ISSN 1175-1584

MINISTRY OF FISHERIES
Te Tautiaki i nga tini a Tangaroa

Analysis of a survey of the prevalence and intensity of *Bonamia* sp. in Foveaux Strait oysters

A. Dunn
K. P. Michael
P. M. Hine
N. L. Andrew
B. K. Diggles
H. J. Cranfield

New Zealand Fisheries Assessment Report 2000/32
September 2000

Analysis of a survey of the prevalence and intensity of *Bonamia* sp. in Foveaux Strait oysters

A. Dunn
K. P. Michael
P. M. Hine
N. L. Andrew
B. K. Diggles
H. J. Cranfield

NIWA
PO Box 14 901
Wellington

New Zealand Fisheries Assessment Report 2000/32
September 2000

Published by Ministry of Fisheries
Wellington
2000

ISSN 1175-1584

Citation: Dunn, A., Michael, K.P., Hine, P.M.,
Andrew, N.L., Diggles, B.K., & Cranfield, H.J. 2000:
Analysis of a survey of the prevalence and intensity of *Bonamia* sp. in Foveaux Strait oysters.
New Zealand Fisheries Assessment Report 2000/32. 28 p.

This series continues the informal
New Zealand Fisheries Assessment Research Document series
which ceased at the end of 1999.

EXECUTIVE SUMMARY

Dunn, A., Michael, K.P., Hine, P.M., Andrew, N.L., Diggles, B.K., & Cranfield, H.J. 2000: Analysis of a survey of the prevalence and intensity of *Bonamia* sp. in Foveaux Strait oysters. *New Zealand Fisheries Assessment Report 2000/32*. 28 p.

A survey in March 2000 of oyster beds in the vicinity of a reported *Bonamia* sp. outbreak confirmed the presence of *Bonamia* sp. infection and described the areal extent of the outbreak. The survey found heavy infection of *Bonamia* sp. and very recent mortality in the northwest corner of Area 2. The low prevalence, but high intensity, of infection to the southeast of Area 2 suggests that this region may experience some *Bonamia* sp. related mortality in the immediate future, though the level and timing are difficult to quantify without further analysis. It is possible that a wave of infection is progressing from the initial point of infection out into the remainder of Foveaux Strait, but the data are inadequate to confirm this hypothesis.

The low prevalence and intensity of infection immediately to the south of Area 2 suggest little *Bonamia* sp. related mortality is likely in that area in the immediate future. However, the *Bonamia* sp. epizootic in the late 1980s also began in a similar initial location, and then proceeded to spread throughout Foveaux Strait over a period of several years. It is possible that the recent outbreak will also spread in a similar manner.

The impact of *Bonamia* sp. related mortality on the commercial (recruited) population is difficult to quantify without further analysis. Estimated recent mortality of recruited oysters (as determined by the proportion of recruit sized new clocks) within Area 2 was about 12% (95% confidence interval 11–13%), with peak mortality of 56% (95% confidence interval 48–64%). A further 10% (95% confidence interval 5–15%) of recruited oysters within Area 2 showed signs of infection. Outside Area 2, recent mortality was 2%, and 2% showed signs of infection. Without knowledge of transmission rates, and the spatial and temporal relationship between infection, intensity, and subsequent mortality, estimates of the future impact are speculative. In addition, little is known about infection and mortality of pre-recruit or smaller oysters, and hence the impact of disease on recruitment.

We discuss four mechanisms for the epizootic: (1) the disease is triggered by high oyster densities; (2) that the occurrence and spread of disease are related to the susceptibility of exposed oysters; (3) that new, virulent strains of *Bonamia* sp. have infected oysters; and (4) that the occurrence and spread of disease results from exposure of infected oysters to stress. However, there is insufficient information of the relationship between *Bonamia* sp. and its oyster host to confirm or discriminate between such mechanisms. Simple theories suggest that high oyster density may be a necessary, but not sufficient, precondition for outbreak of disease; it is probable that several factors may interact in triggering the onset of disease. Whether the disease will spread into other areas of Foveaux Strait with high densities is open to speculation, although previous experience with the *Bonamia* sp. epizootic in the late 1980s suggest that such spread is possible. When more information is available, it can be used to develop predictive models for this and future outbreaks. Without such models, it is impossible to manage *Bonamia* sp. in the fishery in any but a reactive sense.

1. INTRODUCTION

1.1 Objectives

The discovery of the *Bonamia* sp. protozoan in Foveaux Strait oyster fishing beds in early 2000 raised fears of a repeat of the 1986 to 1992 *Bonamia* sp. epizootic. That epizootic reduced the number of oysters in Foveaux Strait to about 10% of the estimated level in 1975, and resulted in the fishery being closed for a number of years. In response to the discovery of the parasite, the Ministry of Fisheries and the Bluff Oyster Management Company (BOMC) contracted NIWA to survey the affected region to determine the prevalence and intensity of *Bonamia* sp. in the surrounding areas within Foveaux Strait.

This report presents information on oyster density, recent mortality, prevalence, and intensity of infection from the *Bonamia* sp. protozoan. The survey began on 23 March 2000 and required three fishing days for field sampling to be completed. A further 14 days were required to read and analyse the heart imprint data. The survey aimed to map the known focus of initial infection and provide data on possible spread of the disease and associated mortality among the recruited oyster population in surrounding areas.

Specifically, the project objectives were (1) to determine the extent of *Bonamia* sp. infected oysters around the area where the infection was first detected; and to establish whether a wave of infection exists and, if so, where the wave front was located; (2) to describe the prevalence and intensity of infection from heart imprints and resulting mortality from the ratio of new clocks and live oysters; (3) to collect information on the density of live recruit (legal sized, i.e., with length of 58 mm and greater) oysters and recruit sized new clocks as an indication of the impact of mortality from *Bonamia* sp. on the commercial population size; and (4) to develop hypotheses about the relationship between the infection by *Bonamia* sp., oyster mortality caused by *Bonamia* sp., and the density of new clocks.

1.2 The history of the Foveaux Strait oyster fishery

The Bluff oyster (*Tiostrea chilensis*) has been commercially fished in Foveaux Strait for more than 130 years. The annual landings of oysters, fishing effort, and the size of the area exploited by fishers steadily increased up until 1986 (Cranfield *et al.* 1999a). The *Bonamia* sp. epizootic between 1986 and 1992 (Doonan *et al.* 1994) devastated the Foveaux Strait oyster population. From the initial focus of infection in the western oyster beds, the disease spread through the population to reached the periphery of oyster distribution in 1992 (Doonan & Cranfield 1992). In 1992, the size of the oyster population in the area surveyed in 1975 was less than 10% of that present in 1975, and future recruitment was considered to be at risk (Doonan *et al.* 1994). The fishery was partially closed to fishing in 1992, and fully closed in 1993, to allow the population to rebuild.

