019
B1	5	0.05	0.04	0.74	4.2	0.0	11.2	78.2	122.1	3.4	9.5	0
B3	3	0.03	0.03	1.00	1.5	0.0	4.8	44.7	105.3	1.5	2.8	5.9
B6	7	0.20	0.10	0.51	6.0	0.0	13.1	30.0	62.8	9.5	10.2	4.9
C1a	3	0.09	0.06	0.62	2.9	0.0	7.1	31.3	51.5	5.6	6.4	0
C2	3	0.04	0.02	0.51	0.9	0.0	2.0	21.9	19.5	4.6	11.2	9
C3	3	0.00	0.00	0.00	0.0	0.0	0.0	32.7	16.5	0.0	1.4	10.4
C5	5	0.10	0.06	0.57	3.8	0.0	8.7	37.7	52.0	7.2	5.1	3.4
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	3.7	0.0	7.2	0
C7	3	0.14	0.11	0.80	4.9	0.0	13.7	36.1	47.3	10.4	2.9	16
C7a	4	0.05	0.03	0.62	1.2	0.0	2.8	23.6	40.2	2.9	7.3	6.4
C8	3	0.17	0.13	0.76	4.5	0.0	12.2	26.8	66.4	6.8	5.3	13.3
C9	3	0.20	0.14	0.66	7.0	0.0	17.7	34.5	114.8	6.1	3.5	9.7
E2	7	0.19	0.08	0.43	8.1	1.3	16.8	42.8	92.8	8.8	3.4	2.8
E4	3	0.02	0.02	1.00	0.5	0.0	1.5	28.0	6.5	7.1	4	0
Core strata	55	0.09	0.02	0.21	45.4	23.9	74.9	491.8	801.4	5.7	5.1	6.4
BK	5	0.00	0.00	1.00	0.6	0.0	1.8	578.4	66.7	0.8	7.8	3.6
Survey total	60	0.04	0.01	0.21	46.0	24.0	77.4	1070.2	868.1	5.3	6.2	5.4

Table 18: The 2021 estimates of recruit-sized oysters with non-fatal infections (category 1 and 2), estimated by heart imprints, scaled to population size in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey total). Columns give the number of stations sampled (No. stns), the mean oyster density per square metre (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n infected, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters, the area of each stratum (Area (km^2)) in square kilometres, by stratum, and the 2020 recruit-sized oyster population size (Popn recruits, shaded grey, millions of oysters) and prevalence in 2021, 2020, and 2019 (Prev (%)).

Stratum	No. stns	Mean density	Density s.d.	CV	Popn infected	Lower 95% CI	Upper 95% CI	Area (km^2)	Popn recruits	Prev (%) 2021	Prev (%) 2020	Prev (%) 2019
B1	5	0.00	0.00	0.00	0.0	0.0	0.0	78.2	122.1	0.0	1.9	0
B3	3	0.03	0.03	1.00	1.5	0.0	4.9	44.7	105.3	1.5	0	0.7
B6	7	0.01	0.01	1.00	0.2	0.0	0.8	30.0	62.8	0.4	1.3	0
C1a	3	0.00	0.00	0.00	0.0	0.0	0.0	31.3	51.5	0.0	0.6	0
C2	3	0.01	0.01	1.00	0.3	0.0	0.8	21.9	19.5	1.3	3.2	0
C3	3	0.00	0.00	0.00	0.0	0.0	0.0	32.7	16.5	0.0	0.3	0
C5	5	0.00	0.00	0.00	0.0	0.0	0.0	37.7	52.0	0.0	0.4	0
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	23.5	3.7	0.0	3.9	0
C7	3	0.00	0.00	0.00	0.0	0.0	0.0	36.1	47.3	0.0	0	0
C7a	4	0.01	0.01	1.00	0.2	0.0	0.6	23.6	40.2	0.5	0.8	0
C8	3	0.03	0.03	1.00	0.9	0.0	2.9	26.8	66.4	1.4	0	0.6
C9	3	0.05	0.05	1.00	1.8	0.0	5.6	34.5	114.8	1.5	1.6	0
E2	7	0.00	0.00	0.00	0.0	0.0	0.0	42.8	92.8	0.0	1.1	0
E4	3	0.01	0.01	1.00	0.2	0.0	0.7	28.0	6.5	3.5	0	0
Core strata	55	0.01	0.01	0.50	5.1	0.1	11.3	491.8	801.4	0.6	0.9	0.1
BK	5	0.00	0.00	0.00	0.0	0.0	0.0	578.4	66.7	0.0	0	0
Survey total	60	0.00	0.00	0.50	5.1	0.1	11.3	1070.2	868.1	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. 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 (C5 and C9) and eastern (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 (see citations in Appendix 6 for details).

Non-fatal infections in 2019 were similarly low and confined to a couple of stations in the central fishery area and a couple of smaller isolated patches in stratum C8 (Figure 26). The distribution of non-fatal infections was more widespread and patchier in 2020 (Figure 27); however, the densities of non-fatally infected oysters remained low.

The distribution of non-fatal infections in 2021 remained similarly low to the last three years, and prevalence has remained low (Figure 28). There was only one site with non-fatal infections in eastern fishery areas, in stratum B6, and no non-fatally infected sites in central strata C1a and C9.

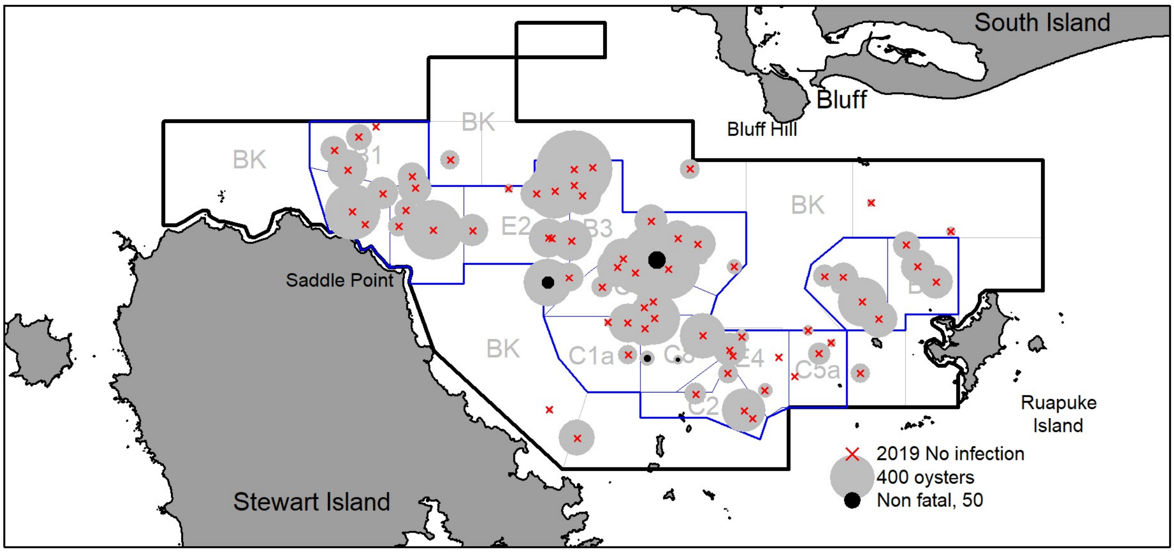

Figure 26: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2019. Stations with no Bonamia infection are shown by red crosses.