By 1995, infection by *Bonamia* sp. had almost completely disappeared (Cranfield *et al.* 1995) and disease was considered unlikely to cause further mortality of oysters in the immediate future. Estimates of the size of the oyster population in 1992 and 1993 suggested that oyster numbers were increasing (Cranfield *et al.* 1993), and by 1995 were large enough to sustain limited commercial fishing (Cranfield *et al.* 1996). The Minister of Fisheries reopened the fishery in 1996 with a quota of 14.95 million oysters. Between 1993 and 1995 the population of recruited oysters in Foveaux Strait had increased from 283 (178–402) million to 639 (448–949) million.

A population survey in 1995 estimated the size of the immediate pre-recruit (50–57 mm in length) oyster population to be 285 (196–418) million (Cranfield *et al.* 1996). Most of these oysters were expected to recruit into the oyster fishery within two years. However, the survey in

1997 found that the size of the recruited oyster population, 630 (395–899) million, was unchanged from that in 1995 (Cranfield *et al.* 1999b). It is not known if the failure to detect any population change over this period is a result of sampling technique or poor recruitment.

Before the *Bonamia* sp. epizootic occurred, fishers had a choice of a up to 50 recognised oyster beds to fish (Cranfield *et al.* 1999b) and employed an informal rotational harvesting strategy, abandoning beds which did not produce a commercially acceptable catch rate (Allen 1979). At this time, oysters were distributed in a number of widely separated, discrete small dense beds considered as stable entities (Cranfield *et al.* 1999b). In 1975–76 the fishery exploited 374 km^2 of the 1200 km^2 of oyster bearing ground delineated in 1962 by Stead (1971). Ninety one percent of the total oyster population in the 1975–76 survey area was located in about 50 small dense beds of oysters that together covered only 12 km^2 of the seafloor (Allen 1979).

Between 1986 and 1992, mortality from *Bonamia* sp. progressively destroyed most of these dense beds of oysters. This catastrophic mortality in the established fishery area (the 1975–76 survey area) forced fishers to expand the size of the area fished. The fishery area expanded to the known limits of the oyster fishery in Foveaux Strait, ahead of the wave of mortality (Doonan *et al.* 1994). The grid surveys between 1990 and 1993 sampled on too coarse a scale to resolve small dense oyster beds (Cranfield *et al.* 1999b). Stratified random surveys in 1995, 1997, and 1999 were aimed at estimating population size, although data from these surveys can be used to look at macro scale distribution of oysters, the distribution of sampling was too widely spread to define small oyster beds. Following the epizootic, the number of oyster beds had declined, catch rates dropped, and fishing focused on fewer remaining oyster beds.

In 1999, the Ministry of Fisheries and the Bluff Oyster Management Company commissioned NIWA to reassess the method for determining the commercially viable population of oysters. Previous estimates had determined the commercial population as that part of the total population contained in beds with recruited densities over 1.95 oysters m^{-2} (400 oysters per standard 0.2 nautical mile tow). The October 1999 population survey determined the commercial population as the total population contained within areas supporting a commercially viable density of oysters. The data from previous surveys and logbooks of fishers from 1999 were used, by fishers, to define the commercial areas, as well as exploratory areas (areas of possible future commercial density) and the remaining non-commercial area. The subsequent survey effort focused on the commercial areas, with the exploratory and non-commercial areas receiving less sampling effort. The TACC for the 2000 fishing year was based on an estimate of the total recruited population within the commercial areas.

The recovery of localised populations since the early 1990s has not been uniform. Dense populations of recruited oysters are still absent in eastern Foveaux Strait and in those western areas most heavily affected by *Bonamia* sp., where dense patches were common before the epizootic. However, survey data from 1990 to 1999 indicate that dense aggregations of oysters are capable of rebuilding to commercial densities over a five to seven year period. There are some signs of rebuilding in western and eastern Foveaux Strait, but the nature and strength of this rebuilding remains poorly described within the published literature.

1.3 The biology and pattern of infection of *Bonamia* sp.

Bonamia spp. are protozoans currently classified in a group called the Haplosporidia and a super group called the Alveolata — which includes apicomplexans (*Toxoplasma*, coccidians, malaria), ciliates, and dinoflagellates (e.g., toxic and non-toxic planktonic algae) (Flores *et al.* 1996). Molecular evidence suggests that haplosporidians are more closely related to dinoflagellates, but the cycle of divisions more closely resembles apicomplexans. *Bonamia* sp. occurs in oysters in southern New Zealand (Dinamani *et al.* 1987), Australia (Hine 1996), and probably Chile (Campalans *et al.* 2000). The biology of the parasite is known only from New Zealand (Doonan

et al. 1994, Hine & Jones 1994, Hine 1996). However, it is very closely related to *Bonamia ostreae* in the United States and Europe. *Bonamia ostreae* infects not only the natural host *Ostrea edulis,* but also *Ostrea angasi* (Bougrier *et al.* 1986), *Ostrea puelchana* (Pascual *et al.* 1991), and *Crassostrea rivularis* (Cochennec *et al.* 1998) introduced into France. It seems likely that *Bonamia* sp. will infect any member of the genus *Ostrea*, but does not infect Pacific oysters, *Crassostrea gigas* (Chagot *et al.* 1992).

In New Zealand flat oysters (also known as Bluff, Dredge, or Foveaux Strait oysters), *Bonamia sp.* is an obligate parasite of haemocytes. It has to be recognised as foreign and phagocytosed by the haemocyte to gain entry into the cell. In order to survive inside the phagocytic oyster haemocytes, it modifies the host membrane, bounding the parasitophorous vacuole and preventing fusion of host cell lysosomes. This prevents the release of acid hydrolases that would usually lead to the destruction of the parasite (Hine & Wesney 1994b). In this, its survival mechanism closely resembles that of *Toxoplasma gondii* and other protozoans that parasitise phagocytes.

After entering the haemocytes *Bonamia* sp. grows and divides, using food phagocytosed by the haemocyte as its food source. One *Bonamia* sp. in a haemocyte may divide to produce 20 *Bonamia* sp. before the haemocyte lyses and releases the progeny. These are then phagocytosed and grow and divide to continue the cycle. To contain the parasite, the oyster stops producing gametes and diverts its energy into producing more haemocytes. To recycle the energy in already formed gametes, the oyster haemocytes phagocytose the gonad and in so doing provide nutrition for the parasite. Eventually the oyster dies of exhaustion. Figure A1 in Appendix A shows the condition of typical oysters with various levels of disease.