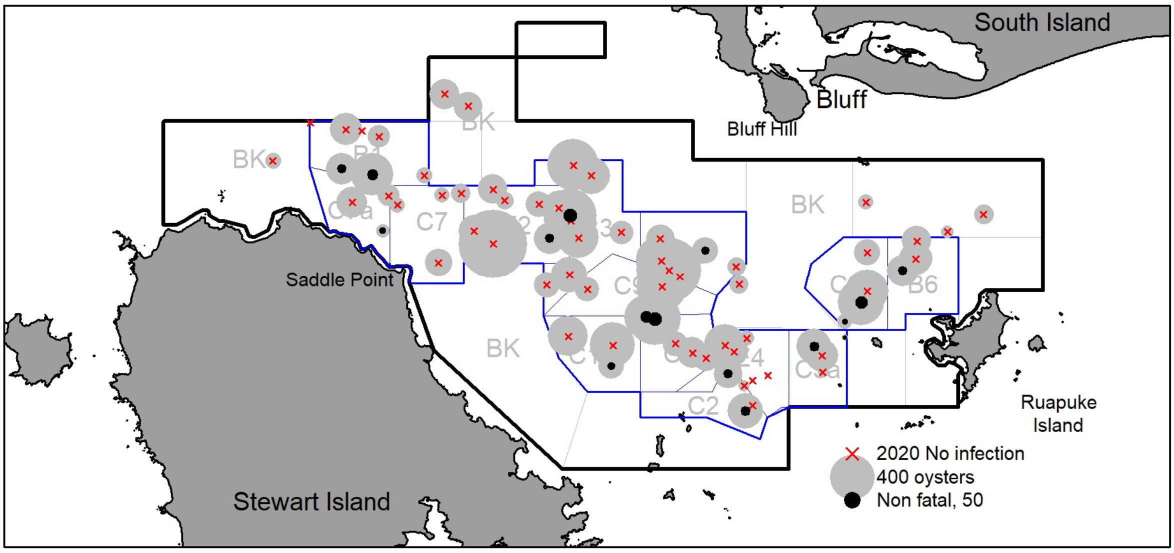

Figure 27: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2020. Stations with no Bonamia infection are shown by red crosses.

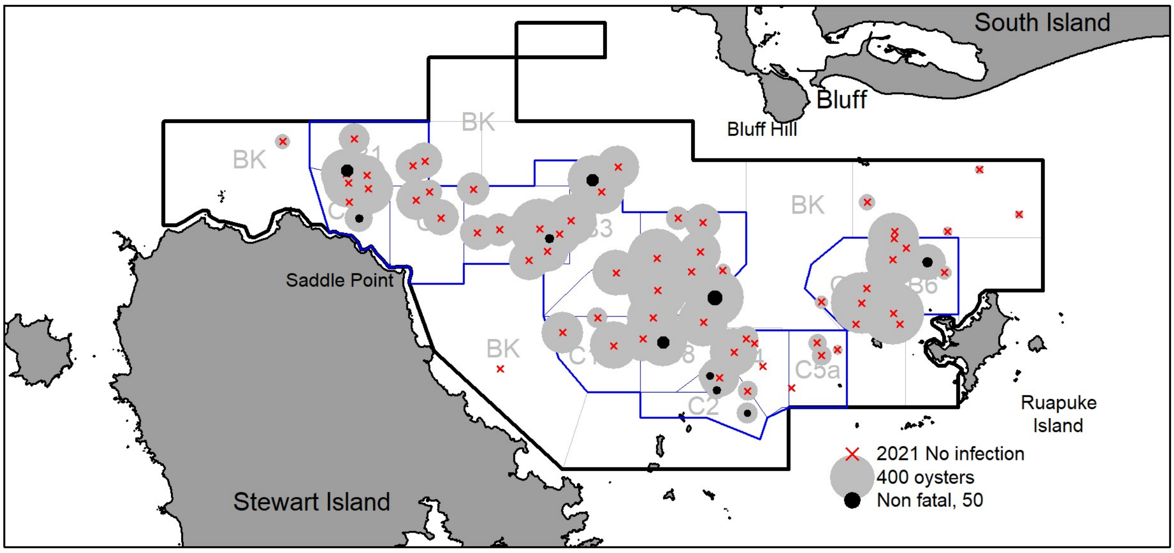

Figure 28: The distribution of recruit-sized oysters (filled grey circles, numbers per standard tow) and oysters with non-fatal (category 1 and 2) infections (filled black circles, the numbers of oysters scaled to the size of the catch) in February 2021. Stations with no Bonamia infection are shown by red crosses.

3.4.6 Summer mortality from Bonamia in the commercial fishery area

Pre-survey mortality was estimated from the population size of recruit-sized new clocks and gapers (see Table 10). In 2021, pre-survey mortality in all core strata combined was estimated to be 5.5 million recruit-sized oysters (95% CI 3.4–8.6, Table 11), 0.7% of the recruit-sized population. The estimated number of oysters killed in 2021 is higher than in 2020 (3.5 million recruit-sized oysters, 95% CI 1.9–5.8); however, the percentage mortality the same as 2020, 0.7%. Projections of post-survey mortality (within about two months of sampling) from the proportion of oysters with categories three and higher (fatal) infections scaled-up to the size of the total recruit-sized oyster population are given in Tables 19–22. Two methods are used to crosscheck the scaled-up estimates of fatal infections: 1, by applying a correction factor to the population estimates derived from the average proportion of fatally infected oysters in the stratum; and 2, post-survey mortality was estimated from the numbers of infected oysters at each sample station scaled to the catch, then to stratum, and to the survey area level. Estimates may differ a little when the numbers of fatal infections and oyster densities are more variable in any given strata.

3.4.7 Projected short-term mortality from Bonamia infections

Post-survey mortality of recruit-sized oysters was estimated for core strata with three or more randomly selected stations. Projected short-term mortality calculated using the correction factor method (Method 1), estimated the difference between the population of recruit-sized oysters at the time of survey less the population size of oysters fatally infected with Bonamia (see Pop.n1 and Pop.n2 in Table 19). Pop.n2 was calculated from the mean oyster density estimated at time of survey less the mean proportion of oysters fatally infected with Bonamia, Table 19). Using this method, post-survey mortality of oysters in 2021 was projected to reduce the recruit-sized oyster population in core strata by 29.2 million oysters (3.6%) from 801.4 million oysters at the time of the survey (February 2021) to 772.2 million oysters (Table 19) by early March 2021 (the beginning of the new oyster season). Post-survey mortality in core strata increased in both numbers of oysters killed and percentage mortality from 12.0 million oysters (2.3%) in 2020, and 6.8 million oysters (1.3%) in 2019. Percentage mortality in 2021was higher than in 2018 (1.5%), and lower than or similar to 2015–2017: 8.7%, 3.8%, and 3.0% respectively. In 2021, post-survey mortality of recruit-sized oysters by stratum (Table 19) ranged from no mortality to 7.0% in B6.

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 the same as that estimated using averaged correction factors (Method 1), 29.2 million oysters (3.6%, Table 20). The estimate of post-survey mortality in core strata using ddPCR relative infection ratios (see Figure A3.3, Appendix 3) and Method 2 was 32.7 million oysters (4.1%), which is higher than for heart imprints (Table 21). Strata B3 and E4 had fatal infections of 1.5% and 33.5%, respectively, where no fatal infections were detected by heart imprints, and stratum C9 had higher prevalence in 2021 (6.1%) compared with 2020 (4.5%, Table 21).

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

Summer mortality was estimated as the percentage of all recruit-sized oyster deaths in the population, from the time mortality began at the beginning of summer to the end of the seasonal mortality (about mid-March). Summer mortality since 2016 has been below 5.1% in the stock assessment survey and Bonamia survey areas, and substantially less than in 2012 (9.2–13.1%) (Table 22). In 2021, this summer mortality was 4.3% of the recruit-sized population in core strata, slightly higher than in 2020 (2.9%), in 2019 (2.0%), and in 2018 (3.7%). Summer mortality in the Bonamia survey area 2001–2020 is shown in 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) in the core strata, background stratum (BK), and for the stock assessment survey area (Survey total) sampled in February 2021. 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 (s.d.) of the density estimate, coefficient of variation (CV) of the oyster density, mean population size at the time of survey (Pop.n1, filled light grey), mean post survey mortality population size (Pop.n2, filled medium grey) in millions of oysters, lower and upper 95% confidence intervals (95% CI) for the post-mortality estimate, losses of oysters (Losses, millions), and the percentage mortality (% Mortality, shaded dark grey) by stratum.