In late winter each year the parasite occurs at very low levels in apparently healthy oysters. It can be found just under the basement membrane of the main gut in the oyster, suggesting this may be a route of entry (Hine 1991a, 1991b). The parasite starts to occur in greater numbers in November to December, when many oysters are going through the male reproductive cycle. By February, *Bonamia* sp. can be found in haemocytes throughout the connective tissue of the digestive gland and in the gills. At about that time most oysters are in the female cycle, though few reach spawning condition, and haemocytes enter the ovary to absorb the lipid-rich ova. *Bonamia* sp. has a lipid-based metabolism and rapidly utilises the lipid from the ova after it has been endocytosed by the haemocyte. It rapidly grows and divides, causing massive proliferation resulting in elevated oyster mortality from March to May (Hine 1991b, Hine & Wesney 1994a). From May to August *Bonamia* sp. enter a late developmental phase, with increasing senescence among the parasite population (Hine 1992, Hine & Wesney 1992), leading to an apparent population collapse of *Bonamia* sp. (Hine 1991b). The relationship between prevalence, intensity, density of oysters, and the probability of an outbreak are poorly understood.

Apart from likely host range, we can assume that some of the information available on *Bonamia ostreae* is also applicable to *Bonamia* sp. *Bonamia ostreae* readily transmits horizontally (i.e., from oyster to oyster) and directly (Culloty & Mulcahy 1996). It has a pre-patent period (when it cannot be detected) of about 10–18 weeks (Grizel *et al.* 1988, Montes 1991). Unpublished information at NIWA suggests that the transmission and pre-patent period are similar for *Bonamia* sp. The 50% infectious dose, determined from inoculation with 10–10^6 *Bonamia ostreae*, is 80 000 *Bonamia ostreae* per oyster for a three year-old (Hervio *et al.* 1995). Oysters differ in their susceptibility to *Bonamia ostreae* (Naciri-Graven *et al.* 1998), with this difference in susceptibility probably being genetically determined. When oysters are stressed by interference such as fishing, the prevalence and intensity of the parasite can increase (van Banning 1991). However, the extent of this additional infection rate is not well known, and may not be high. Oysters resistant to the disease are less affected by stress than those that are susceptible (Baud *et al.* 1997). There is some evidence that oyster boats may spread the infection (van Banning 1991, Howard 1994).

Bonamia sp. has probably parasitised oysters in southern Australia, New Zealand, and Chile for several million years. The annual pattern of infection suggests that the oyster and *Bonamia* sp. are well adjusted to each other and probably a large proportion of oysters in Foveaux Strait have the parasite at low levels throughout the year.

1.4 The preliminary study of *Bonamia* sp. prevalence and intensity

Reports in early March 2000 of high numbers of freshly dead shells (new clocks) in the north-west of the largest and densest beds (labelled as area 2 in Figure A2) and the confirmation of a very high level of infection in a single oyster sampled from Area 2, led to a preliminary study of oysters from the affected region. This was carried out by NIWA on 16 March 2000. Sixty recruit sized oysters (58 mm and greater) were sampled from the middle of the reported area of mortality (Station 3, Figure A2), and again at four sites at a distance of about 0.5–0.7 nautical miles around this point (Stations 1, 2, 4, 5, Figure A2 in Appendix A).

The samples were taken on the F.V. *Lucy Star* using commercial dredges. At each site a single 0.2 nautical mile straight line tow was used to obtain the samples, except at station 5: the crew of *Lucy Star* reported difficulty in obtaining a sample of 60 oysters from this station and made repeated tows until a full sample was obtained (indicating that the station may be located at a site of low density). No such difficulties were reported at the other four sites. At each site, an estimate of the proportion of new clocks was made from visual inspection of the dredge. Estimates of oyster density at each of the five sites are not available.

Each set of samples was returned to NIWA (Greta Point) the following day, the heart removed, and imprints of host haemocytes made on slides. The slides were read and classified into one of six categories (*see* Section 2.2 below for detail of the classification system employed). For each site, prevalence and mean intensity were calculated (*see* Section 2.2 below for the definitions of these terms). The results are shown in Table 1.

Table 1: The prevalence, intensity, and new clock ratios from the preliminary study of oysters from the five stations in Area 2

Station	Sample size	Prevalence (95% CI)[1]	Mean intensity[2]	New clocks (%)[3]
1	60	33 (22–47)	3.1	60–70
2	60	22 (12–34)	2.6	60–70
3	60	5 (1–14)	3.3	More than 75
4	60	15 (7–27)	2.6	60–70
5	60	0 (0–6)	–	10

1. Percent of infected (i.e., stage 1–5) oysters in the sample
2. The sum of intensity of infection for all oysters divided by the number of infected oysters
3. Percent of freshly dead (articulated and not fouled) recruit sized shells in the total recruit sized catch (estimated by visual inspection of the dredge)

Only 3 of the 60 oysters from the central station (Station 3) were infected, although mean infection intensity was relatively high. The high clock ratio suggested that the epizootic may have almost finished at Station 3 — a high proportion of remaining oysters were uninfected. The uninfected oysters may possibly be those with some natural or otherwise enhanced resistance to infection. Stations 1, 2, and 4 appeared to have experienced high mortality, with high new clock ratios, and the presence of a several stage 3–5 oysters at these stations suggests mortality may increase. Station 5 appeared to be uninfected, and had a corresponding low proportion of new clocks.

2. METHODOLOGY

2.1 Sampling design and operational procedure

Anecdotal reports from fishers and results of the preliminary study (Section 1.4) suggested that there was high infection of *Bonamia* sp. near the centre of Area 2. The location of Area 2 and the tow midpoints of the sampling sites are shown in Figure A2. Station numbers and locations of tow midpoints are given in Table C1 in Appendix C. Fishers did not report any other regions within Foveaux Strait as having high levels of new clocks or other signs of *Bonamia* sp. related mortality. On instructions from the Ministry of Fisheries, sampling was concentrated on Area 2 at a spatial scale designed to map prevalence and intensity of infection. Additional sample sites were chosen at regular intervals along four transect lines (Figure A2) to map possible disease spread across areas of high density along the direction of the assumed tidal flow. Three additional sites were located in Area 6, and one site directly west of Area 2, where high densities of live recruited oysters were found in the October 1999 survey.

Sampling effort was restricted to three days fishing, effectively constraining the coverage and spatial scale of sampling, and resulted in a sample size of 71 tows. Most of the sampling effort (39 tows) was allocated to grid mapping Area 2, around the assumed focus of initial infection. The remaining tows were allocated to the four transect lines (28 tows) and to selected 1999 high density stations (4 tows).

Sampling followed a similar procedure to that used for previous surveys in 1990, 1992, 1993, 1995, and 1997 (Cranfield *et al.* 1991, Doonan & Cranfield 1992, Cranfield *et al.* 1993, Cranfield *et al.* 1996, 1999b). A commercial oyster vessel, F.V. *Karaka*, was used for the survey. It used satellite derived differential GPS position fixing to ensure precise navigation. Differential corrections from a Fugo Omnistar receiver were interfaced to a Furuno GP-31 by cable using NMEA 0183 data format. NIWA staff provided the navigation and ensured continuity of sampling.

At each station, a standard commercial dredge (530 kg, 3.35 m wide) was towed in a straight line for about 0.1 nautical miles (185 m). The start of tow position was recorded when the towing warp became tight after the winch brake had been applied. Tow length was controlled by using the distance travelled and the GPS alarm features. The position of the end of the tow was recorded from the point the winch began retrieving the dredge.

Only the forward dredge was used to sample stations. Dredges were landed onto the forward cultching benches after washing (dipping the dredge). Dredges were washed to reduce the volume of bycatch and to remove small oysters (reducing the sorting time of each sample, and increasing the station sampling rate). The fullness of the dredge was visually estimated after washing.

The catch from each survey tow was sorted into recruited live oysters, recruit-sized new clocks, and recruit-sized gaping oysters, where size was determined by the failure of the oyster to pass through a 58 mm diameter reference ring, and the numbers of recruited live oysters, recruit-sized new clocks, and recruit-sized gapers were counted. The data recording form is given as Appendix B. A sample of 65 recruited oysters (or less when the total catch was less) was randomly selected from each catch, bagged, and shipped to NIWA (Greta Point) for the heart imprints and histology samples. When less than 65 recruited oysters were caught, oysters down to a size of 50 mm were added (where available) to make up numbers to at least 50 (Table 2). The data recorded at each station included start and finish location of the tow; numbers of oysters, new clocks, and gapers caught; percentage fullness of the dredge; wind force; and sediment type. No data on pre-recruit-sized oysters (50–57 mm), small oysters (less than 50 mm), or old clocks was recorded.

Table 2: Stations, location, and samples for stations where additional pre-recruit sized oysters were included in cytology analyses

Station number	Longitude	Latitude	Live recruits	Additional samples	Total samples
77	168°14.789' S	46°46.859' E	35	15	50
80	168°10.213' S	46°45.187' E	21	15	36
87	168°09.058' S	46°45.188' E	7	10	17
91	168°09.258' S	46°47.067' E	42	8	50
140	168°10.167' S	46°41.223' E	25	3	28
145	168°09.382' S	46°40.497' E	28	22	50

New clocks are the articulated shells of recently dead oysters with the ligament attaching the two valves intact, and typically defined as those that are clean and without any fouling on the inner surface. When surveys are carried out during October in each year, new clocks are the shell of those oysters that died since previous summer (i.e., within the six month period immediately before the survey). The shells of oysters that are fouled are termed old clocks. Old clocks are covered in fouling organisms on both external and internal surfaces, and as the ligament of oysters breaks down over a three-year period, old clocks represent oysters that died between 6 months and 3 years ago (Cranfield *et al.* 1991).

Anecdotal reports of large numbers of new clocks present at a single site in Area 2 during January 2000 suggests that some mortality may have occurred during the periods when fouling organisms settle. In addition, sampling suggested that a number of clocks were new (as determined by colour and condition) but also had a small amount of fresh fouling. Hence, for this survey, new clocks were defined as the articulated shells with shiny fresh green inner shells (nacreous layer) and very light levels of fresh fouling.

Gapers are oysters in which the two shells are parted, and when the shell is tapped, the shell does not close, i.e., where the adductor muscle has lost its ability to contract. The numbers of gapers found at all sites was minimal, with only 27 gapers recorded in total. We assume that gapers are exhausted and close to death, and combined these with the number of new clocks in all analyses below. Hence, we assume that new clocks reflect the mortality of oysters over the period between late 1999 and early 2000.

2.2 Determining the prevalence and intensity of *Bonamia* sp.

From the sampled 65 oysters from each station, a random sub-sample of 50 was selected after removing any obviously dead oysters. These were opened, the heart removed, and imprints of host haemocytes made on slides. From every fifth oyster, a section was taken through the digestive gland and fixed in Davidson's solution for histology (but not analysed). Another small piece of digestive gland was fixed in 70% ethanol for later *Bonamia* sp. DNA probe development.

The heart imprints from each oyster were stained with Hemocolor®, oven dried, and examined under the microscope. The infection observed was then classified into one of six categories:

Stage 0 Not infected.
Stage 1 One *Bonamia* sp. observed after examining an imprint.
Stage 2 More than 1, but less than 10, *Bonamia* sp. observed after examining an imprint.
Stage 3 More than 10 *Bonamia* sp. present in the imprint, but few parasites in each haemocyte.
Stage 4 *Bonamia* sp. present in many haemocytes of each imprint, and many parasites in each haemocyte.

Stage 5 *Bonamia* sp. present in nearly all haemocytes of each imprint, many parasites in each haemocyte and extracellularly.

Previous correlation studies with histopathology (NIWA, unpublished data) suggest that stages 1 and 2 are relatively light focal infections and do not appear to affect the health of the host. Stage 3 infections are elevated and systemic, with minor tissue damage throughout the host. It appears likely they will 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.

Examination of imprints is less sensitive than histology, but whereas histology is time consuming and expensive, heart imprints can be rapidly screened and are comparatively cheap. The correlation studies with histopathology have shown that the prevalence estimated from heart imprints can underestimate the true infection rate by about 10%, . All histology samples have been archived at NIWA, and are available for any future work.

For each station, **prevalence** is defined as the proportion of oysters in a sample with at least one *Bonamia* sp. cell observed (i.e., the number of stage 1–5 oysters divided by the number of all oysters examined in the sample). Mean **intensity** is defined as the mean frequency of stage 1–5 oysters (i.e., the mean stage of all oysters examined that had at least one *Bonamia* sp. cell observed). The inclusion of the additional smaller oysters at sites where low numbers of recruited oysters were caught is likely to introduce a bias into estimates of prevalence and intensity. Sites where additional small sized samples were included were distant from focal point of infection and had low densities of recruits — so any such bias is likely to be small.

Exact 95% confidence intervals are given for prevalence and for the proportion of new clocks, determined from the *F*-distribution, i.e., for a proportion π, where $\pi = r/n$, then the 95% confidence interval is determined by

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

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

2.3 Density surface estimation

The estimates of population and *Bonamia* sp. infection density surfaces were made using kriging (Ripley 1981, Cressie 1991), i.e., we assume a state space process $Z(t)$: $t \in T$, defined over some rectangular region A, with mean defined as $m(t) = \mathrm{E}[Z(t)]$ and variance $C(u,v)=\mathrm{Cov}[Z(u),Z(v)]$. Further, we assume a covariance function $C(r) = c(d(r))$ that is some function of the Euclidean distance r. Surfaces were fitted without trend, with an exponential covariance function $C(r) = \sigma^2 e^{-r/d}$ and distance parameter typically $d = 0.7$ nautical mile (determined from the visual inspection of semivariogram diagnostic plots). Models were fitted using log transformed density estimates, and maps produced by back-transforming the kriged estimates. We assume no nugget effect, and hence employ the method as an exact interpolator, although the log transformation introduces a bias into interpolated estimates.