Core Strata	Area (km^2)	No. stns	Correction factor	Mean density	Density s.d.	CV	Pop.n1	Pop.n2	Lower 95% CI	Upper 95% CI	Losses	% Mortality
B1	78.2	5	0.966	1.51	0.25	0.17	122.1	118.0	68.8	188.5	4.2	3.4
B3	44.7	3	1.000	2.36	0.14	0.06	105.3	105.3	70.5	154.9	0.0	0.0
B6	30.0	7	0.930	1.95	0.52	0.27	62.8	58.5	25.3	102.5	4.4	7.0
C1a	31.3	3	0.940	1.55	0.55	0.35	51.5	48.4	14.0	93.5	3.1	6.0
C2	21.9	3	0.973	0.87	0.43	0.50	19.5	19.0	0.7	41.7	0.5	2.7
C3	32.7	3	1.000	0.50	0.35	0.69	16.5	16.5	0.0	42.2	0.0	0.0
C5	37.7	5	0.962	1.33	0.42	0.31	52.0	50.0	17.4	92.9	2.0	3.8
C5a	23.5	3	1.000	0.16	0.11	0.67	3.7	3.7	0.0	9.4	0.0	0.0
C7	36.1	3	0.931	1.22	0.29	0.24	47.3	44.0	21.4	75.5	3.2	6.9
C7a	23.6	4	0.982	1.67	0.73	0.44	40.2	39.5	4.9	81.6	0.7	1.8
C8	26.8	3	0.986	2.44	0.17	0.07	66.4	65.5	43.3	97.7	0.9	1.4
C9	34.5	3	0.954	3.17	0.49	0.15	114.8	109.5	64.4	174.5	5.3	4.6
E2	42.8	7	0.947	2.06	0.37	0.18	92.8	88.0	49.5	142.4	4.9	5.3
E4	28.0	3	1.000	0.23	0.19	0.84	6.5	6.5	0.0	18.7	0.0	0.0
Core strata	491.8	55		1.57	0.10	0.06	801.4	772.2	516.6	1152.8	29.2	3.6
BK	578.4	5	1.000	0.12	0.04	0.33	66.7	66.7	22.0	125.9	0.0	0.0
Survey total	1070.2	60	-	0.78	0.05	0.06	868.1	838.9	553.6	1230.0	29.2	3.4

Table 20: Scaled up estimates of the population size of recruit-sized oysters with fatal infections (category 3–5) estimated by heart imprints in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey area) sampled in February 2021. 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 per square metre expected to die (Mean density), the standard deviation of the mean density (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size at the time of survey (Popn recruits, filled light grey), 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 2021, 2020, and 2019 (% Mortality, shaded dark grey) by stratum.

Stratum	Area (km^2)	No. stns	Mean density	Density s.d.	CV	Popn recruits	Losses	Lower 95% CI	Upper 95% CI	Mort (%) 2021	Mort (%) 2020	Mort (%) 2019
B1	78.2	5	0.05	0.04	0.74	122.1	4.2	0.0	11.2	3.4	5.4	0
B3	44.7	3	0.00	0.00	0.00	105.3	0.0	0.0	0.0	0.0	1.8	0.8
B6	30.0	7	0.15	0.08	0.53	62.8	4.4	0.0	9.8	7.0	0	2
C1a	31.3	3	0.10	0.10	1.00	51.5	3.1	0.0	9.8	6.0	5.2	0
C2	21.9	3	0.02	0.02	1.00	19.5	0.5	0.0	1.6	2.6	4	5.4
C3	32.7	3	0.00	0.00	0.00	16.5	0.0	0.0	0.0	0.0	1.1	0.8
C5	37.7	5	0.05	0.03	0.63	52.0	2.0	0.0	4.9	3.8	3.2	1.2
C5a	23.5	3	0.00	0.00	0.00	3.7	0.0	0.0	0.0	0.0	0	0
C7	36.1	3	0.09	0.06	0.70	47.3	3.2	0.0	8.4	6.9	1.9	3.2
C7a	23.6	4	0.03	0.03	1.00	40.2	0.7	0.0	2.3	1.8	6.5	2.2
C8	26.8	3	0.03	0.03	1.00	66.4	0.9	0.0	2.9	1.4	1.1	3.1
C9	34.5	3	0.15	0.09	0.58	114.8	5.3	0.0	12.4	4.6	2	1.9
E2	42.8	7	0.11	0.09	0.79	92.8	4.9	0.0	13.5	5.3	0.1	0.6
E4	28.0	3	0.00	0.00	0.00	6.5	0.0	0.0	0.0	0.0	0	0
Core strata	491.8	55	0.06	0.02	0.26	801.4	29.2	12.9	50.5	3.6	2.3	1.4
BK	578.4	5	0.00	0.00	0.00	66.7	0.0	0.0	0.0	0.0	3.9	0.8
Survey total	1070.2	60	0.03	0.01	0.26	868.1	29.2	12.9	50.5	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 in the core strata, background stratum (BK), and for the whole 2007 stock assessment survey area (Survey area) sampled in February 2021. 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 per square metre expected to die (Mean density), the standard deviation of the mean density (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size at the time of survey (Popn recruits, filled light grey), 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 2021 by ddPCR (Mort (%) 2021 (ddPCR), shaded dark grey) and by heart imprints (Mort (%) 2021 (Heart imp.), shaded light blue grey), by stratum.

Stratum	Area (km^2)	No. stns	Mean density	Density s.d.	CV	Popn recruits	Losses	Lower 95% CI	Upper 95% CI	Mort (%) 2021 (ddPCR)	Mort (%) 2021 (Heart imp.)
B1	78.2	5	0.05	0.04	0.74	122.1	4.2	0.0	11.2	3.4	3.4
B3	44.7	3	0.03	0.03	1.00	105.3	1.5	0.0	4.8	1.5	0.0
B6	30.0	7	0.15	0.08	0.53	62.8	4.4	0.0	9.8	7.0	7.0
C1a	31.3	3	0.10	0.10	1.00	51.5	3.1	0.0	9.9	6.0	6.0
C2	21.9	3	0.02	0.02	1.00	19.5	0.5	0.0	1.6	2.6	2.6
C3	32.7	3	0.00	0.00	0.00	16.5	0.0	0.0	0.0	0.0	0.0
C5	37.7	5	0.05	0.03	0.63	52.0	2.0	0.0	4.8	3.8	3.8
C5a	23.5	3	0.00	0.00	0.00	3.7	0.0	0.0	0.0	0.0	0.0
C7	36.1	3	0.09	0.06	0.70	47.3	3.2	0.0	8.4	6.9	6.9
C7a	23.6	4	0.03	0.03	1.00	40.2	0.7	0.0	2.3	1.8	1.8
C8	26.8	3	0.03	0.03	1.00	66.4	0.9	0.0	2.9	1.4	1.4
C9	34.5	3	0.20	0.14	0.66	114.8	7.0	0.0	17.7	6.1	4.6
E2	42.8	7	0.11	0.09	0.79	92.8	4.9	0.0	13.5	5.3	5.3
E4	28.0	3	0.01	0.01	1.00	6.5	0.2	0.0	0.7	3.5	0.0
Core strata	491.8	55	0.07	0.02	0.26	801.4	32.7	14.8	56.6	4.1	3.6
BK	578.4	5	0.00	0.00	0.00	66.7	0.0	0.0	0.0	0.0	0.0
Survey total	1070.2	60	0.03	0.01	0.26	868.1	32.7	14.8	56.6	3.8	3.4

Table 22: Summer mortality for 2018–2021 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	2018	2019	2020	2021
Recruit-sized new clocks (NC, millions)	3.4	9.2	8.5	5.5	2.9	4.1	3.5	5.5
Post-survey mortality								
Correction factor (millions of oysters)	23.1	9.4	24.9	29.2	15.7	6.8	12.0	29.2
Scaled catch (millions of oysters)	10.7	9.4	25.5	29.2	7.3	6.8	12.0	29.2
Combined summer mortality								
Correction factor +NC (millions of oysters)	26.5	18.6	33.4	34.7	18.6	10.9	15.5	34.7
Scaled catch +NC (millions of oysters)	14.1	18.6	34.0	34.7	10.2	10.9	15.5	34.7
Population before summer mortality								
Recruit-sized oysters +NC (millions of oysters)	886.7	868.0	887.8	873.6	497.0	542.5	533.4	806.9
Percent summer mortality								
Correction factor +NC (%)	3.0	2.1	3.8	4.0	3.7	2.0	2.9	4.3
Scaled catch +NC (%)	1.6	2.1	3.8	4.0	2.1	2.0	2.9	4.3