Surface density estimates have been presented into units of oysters per metre^2 computed from the number of oysters found (N), the estimated length of the tow (d), an assumed dredge

efficiency of $q = 0.1656$ (Doonan *et al.* 1994), and dredge width of $W = 3.35$ m, and hence calculating the absolute density as $N/(qWd)$.

Kriging is a statistical methodology that produces visually appealing contour and surface plots from regularly or irregularly spaced data. The procedure predicts values suggested by the data onto a regular grid by modelling the relationship between the location and values of the samples (Ripley 1981, Cressie 1991). However, this method requires some assumptions of the nature of this relationship and can, particularly in sparsely sampled locations, make predictions with little supporting data. In addition, the distance parameter d suggests that the covariance operates over a distance that is close to the nyquist frequency, and hence the small scale resolution is unlikely to be accurate. In addition, assumptions of stationarity are unlikely, and any long range correlations have been ignored in the kriged estimates. Consequently, some caution is required when interpreting the density maps and subsequent derived data. Density maps estimated using kriging have been masked so that interpolated points at a distance of more than 1 nautical mile from the nearest sample location are not shown.

The use of kriging methods in this report differs from the summaries of the spatial pattern previously used, where surface plots were estimated using kernel density smoothing methods (Wand & Jones 1995). Kernel density methods produce surface maps that can account for macro scale variation, but smoothes the small scale spatial variation.

3. RESULTS

3.1 Prevalence and intensity of infection

Seventy one sites were sampled during the survey. The data recorded for each site and summary figures of collected variables are given as Table C1 and Figures C1–C6 in Appendix C. Wind and sea conditions were recorded as light to moderate. Tow lengths, calculated from the differential GPS longitude and latitude of the start and end of tow, were all close to the length specified by the survey design. The fullness of the dredge ranged from two to 70%, with indications of a small degree of saturation of the dredge on high density tows.

Heart imprints showed infection of *Bonamia* sp. in about 50% of all sites, with the prevalence exceeding 40% in three sites. In sites with *Bonamia* sp. the mean intensity was 2.12. About three-quarters (78%) of the sites strictly within Area 2 (*see* Figure A2) surveyed showed indications of presence of *Bonamia* sp., and had mean intensity 2.15. Table 3 shows summary statistics for all stations, and for those stations that lie strictly within the boundary of Area 2. Summary data of the results of the heart imprint reading are given in Table C1 (Appendix C).

Moderate levels of intensity were found throughout Area 2 and down to the south-east along Transect 1, with much reduced levels to the south of Area 2. Tabulated numbers of sites by prevalence and intensity are given in Table 4. Circle plots showing the distribution of prevalence and intensity across the survey region are given in Figure 1.

Figure 2 shows the density of new clocks and estimated density of infected oysters by site. The proportion of new clocks was high in the northwest of Area 2, declining to the east. The proportion of new clocks was close to zero in all stations outside Area 2 — suggesting that recent mortality was restricted to Area 2. The picture is similar for the density of infected oysters (Figure 2b), except for some indications of infected oysters along Transect 2, southeast of Area 2.

Table 3: Summary statistics for all stations, and for those stations within Area 2 (*see* Figure A2)

	Area 2	All except Area 2	All sites
Number of sites sampled	27	44	71
Mean density of live recruits (oysters m^{-2})	3.28 (2.51–4.05	2.76 (2.25–3.27)	2.96 (2.52–3.39)
Mean density of new clocks (oysters m^{-2})	0.44 (0.20–0.68	0.05 (0.02–0.09)	0.20 (0.10–0.30)
Number of sites with *Bonamia* sp.	21	15	36
Mean prevalence of *Bonamia* sp. (%)	10.0 (5.0–15.0)	1.9 (0.0–3.9)	5.0 (2.6–7.4)
Mean intensity of *Bonamia* sp.	2.15	2.07	2.12
Mean density of infected oysters (oysters m^{-2})	0.33 (0.16–0.51	0.05 (0.01–0.11)	0.16 (0.08–0.24)

The October 1999 population survey of Foveaux Strait found the highest density of recruited oysters in Area 2, with another high density, but smaller, patch in Area 5. The estimated density of live recruited oysters from the October 1999 survey is given in Figure A3 (live recruits 1999). This shows the kriged surface of oyster density estimated from the counts of live recruited oysters from that survey. Similarly, Figure A4 shows the kriged surface of density estimated from the counts of recruit-sized new clocks (new clocks 1999). The October 1999 survey found only very low proportion of new clocks (less than 0.05%) throughout Foveaux Strait, except at one site at the southwest of the centre of Area 4, about 6%.

When we compare the estimates of oyster density and new clock density from October 1999 with the results of the March 2000 survey, some patterns emerge. Figure A5 shows the kriged surface estimated from the counts of live recruits (live recruits 2000), and Figure A6 shows the kriged surface from the counts of new clocks (new clocks 2000). A comparison of Figure A3 (live recruits 1999) with Figure A5 (live recruits 2000) suggests that the distributions from both surveys, as would be expected, are similar. There was perhaps some indication that there are fewer oysters in March 2000 in the northwest corner of Area 2 than were found in 1999. However, the resolution of sampling is coarse when compared to the small-scale variation in oyster density, and makes localised comparisons circumspect.

A clearer picture arises from comparing estimated surfaces shown in Figure A4 (new clocks 1999) with Figure A6 (new clocks 2000). There was a large change in the proportions of new clocks observed between the two surveys. In 1999, a maximum proportion of 6% new clocks was observed. In 2000, this had increased to about 56% (95% confidence interval 48–64%), with most of the recent mortality, as represented by new clocks, found in the northwest corner of Area 2. As Figure A7 confirms, the sites of high recent mortality are in a similar location to the sites with the highest prevalence of *Bonamia* sp. Figure A8 shows the spatial distribution of infection intensity (*see* also Figure 1). Moderate levels of intensity were found across most of Area 2 and along Transect 1 — even though the estimated prevalence was low outside of Area 2.

Evaluation of the prevalence and recent mortality along the four sampling transects (projected through Area 2, *see* Figure A9), gives some indication of the relationship between prevalence and associated mortality among the recruited population. Arbitrary lines (and labelled transects 1–4) placed along the sites from the four sampling transects and projected into Area 2 were drawn, and the values of various parameters evaluated from the kriged surface along these lines.

Figure 3 shows the estimated proportion of new clocks, estimated proportion of infected live recruits, and estimated density of live recruits, evaluated along a line through the estimated (kriged) surfaces. There was a positive association between the estimated proportion of new clocks and prevalence of *Bonamia* sp.; at high levels of prevalence a high proportion of new clocks are found and vice versa. Less clear was any relationship between the level of infection and the density of live recruits, with no obvious association between density and prevalence. These relationships need to be explored in more detail and require further analysis.