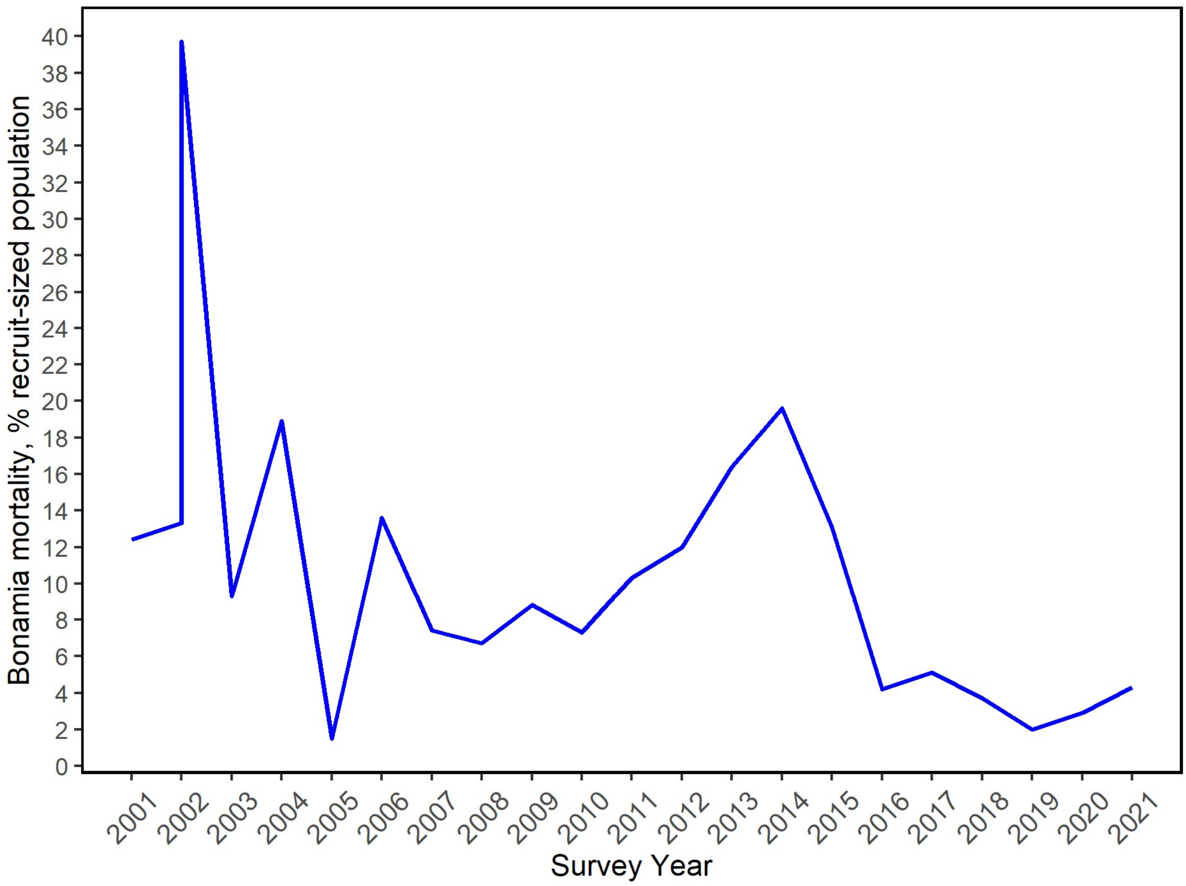

Figure 29: Percentage mortality of the recruit-sized oyster population in the Bonamia survey area between 2001 and 2021.

3.5 The status of the OYU 5 fishery in 2021 and future trends

The 2017 stock assessment for OYU 5 suggested that an annual commercial harvest of up to 30 million oysters is not likely to have a significant effect on the future (1–5 years) status of the stock (Figure 30), (Large et al. 2021). Disease mortality and recruitment to the fishery are the main drivers of future stock size in the OYU 5 fishery. Since 1985, OYU5 has shown cyclic trends in oyster abundance.

Between 1993 and 1999, the fishery rebuilt rapidly from an 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 during the February 2018 survey. Bonamia mortality was low (2–3%) over the summer of 2017–18. This upward trend in recruit-size stock abundance and recruitment, and low Bonamia mortality, continued into the summer of 2018–19. The numbers of spat per collector were similar to the previous summer, small oysters per recruit continued to increase, and the recruit-sized population increased by around 9.8% between 2018 and 2019, as did pre-recruit 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% (see Table 8). Assuming the Bonamia survey area (core strata) shows the same trend as the stock area, the February 2020 survey showed increases of 28.3%, 51.2%, 83.6%, and 3.7% for commercial-sized, recruit-sized, pre-recruit oysters, and small oysters. If the substantial increases in the recruit-sized and pre-recruit oysters, and a consistently high population of small oysters, are unaffected by Bonamia mortality, the population size and density of commercial-sized oysters should increase rapidly.

The future status of the fishery is best represented by series 'a' in Figure 30 which assumes no Bonamia mortality; actual mortality was 4.3%, lower than the midpoint between the two projections based on zero and 10% mortality. Moreover, non-fatal infections are low (0.7% of the recruit-sized population), suggesting low *B. exitiosa* mortality in 2022.

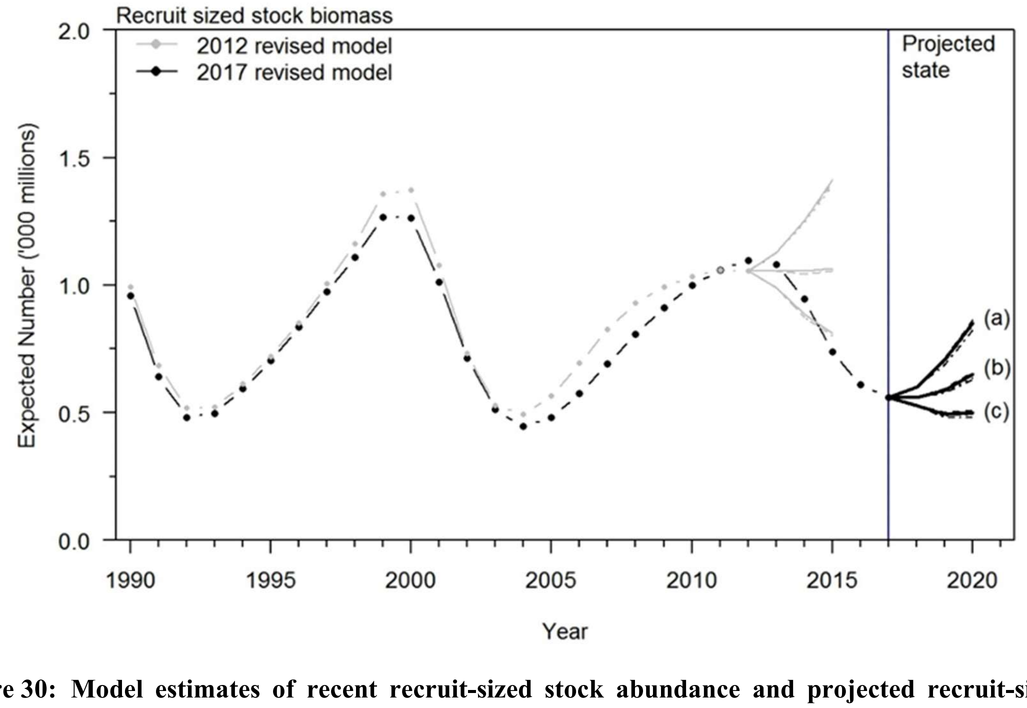

Figure 30: Model estimates of recent recruit-sized stock abundance and projected recruit-sized stock abundance with catches of 7.5 (solid line), 15 (dash dot), and 30 million oysters (dashed line) under assumptions of (a) no disease mortality, (b) disease mortality of 0.10 y^{-1}, and (c) disease mortality 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. This forecast also updates the status of infection and estimates of disease mortality, together with estimates of recruitment from spat monitoring, catch sampling, and survey estimates, which are important in determining the trajectory of the stock. The limited sampling in the background stratum also allows these data to be incorporated into stock assessments. These surveys achieved low CVs for population estimates, well below the 20% target set by Fisheries New Zealand for stock assessment surveys. A CV of 6% was obtained for estimates of recruit-sized oysters in the Bonamia survey area in 2021.