Table 4: Number of stations by prevalence and intensity of *Bonamia* sp. for all stations and for those stations within Area 2 (*see* Figure A2)

Prevalence (%)	Area 2	All except Area 2	All sites	Intensity	Area 2	All except Area 2	All sites
0	6	29	35	Not applicable	6	29	35
1–10	13	14	27	1–1.9	10	7	17
11-20	4	0	4	2–2.9	11	6	17
21–40	2	0	2	3–3.9	0	2	2
41–60	2	1	2	4–4.9	0	0	0

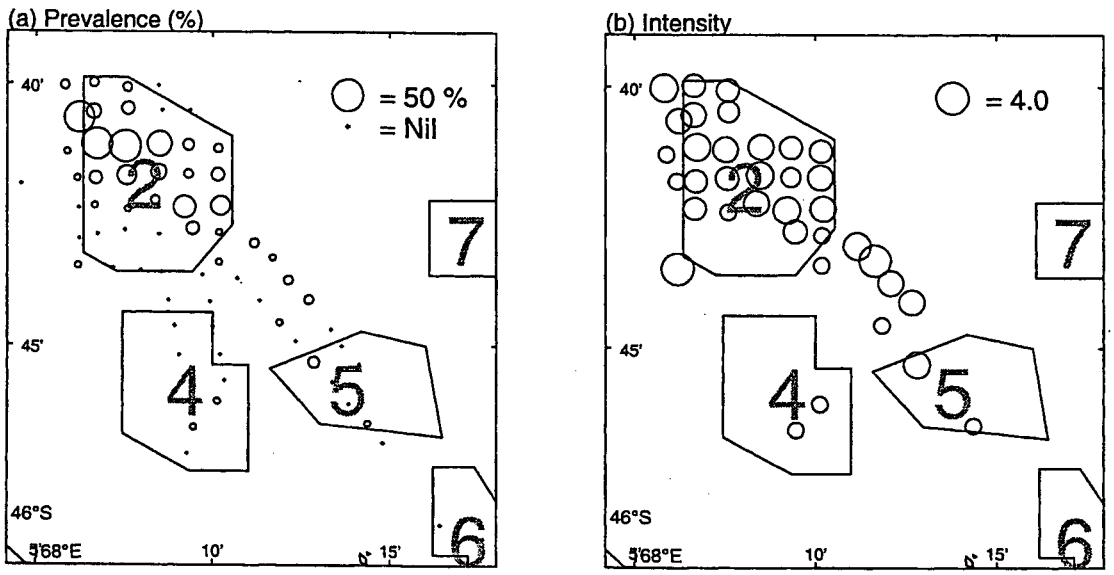

Figure 1: Circle plots of (a) the prevalence of *Bonamia* sp. and (b) mean intensity of infection, with symbol area proportional to plotted value, March 2000 survey.

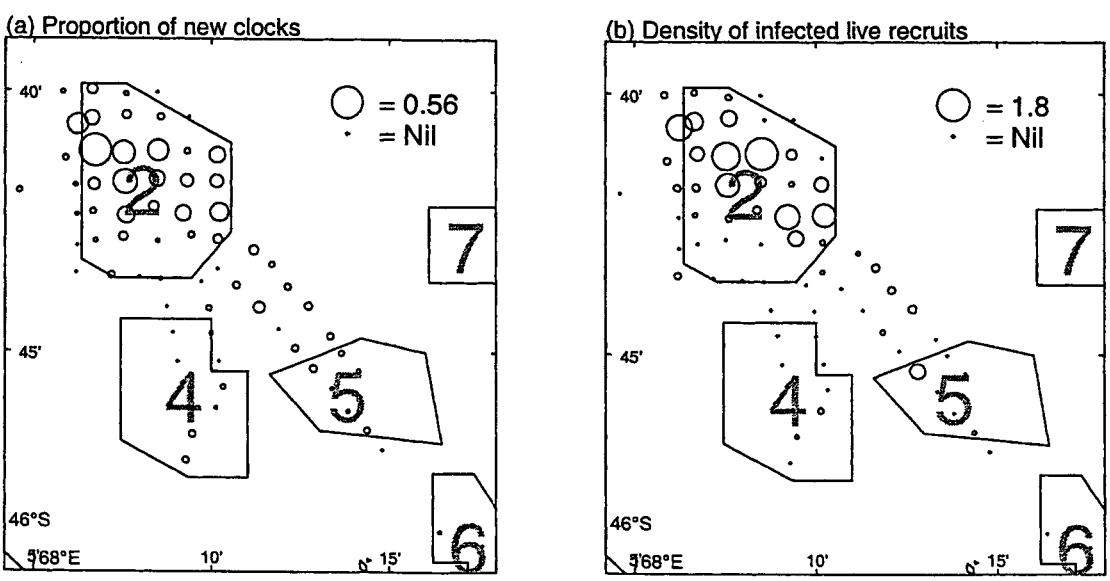

Figure 2: Circle plots of (a) new clocks as a proportion of the number of live recruits plus new clocks, and (b) density of infected oysters (oysters m^{-2}), with symbol area proportional to plotted value, March 2000 survey.