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 standard Bonamia survey area, was established in 2014 to make these surveys comparable from year to year. The February 2021 survey is the eighth in this new time series. Because both estimates of new clocks and fatal infections are scaled to the size of the oyster population, better estimates of oyster density from randomised, two-phase sampling are likely to give more precise estimates of total summer mortality.

4.1 Survey results

This survey used the same vessel, skipper, and crew, and standard sampling methods as for previous Foveaux Strait surveys. Sampling conditions during the February 2021 survey were good, and not expected to have affected dredge efficiency. Additionally, the CVs obtained for population estimates from the survey were much lower (6%) than those predicted for recruit-sized oysters (CV of 11%), probably due to the rebuilding of localised populations which has decreased the variation in oyster densities. There is a possibility that inter-survey variation, whether random stations on average land on high- or low-density areas in a patchily distributed population, may have contributed to the substantial increase in the estimates of population size. However, it is more likely that some of the large number of small oysters have grown into the recruit-sized oyster group over one summer.

The timing of Bonamia surveys coincides with a period of peak seasonal mortality from Bonamia and the shedding of infective particles. In 2021, 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 which also provides for quantification of infection. Overall, the new method provides correspondence of normalised quantification to histological scorings, a high level of precision and repeatability, superior levels of sensitivity and detection, and cost-effectiveness. The use of ddPCR since 2018 most likely improved the detection of low-level infection, increasing our estimates of prevalence in the Foveaux Strait population. Prevalence was still relatively low in 2021 at 4.3% of recruit-sized oysters in the Bonamia survey area.

Stock assessment and Bonamia surveys estimate oyster densities and mean population sizes by stratum, for the Bonamia survey and stock areas. The 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. Even within core strata, 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 estimates of oyster densities, and the status of *B. exitiosa* prevalence, intensity, and mortality at the levels of stratum, all core strata combined, and the stock area.

The design of the surveys does not describe the spatial structure of the stock well, especially the distribution of high-density patches of large oysters important to fishers. Oyster density and meat quality in the highest-density patches determine commercial catch rates. Strata with high density patches (‘oyster beds’) are best represented by those with commercial-sized and recruit-sized oyster densities greater than 400 oysters per tow (1.0 oysters m^{-2}). In 2021, four strata had densities of commercial-sized oysters over 1.0 oysters m^{-2} (B3, C8, C9, and E2). All core strata, except for C3, C5 C5a, and E2 had recruit-sized oyster densities greater than 1.0 oysters m^{-2} in 2021. More strata (10) had commercial densities in 2021, than in 2020 (seven, B1, B3, C1a, C3, C5, C9, and E2), and in 2019 (six, B3, C5, C7, C7a, C8, and E2). In the Bonamia survey area, the recruit-sized oyster population above the threshold of greater than 400 oysters per tow was 519.3 million oysters (95% CI 321.9–801.4), 2.2 times more than in 2020. The lower percentages of commercial-sized oysters in 2021 reflects substantial increases in recruit-sized oysters rather than a decrease in commercial-sized oyster densities.

4.2 Fishery trends

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 (estimated at 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, there have been substantial increases in recruit-sized, pre-recruit, and small oysters (see Figure 7 and Table 8). The population size of recruit-sized oysters in the Bonamia survey area is 2.2 times that in 2017 (see Table 8).

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 (sacks h^{-1}) in 2010 to 2.3 sacks h^{-1}(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 2.8 sacks h^{-1} (95% CI 2.7–2.9) in 2020.

4.3 Outlook for the 2021 oyster season

All the key indicators for the February 2021 survey are strongly positive for the continued rebuilding of the fishery. 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. 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 was high (1091.2 million oysters, 95% CI 726.3–1637.0) in 2021. The peak in population size for small oysters was 1410 million oysters in the stock assessment area, in 2001, the highest since surveys of small oysters began in 1993. The population size of recruit-sized and pre-recruit oysters started to increase from 2017 and both size groups increased substantially between 2020 and 2021 as small oysters grew into these size groups. Recruit-sized oysters increased by 51.2% to 801.4 million oysters (95% CI 536.2–1196.7) and pre-recruits increased by 83.5% to 487.0 million oysters (95% CI 320.9–733.6). Commercial-sized oysters also increase between 2020 and 2021 by 28.3% to 405.6 million oysters (95% CI 271.0–606.6). In the absence of significant mortality, continued rapid increases in commercial-sized and recruit-sized oysters driven by the large numbers of small and pre-recruit oysters is highly likely. Oysters growing to recruit size in 2021 may not grow to commercial size for another 1–2 years. Catch rates of commercial-sized oysters for the 2021 season are expected to remain similar to, or slightly higher, than the 2020 levels.

Future levels of disease mortality in the fishery are uncertain. *B. exitiosa* mortality has been low since 2018. In 2021, the numbers of gapers and new clocks were low and similar to base levels for natural mortality, regardless of cause of death. The prevalence of *B. exitiosa* infection by ddPCR was 5.7%, 45.4 million oysters (95% CI 23.9–74.9), higher than 5.1% in 2020, but lower than 6.4% in 2019. The infected population of recruit-sized oysters in the Bonamia survey area estimated using ddPCR is 32.4% higher than that estimated using heart imprints in 2021. Mortality in the Bonamia survey area over the summer of 2020–21 was 4.3% (34.7 million recruit-sized oysters), comprised mostly of post survey mortality of fatally infected oysters. Non-fatal infections of *B. exitiosa* were 0.7% of the recruit-sized oyster population in 2021, and mortality from *B. exitiosa* is expected to be low in 2022. However, the status of *B. ostreae* detected in the Foveaux Strait oyster fishery (OYU 5) in March 2021 is unknown, and this pathogen 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 2.9% of the portion of the stock above 400 oysters per tow (519.3 million oysters), and 1.7% of the recruit-sized population in core strata alone. In 2020, the oyster industry landed 8.1 million oysters representing 1.0% 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.

5. ACKNOWLEDGEMENTS

We thank Graeme Wright (BOMC) for his support in facilitating the survey and David Skeggs for the support of BOMC, Stephen Hawke and his crew (Victoria Pearsey, Russel Dixon, Scotty Smith, Mark Strange, and Ethan Carstensen), and Graeme Moss (NIWA) for the long days at sea and tremendous effort put in to complete the survey to a high standard.

Thanks also to Sadie Mills, Dean Stotter, Diana MacPherson, Peter Notman, Josette Delgado, Valeria Mobilia, Savannah Goode, Kushboo Jhugroo, Owen Anderson, Graeme Moss, and others who helped process oyster samples at NIWA Wellington (Greta Point). We thank Alistair Dunn and Dan Fu who developed scripts for the analyses of these data, and Chloe Hauraki, Darren Parsons, and Marine Pomarède (Fisheries New Zealand) for reviewing this report and comments on the manuscript. This investigation was funded by Fisheries New Zealand under Project OYS2020/01, objectives 1–4.

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APPENDIX 1: SURVEY STATION FORM AND DREDGE SAMPLING METHODS

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*

Bycatch data form, presence absence.

Foveaux Strait substrate data	code	present		Species	code	present
Sand-subtrate	1			Notoplax sp.	55	
Mud-subtrate	2			Notoplax sp. #2	56	
Shell hash/grit-subtrate	3			Crypotoconchus porosus	57	
Bryozoan hash-subtrate	4			Eudoxochiton nobilis	58	
pea gravel-subtrate	5			Rhyssoplax canaliculata	59	
Cobbles-subtrate	6			CH1	60	
Oyster shell	7			*CH2*	61	
Longimactra elongata shell	8			*Sigapatella novaezelandiae*	62	
Panopea zealandica shell	9			*Crepidula monoxyla*	63	
Glycemeris modesta (small) she	10			*Astraea heliotropium*	64	
Glycemeris laticostata (large) she	11			*Modelia granosa*	65	
Barnicle shell	12			*Xymene pusillus*	66	
Circular saw shell	13			*Charonia sp.*	67	
maoriculpus shell	14			*Cabestana spengleri*	68	
Other shell	15			*Argobuccinum pustulosum tu*	69	
Red Algae	16			*Maoricolpus roseus roseus*	70	
kelp	17			*Haliotis virginea*	71	
				Buccinulum linea?	72	
Ophiopsammus maculata	18			*Cominella nassoides*	73	
Ophiopeza gracilis	19			*Alcithoe arabica*	74	
Ophiopterus papillosum	20			*Barbatia noveaezealandiae*	75	
BTST 2 (alphabet)	21			*Modiolarca impacta*	76	
BTST 3	22			*Modiolus areolatus*	77	
btst4 bright red (4060)	23			*Aulacomya atra maoriana*	78	
Coscinasterias muricata	24			*Cardita aoteana*	79	
Allostichaster insignis	25			*Venericardia purpurata*	80	
Sclerasterias mollis?	26			*Ostrea chilensis*	81	
LGST 1 Purple	27			*Chlamys spp.*	82	
Patiriella regularis	28			*Pecten novazealandiae*	83	
Pentagonaster pulchellus	29			*Talcochlamys*	84	
Asterodon miliaris	30			*Mesopplum convexum*	85	
Apatopygus recens	31			*Panopea sp.*	86	
Goniocidaris umbraculum	32			*Glycymeris modesta*	87	
Pseudechinus albocinctus	33			*Tucetona laticostata*	88	
Pseudochinus huttoni	34			*Dosina zelandica*	89	
Pseudechinus novaezealandie	35			*Tawera spissa*	90	
Evechinus chloroticus	36			*Pseudoxyperas elongata*	91	
Australostichopus mollis	37			Other bivalve	92	
Holo other	38					
*Chondropsis topsentii**	39			*Octopus huttoni*	93	
*Crella incrustans**	40			*Enteroctopus zealandicus*	94	
*Dactyllia palmata**	41					
*Nemertesia elongata**	42			*Othoscuticella fusiformis*	95	
UNKNOWN	43			*Celleporaria agglutinans*	96	
Other hydroids	44			*Cinctipora elegans**	97	
Barnacle	45			*Retehornera foliacea**	98	
Metacarcinus novaezealandie	46			*Tetrocycloecia neozelanica**	99	
Pagurus novizealandiae	47			Disporella sp.*	100	
Eurynolambrus australis	48			BR1	101	
Nectocarcinus sp.	49			*Neothyris compressa*	102	
unknowncamouflage crab	50			*Neothyris lenticularis*	103	
Callochiton sulculatus	51			*Botryloides leachi*	104	
Callochiton crocinus?	52			*Pyura carnea*	105	
Callochiton sp.	53			*Pyura pulla*	106	
Craspedochition rubiginosus	54			*Pyura pachydermatina*	107	
				Pseudodistoma	108	
				Corella eumyota	109	
				Calliostoma	110	

Except for minor variations, dredge sampling has followed standard procedures for stock assessment and Bonamia surveys since October 2002: standard dredge sampling methods, standard methods for sorting the catch and recording data, and standard methods for sampling oysters to determine the status of Bonamia. Two commercial oyster vessels have been used for surveys since 1999, F.V. *Golden Lea* 1999–2010 and F.V. *Golden Ques*t 2011–2020, except in 2016 when the F.V. *Golden Lea* was used due to the unavailability of the F.V. *Golden Quest*. Stephen Hawke has skippered the survey vessel since 2011, to maintain consistency in the time series. Survey stations were sampled with the standard survey dredge (commercial dredge 3.35 m wide and weighing 430 kg) used since 1993 and rebuilt in 2014 to the same specifications. A traditional friction winch used to deploy the dredge on F.V. *Golden Quest* was replaced with a hydraulic winch system in 2014.

Navigation

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

Survey tows

Where the start of the tow could not be made on position because of weather, tide, or boundary constraints, the tow direction was reversed, and the tow finished on position. Oyster surveys use straight-line tows to enable the area sampled by the dredge to be calculated. This differs to the elliptical tows used by commercial oyster fishers, who fish down tide, then tow back to the start position to enable them to stay on oyster patches. Straight-line tows were made down tide for a distance of 0.2 nautical mile (370 m) at each station. The start of the tow was recorded at the time when the winch brake was applied, and tension came onto the warp. The distance towed was monitored against a 0.2 nautical mile range ring on SEAPLOT. Once the dredge had travelled 0.2 nautical miles, the end of tow position was recorded, the winch brake released, and the dredge hauled aboard without washing. The area swept by a standard dredge tow is 1240 m^2, the tow length (370 m) multiplied by the dredge width (3.35 m).

When it was possible in 2021, fixed tows were repeated over the same tow line and in the same tow direction as in previous surveys and started on station position where possible.

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

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

Sorting the catch

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

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

In this February survey, new clocks were defined as those that had clean inner valves that had retained their lustre but may have had some minor speckling of fouling organisms (Figure A1.1). For this analysis, we assumed that new clocks were only those oysters that had died since summer mortality from Bonamia began, and oysters that died before this were categorised as old clocks.

The shells of oysters that are fouled or in which the inner valves have lost their lustre are termed old clocks (Figures A1.2 and A1.3). Old clocks can be covered in fouling organisms on both external and internal surfaces, and as the ligaments of oysters are thought to break down over about a three-year period, old clocks represent oysters that died between 1 and 3 years previously (Cranfield et al. 1991). The classification of old clocks may vary depending on habitat. Old clocks from sand habitats may be older as they may be filled with sand preventing the settlement of fouling organisms and reducing physical forces on the hinge and prolonging the time that both valves remain attached beyond three years. Gravel habitats are usually shallower with stronger tidal currents and higher swell energy and the valves of old clocks there may be disconnected much more quickly than three years or the clocks (new and old) may be transported out of the fishery area by the strong tides.

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

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

Figure A1.1: New clock (with hinge intact), glossy inner valve with no fouling except a few white coralline specks.

Figure A1.2: Recent old clock (with hinge intact), glossy inner valve with light fouling.

Figure A1.3: Old clock with hinge intact. No gloss on inner valve and heavy fouling.

APPENDIX 2: BONAMIA SAMPLING FORM

Bonamia Testing: Laboratory Sampling Record

Page___of____

Project code:

Site:

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

Oyster no	Length (mm)	Height (mm)	Size category	Gaper	Colour of larvae	Gonad section	Heart Imprint Score	Heart/Gill plate#	Extra Gillx2 plate #
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									

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

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

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

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

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

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

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

Sampling of oyster tissues

Samples of up to 30 randomly selected recruit-sized oysters are required from each station to determine the status of Bonamia infection. When there were insufficient recruit-sized oysters in the catch, pre-recruit and small oysters were used to fill the sample size, or the whole catch was retained for processing. Samples were bagged, labelled with station number, date, and time on waterproof labels and the sacks tied securely. The oysters for Bonamia samples were kept cool and damp in oyster sacks, transferred to poly bins, and flown to NIWA Wellington for processing. Oyster samples generally arrived in Wellington within 36 hours of capture and were mostly processed on the day of arrival. The samples were held in poly bins under cool conditions (about 8–12 °C) in the aquarium. Oyster samples not processed 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), and the measurement was truncated to the lower whole millimetre. If samples contained insufficient recruit-sized oysters, pre-recruits were used in preference to small oysters. Recruit-size oysters were denoted with an R, pre-recruit oysters with P, and small oysters with an O. Gaping oysters with valves of the shell apart, but which closed when tapped, were marked with an asterisk alongside the corresponding oyster number. Oysters incubating larvae were recorded as either white (early-stage) larvae, grey (late-stage) larvae, yellow (almost ready to settle) larvae, or with no larvae present (coded NA).