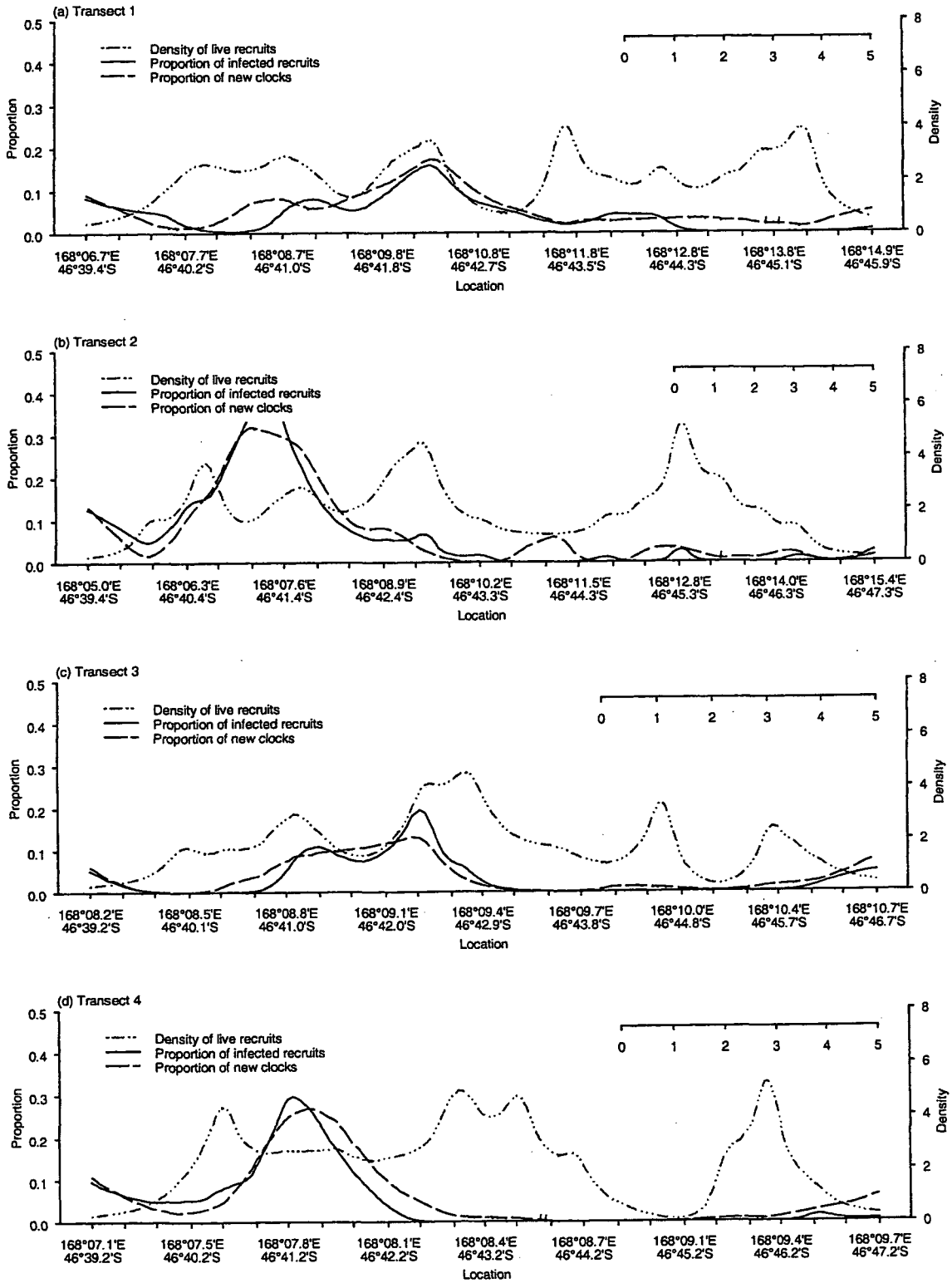

Figure 3: Estimated new clocks (recruit size) and estimated infected oysters as a proportion of total recruited live oysters plus new clocks (left axis), and estimated density of live recruits (right axis), for lines drawn along the four sampling transects and projected into Area 2, March 2000 survey. These are labelled transects 1–4 (*see* Figure A9 for detail). The scale for each figure is given in kilometres.

4. DISCUSSION

The survey found a high rate of infection of *Bonamia* sp. and very recent mortality in the north-west corner of Area 2. Here, the proportion of recruit-sized new clocks reached the maximum observed value of 56% (95% confidence interval 48–64%). The presence of heavily infected oysters to the southeast of Area 2 suggests that this region may experience some *Bonamia* sp. related mortality in the immediate future, though the dynamics cannot be quantified without further analysis. It is possible that a wave of infection is progressing from the initial point of infection out into the remainder of Foveaux Strait, but the data are inadequate to confirm this hypothesis. The low prevalence and intensity of infection immediately to the south of Area 2 suggest little *Bonamia* sp. related mortality is likely in this area in the immediate future, but it is possible that this are will become highly infected over the next few years.

The impact of *Bonamia* sp. related mortality on the total commercial population is difficult to quantify without a more detailed analysis. Estimated recent mortality (as determined by the proportion of new clocks) within Area 2 was about 12% (95% confidence interval 11–13%), with a peak mortality of 56% (95% confidence interval 48–64%). A further 10% (95% confidence interval 5–15%) of recruited oysters show signs of infection. Outside Area 2, recent mortality was 1.9% (95% confidence interval 1.6–2.1%), with peak mortality of 23% (95% confidence interval 19–28%), and a further 2.0% (95% confidence interval 1.7–2.2%) of recruited oysters showed signs of infection. Without knowledge of transmission rates, and the spatial and temporal relationship between infection, intensity, and subsequent mortality, estimates of the future impact are speculative. In addition, little is known about infection and mortality of pre-recruit or smaller oysters, and hence the impact of disease on recruitment.

Care must be taken when interpreting these results as disease transmission and spread are dynamic processes. For example, no new clocks and high infection levels suggest a mortality event is about to occur; moderate numbers of new clocks and high or moderate levels of infection suggest a mortality event is occurring; and high numbers of new clocks but moderate or low levels of infection suggest a mortality event has occurred but is now waning. Repeated sampling at selected locations over a period of time would help test hypotheses of disease transmission.

As *Bonamia* sp. transmits horizontally and directly from oyster to oyster, infection probably depends on how many infectious particles a healthy oyster is exposed to as well as the susceptibility of the oyster to the parasite. The latter may be a combination of the oyster genotype and environmental stress (e.g., environmental conditions, fishing pressure, starvation). The causes of this outbreak are unknown. Below we summarise four non-exclusive hypotheses concerning the onset and spread of *Bonamia* sp.

Hypothesis 1: That the occurrence and spread of disease is triggered by high oyster densities.

> This appears to be a credible explanation, as it is known that the parasite transmits directly from oyster to oyster. The infection levels build up in the dense oyster beds, for unknown reasons, until they reach a threshold. At this point, some die of the disease, releasing *Bonamia* sp. and hyper-infecting nearby oysters. These then die and mortality cascades through the population. This was suggested as a plausible factor in the mortality starting in 1986 (Hine 1996). However, the threshold densities that may be required are unknown. Alternative and confounding explanations may also exist. For example, it is possible the *Bonamia* sp. and oyster relationship follows a predator-prey like cycle, and oyster population crashes may be an episodic feature of natural population regulation.

This hypothesis could be investigated by determining the distance over which the parasite may be transmitted, and determining differences in its rate of spread among oysters at high and low density. Such information can be ascertained by relatively simple experiments, and validated by studies on wild stocks.

Hypothesis 2: That the occurrence and spread of disease are related only to the susceptibility of exposed oysters.

There is a wealth of evidence that oysters vary greatly in their susceptibility to disease, although much less is known about variations in the pathogenicity of parasites. The observed low prevalence with a few highly infected oysters (as found in many sites in eastern Area 2 and to the southeast) may be due to susceptibility of a few individuals with the remaining oysters having some resistance. After the high mortality in the late 1980s and early 1990s, susceptible oysters would have died before resistant oysters, so many oysters surviving at the end of the event would have a high degree of resistance. Changes in susceptibility are usually genetic, though changes in environmental conditions can contribute.

In Europe, oysters are being selectively bred for their resistance to *Bonamia ostreae*, and in the United States, oysters resistant to *Haplosporidium nelsoni* (closely related to *Bonamia* sp.) have been bred for the commercial industry for many years. When flat oysters from Bluff, Tasman Bay, and Port Chalmers, being held under identical conditions at Mahanga Bay, were infected with *Bonamia* sp. from oysters in Wellington Harbour, the Bluff oysters succumbed first, the Tasman Bay oysters next, and the Port Chalmers last (M. Hine, unpublished data).