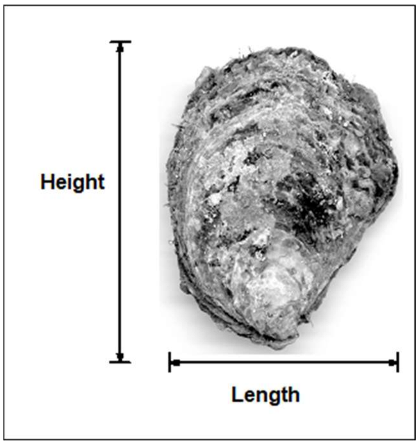

Figure A3.1: An oyster showing length (anterior-posterior axis) and height (dorsal-ventral axis) dimensions.

Droplet digital polymerase chain reaction (ddPCR) testing method

Procedures were implemented to prevent contamination of the ddPCR samples. Laboratory staff replaced gloves and rinsed solutions for every station. Pre-labelled 96 well plates covered with plastic film were placed on the chill blocks to keep samples cool. These chill blocks were stored at -20 °C 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 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

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). Oyster tissues were digested with Proteinase K and the digest was diluted to 1:20. Sample volumes of 4 µl of the tissue digests were combined with 19 µl of BioRad ddPCR SuperMix, primers, and probes to a total volume of 23 µl. Samples were tested on a 96 well plate. 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 de-ionised distilled water was used as a negative template control.

The ddPCR data from tested survey samples were analysed using QuantaSoft Pro and positive/negative thresholds for both FAM and HEX channels were set at 2000 relative fluorescence units. Each ddPCR reaction was assessed to ensure that it contained a minimum of 103 droplets and that at least one droplet was negative for each target, as required for Poisson-based calculations of sample concentration. Reactions with less than 103 total droplets were repeated. In some cases, highly concentrated samples (particularly β-actin) displayed zero negative droplets and were repeated using a further 1:1 dilution of the same 1:20 tissue digest dilution. Samples displaying a minimum of five positive droplets were classed as positive for either target (Bonamia or oyster β-actin). Any sample with fewer than five positive droplets for the β-actin internal control was repeated by creating a new 1:20 dilution of tissue digest from both heart and gill samples and using both in a repeated ddPCR reaction. The repeated ddPCR scorings were used in the analysis for presence/absence and quantification.

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

Heart imprint methods

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

Analysis of oyster heart imprint data

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

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

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

Stage	Criteria
0	No 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.

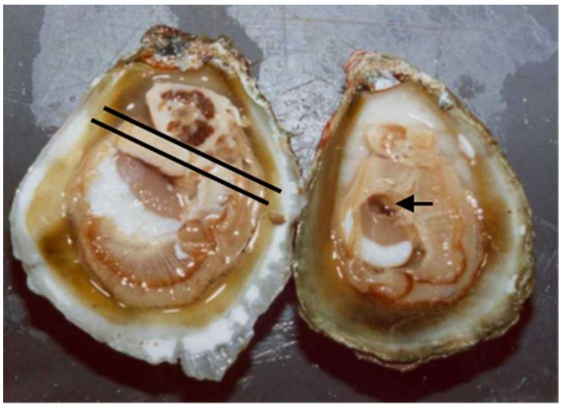

Figure A3.2: Lines on left oyster show location of 5 mm thick standard section taken for histology. The arrow on the oyster on the right shows the heart, a black organ adjacent to the adductor muscle.

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 shown in Figure A3.3 where heart imprints were not able to detect infection (score 0). At intensifying levels of infection (heart imprint score 3) and fatal infections (scores 4 & 5), the relative level of infection from ddPCR increases rapidly (Figure A3.3).

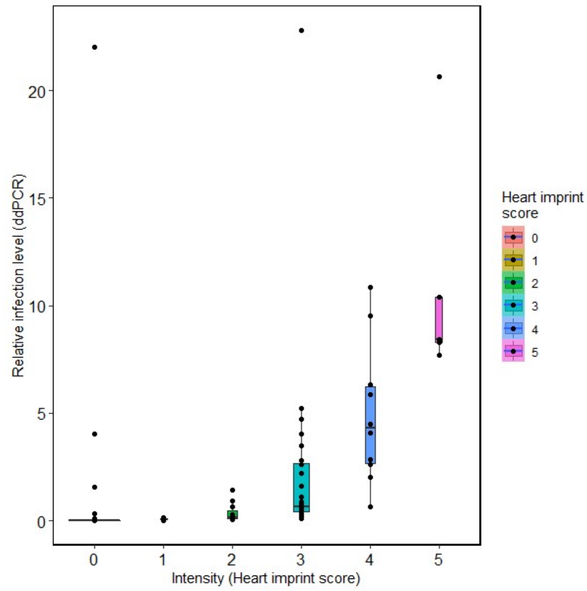

Figure A3.3: Boxplots of the intensity of *Bonamia exitiosa* from ddPCR calculated as the ratio of the concentration of Bonamia targets to the concentration of β-actin targets in each sample plotted by the categorical intensity of infection from heart imprints using the methods of Diggles et al. (2003).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

APPENDIX 4: FEBRUARY 2021 SURVEY CONDITIONS AND COMPARABILITY

Dredge tow lengths in February 2021 were almost all 0.2 nautical miles (370 m, 5th percentile 0.2, and 95th percentile 0.21) in length (Figure A4.1). All oyster and clock densities were standardised to a 0.2 nautical mile standard tow length for analysis. Most of the survey stations were sampled in wind conditions less than 10 knots (Figure A4.2). The median wind force was 1 on the Beaufort scale (1−2 knots), with 5th and 95th percentiles of Beaufort scale 0 (calm) and 3 (7−10 knots) respectively. Maximum wind speed during sampling was about 15 knots. Operational limits for dredge sampling of 20 knots and Figure A4.2 show the February 2021 survey was undertaken in similar conditions to previous February surveys.

Oyster dredges are considered saturated and cease fishing before the end of tow when they are more than 80% full on landing (J. Cranfield pers. comm.). Dredge saturation may lead to an underestimate of oyster density. No dredge was landed more than 80% full. Dredge fullness ranged from 1% to 70% with a median fullness of 50%, similar to 2014–2019, but higher than in 2020 (40%). Differences in dredge fullness are in part related to levels of pre-survey mortality from Bonamia which increases the quantities of dead shell. Dredge saturation is not likely to have had a large effect on sampling effectiveness in the 2021 survey (Figure A4.3). Observations and anecdotal evidence from video data recorded during dredge trials suggest that dredge saturation may occur in dredges landed less than 80% full; however, when this occurred, the dredge contents were unevenly but symmetrically spread, with contents lower in the middle of the dredge than at the edges of the dredge ring bag. This was not recorded in the 2021 survey data; future surveys will identify stations with this pattern in the distribution of catch.

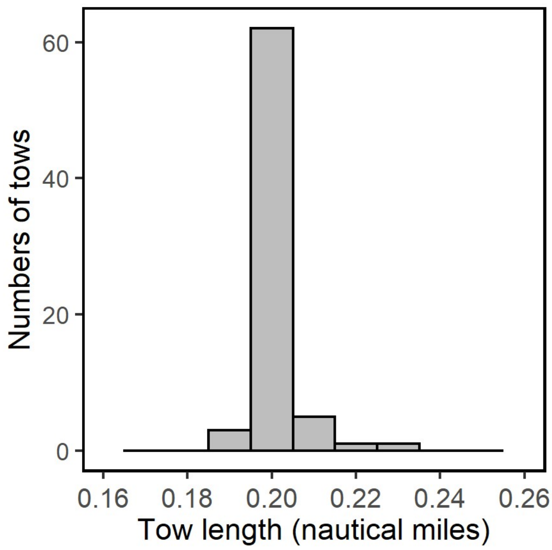

Figure A4.1: Distribution of dredge tow lengths from the February 2021 survey. The standard tow length was 0.2 nautical mile (370 m).

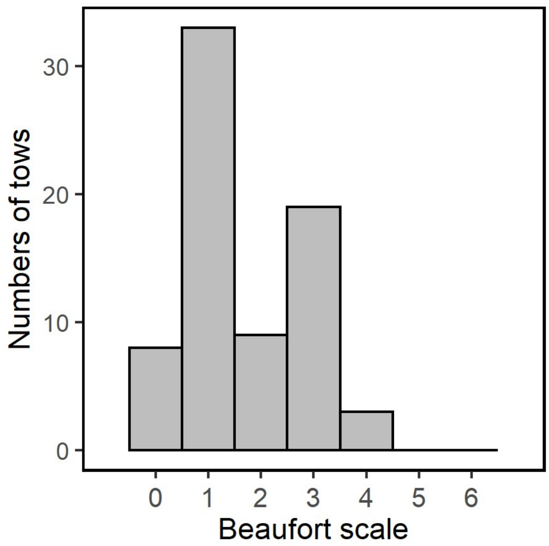

Figure A4.2: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2021. Beaufort scale: 0, < 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

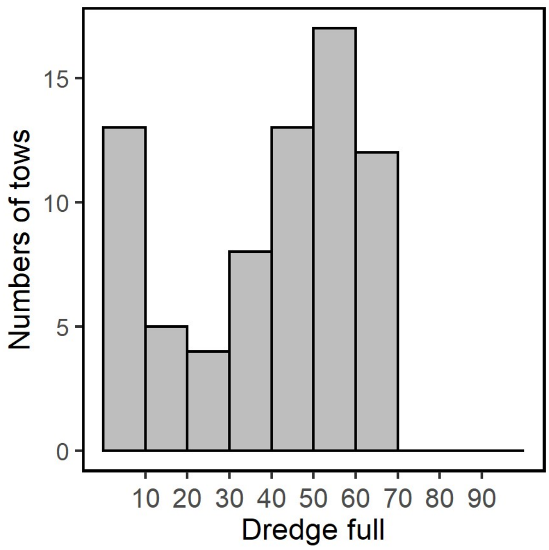

Figure A4.3: Distribution of dredge fullness (%) recorded for survey tows in February 2021. No tows were landed with a dredge fullness of greater than 80%. Unpublished video data suggests that dredge saturation may occur below 80% full.

APPENDIX 5: 2021 SURVEY CATCH AND INFECTION DETAILS

Table A5.1: Details of recruit-sized oysters (Recruits) and densities m^{-2} (Density) by stratum (Str) and station (Stn); the numbers of oysters tested (Total) and numbers of uninfected (Un.inf) samples, samples with non-fatal infections (NF.inf) and fatal infections (Fat.inf) based on category 3 higher infections. 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 2021 survey.

Str	Stn	Recruit	Density	Total	Un.inf	NF.inf	Fat.inf	%Prev	% ddPCR	Diff.f
B1	1	293	0.24	25	293	0	0	0	0	0
B1	2	557	0.45	25	512	0	45	8	8	0
B1	3	270	0.22	25	270	0	0	0	0	0
B1	4	216	0.17	25	216	0	0	0	0	0
B1	5	337	0.27	25	324	0	13	4	4	0
B3	6	423	0.34	25	423	0	0	0	0	0
B3	7	477	0.38	25	477	0	0	0	0	0
B3	8	528	0.43	25	507	21	0	4	4	0
B6	9	875	0.70	25	770	0	105	12	16	4
C1a	12	399	0.32	25	399	0	0	0	4	4
C1a	13	103	0.08	25	103	0	0	0	0	0
C1a	14	510	0.41	25	449	0	61	8	8	0
C3	18	238	0.19	25	238	0	0	0	0	0
C3	19	39	0.03	25	39	0	0	0	0	0
C3	20	25	0.02	25	25	0	0	0	0	0
C5	21	136	0.11	25	136	0	0	0	12	12
C5	22	46	0.04	25	46	0	0	0	0	0
C5	23	399	0.32	25	367	0	32	8	16	8
C5	24	542	0.44	25	520	0	22	4	4	0
C5	25	291	0.23	25	291	0	0	0	0	0
C5a	26	76	0.06	25	76	0	0	0	0	0
C5a	27	22	0.02	25	22	0	0	0	0	0
C7	29	300	0.24	25	288	0	12	4	4	0
C7	30	367	0.30	25	323	0	44	12	20	8
C7	31	148	0.12	25	148	0	0	0	0	0
C7a	32	594	0.48	25	570	0	24	4	4	0
C7a	34	573	0.46	25	573	0	0	0	0	0
C8	35	440	0.35	25	440	0	0	0	4	4
C8	36	530	0.43	25	488	21	21	8	16	8
C8	37	562	0.45	25	562	0	0	0	0	0
C9	38	483	0.39	25	483	0	0	0	0	0
C9	39	807	0.65	25	775	0	32	4	4	0
C9	40	775	0.62	25	682	31	62	12	12	0

Table A5.1: Continued.

Str	Stn	Recruit	Density	Total	Un.inf	NF.inf	Fat.inf	%Prev	% ddPCR	Diff.f
E2	41	438	0.35	25	438	0	0	0	4	4
E2	42	367	0.30	25	352	0	15	4	12	8
E2	43	584	0.47	25	584	0	0	0	4	4
E2	44	242	0.19	25	242	0	0	0	16	16
E2	45	247	0.20	25	247	0	0	0	0	0
E2	46	402	0.32	25	386	0	16	4	4	0
E2	47	781	0.63	25	656	0	125	16	16	0
E4	48	14	0.01	25	14	0	0	0	0	0
E4	49	1	0.00	10	1	0	0	0	10	10
E4	50	126	0.10	25	121	5	0	4	8	4
BK	51	18	0.01	25	18	0	0	0	0	0
BK	53	49	0.04	25	49	0	0	0	0	0
BK	54	16	0.01	25	16	0	0	0	0	0
BK	55	33	0.03	25	33	0	0	0	4	4
B6	68	189	0.15	25	189	0	0	0	0	0
B6	70	46	0.04	25	46	0	0	0	0	0
B6	72	308	0.25	25	296	12	0	4	4	0
B6	74	301	0.24	25	301	0	0	0	0	0
C2	82	95	0.08	25	95	0	0	0	0	0
C2	83	88	0.07	25	81	7	0	8	12	4
C2	84	360	0.29	25	346	0	14	4	4	0
C5a	102	1	0.00	6	1	0	0	0	0	0
C7a	117	2	0.00	19	2	0	0	0	0	0
C7a	118	185	0.15	25	178	7	0	4	8	4
BK	148	3	0.00	12	3	0	0	0	0	0
B6	157	584	0.47	25	537	0	47	8	8	0
B6	158	685	0.55	25	630	0	55	8	12	4
C5a	T1	97	0.08	25	97	0	0	0	0	0
B1	T2	252	0.20	25	232	20	0	8	8	0
E2	T3	289	0.23	25	289	0	0	0	0	0
E2	T4	199	0.16	25	191	8	0	4	24	20
C8	T5	589	0.47	25	565	0	24	4	16	12
B6a	T6	20	0.02	25	25	0	0	0	4	4
C2	T7	110	0.09	25	97	4	9	12	12	0
C3	T8	854	0.69	25	854	0	0	0	0	0
C5	T9	780	0.63	25	749	0	31	4	8	4
C6a	T10	58	0.05	25	58	0	0	0	4	4
E4	T11	143	0.12	25	143	0	0	0	0	0
E4	T12	512	0.41	25	512	0	0	0	0	0

APPENDIX 6: REFERENCES FOR SURVEYS 2010–2020

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*, OYU5) 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 (OYU5) 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 (OYU5) 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 (OYU5) 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.