There is no published information about the relative susceptibility of oysters. It could be investigated by exposing oysters from different locations around New Zealand to a uniform challenge with *Bonamia* sp., as has been done for *Bonamia ostreae* (see Hervio *et al.* 1995).

Hypothesis 3: That strains of *Bonamia* sp. exist, that some are more virulent than others, and that the epizootics are due to increase in numbers of virulent strains.

It is well known from human and veterinary medicine that many micro-organisms exist as a variety of strains, and that some strains are more virulent than others. Virulence is genetically determined, and many virulence genes have been identified. However, distinct and different strains usually arise when populations of the micro-organism become isolated, due to genetic drift (the slow change from one distinct type — a genotype — into a genetically different genotype) and in-breeding. Such populations are usually isolated by geographical distance, as in the case of *Perkinsus marinus*, a dinoflagellate-like parasite of eastern oysters (*Crassostrea virginica*), which extends south from Maine, northeast USA, to Venezuela, and which over that range exists as four genotypes. Disease becomes particularly severe when different host and parasite genotypes are mixed. This is because within a limited and stable range (no introductions or other movements of stocks) the host and parasite are usually adapted to each other and no disease results. However, when genotypes are mixed, there is no adaptation and disease can result.

The small size of Foveaux Strait make it very unlikely that new strains of *Bonamia* sp. will arise within its oyster population. Although it is possible to purify *Bonamia* sp. from oysters from different locations around the South Island and lower North Island, the historic widespread movement of oysters between these areas in relatively recent

times, and the remote probability that new strains have arisen, make such research of low priority.

Hypothesis 4: That the occurrence and spread of disease results from exposure of infected oysters to stress.

It is thought *Bonamia* sp. and the New Zealand flat oyster have co-existed for many centuries, and that they are therefore highly adapted to each other. Consequently they may normally exist in a steady state in which the parasite is tolerated by the host, and does not proliferate to cause disease. In nearly all reported epizootics, mortality has directly or indirectly occurred in association with human activity, and there is some evidence that simply handling oysters subjects them to stress, making them susceptible to pathogens. The evidence for this is not well founded, although the belief is widespread. In the current case it seems that those beds in which the disease has occurred have been subject to only light fishing in the 6 months before the start of the fishing season. Whether this light fishing was a causal or contributing factor in the current outbreak is unknown. Alternatively, oysters may be stressed by natural environmental events, such as storms or food availability, or changes in the natural environment (see, for example, Cranfield *et al.* 1999a), and these events may trigger the onset of disease.

There is no current information to support or contradict this hypothesis. Experiments to test the effects of stress on the oyster/*Bonamia* sp. interaction could be easily conducted.

There is insufficient known of the relationship between *Bonamia* sp. and its oyster host to discriminate between causal mechanisms. High oyster density may be a necessary but not sufficient precondition for outbreak of disease. Whether the disease spreads into other areas of Foveaux Strait with high densities is open to speculation, but the previous *Bonamia* sp. epizootic spread from an similar initial point of infection throughout most of Foveaux Strait over several years (Doonan *et al.* 1994). The high correlation between new clocks and prevalence suggests that the data currently being collected by fishers in logbooks will be an important source of new data. In general, more information is needed to understand such situations, and when available, can be used to develop predictive models. It is impossible to manage *Bonamia* sp. in the fishery in anything but a reactive way, until such predictive models have been developed.

5. ACKNOWLEDGMENTS

We are grateful for the participation and help of many people who gave their time and effort, often at short notice, in assisting in the collection, sampling, and preparation of the data for this report. From NIWA, we thank David Fisher for his efforts in overseeing the sampling in Foveaux Strait, and Mike Tait, Terese Kendrick, Peter Marriott, Peter Notman, Peter Gerring, Reyn Naylor, Owen Anderson, Dean Stotter, and Jeff Forman for their help in the preparation of the heart imprints. Don Robinson and Ian Doonan provided helpful comments on the manuscript, and Alan Blacklock provided the photograph of healthy and diseased oysters. In addition, we thank Ben Tait, Marty Sharpe, Lisa De Garis, Caroline Jones, Anjali Panade, Kerstin Kroeger, George Michael, Jenny Mauger, Lynda Angus, Joanne Long, Lucy Banks, and Johanna White for help in preparing the heart imprints. From F.V. *Karaka*, we thank the skipper, Terry Dixon, and his crew of Chuck, Tiki, John, Lynne, Jason, and Jason. We thank Fisheries Officer Shaun Hogan for his advice and assistance in conducting the survey. We thank Karl Ryan and the crew of *Lucy Star* for obtaining the preliminary samples from Area 2. From Johnson's Oysters Ltd., we thank Ben Calder and his staff for packaging and delivering the

oyster samples to Greta Point. This project was funded by the Ministry of Fisheries under project code MOF 1999/04.

6. REFERENCES

Allen, R.L. 1979: A yield model for the Foveaux Strait oyster (*Ostrea lutaria*) fishery. *Rapports et Procès-verbaux des réunions Conseil Permanent International pour l'Exploration de la Mer 175*: 70–79.

Baud, J.P., Gerard, A., & Naciri-Graven, Y. 1997: Comparative growth and mortality of *Bonamia ostreae* – resistant and wild flat oysters, *Ostrea edulis* in an intensive system. 1. First year of experiment. *Marine Biology 130*: 71–79.

Bougrier, S., Tigé, G., Bachère, E., & Grizel, H. 1986: *Ostrea angasi* acclimatization to French coasts. *Aquaculture 58*: 151–154.

Campalans, M., Rojas, P., & Gonzalez, M. 2000: Haemocytic parasitosis in the farmed oyster *Tiostrea chilensis*. *Bulletin of the European Association of Fish Pathologists 20*: 31–33.

Chagot, D., Boulo, V., Hervio, D., Mialhe, E., Mourton, C., & Grizel, H. 1992: Interactions between *Bonamia ostreae* (Protozoa: Ascetospora) and hemocytes of *Ostrea edulis* and *Crassostrea gigas* (Mollusca: Bivalvia): entry mechanisms. *Journal of Invertebrate Pathology 59*: 241–249.

Cochennec, N., Renault, T., Boudry, P., Chollet, B., & Gerard, A. 1998: *Bonamia* – like parasite found in the Suminoe oyster *Crassostrea rivularis* reared in France. *Diseases of Aquatic Organisms 34*: 193–197.

Cranfield, H.J., Doonan, I.J., & Michael, K.P. 1991: Assessment of the effects of mortality due to *Bonamia* sp. on the oyster population of Foveaux Strait in 1990 and the outlook for management in 1991. N.Z. Fisheries Assessment Research Document, 91/18. 36 p. (Unpublished report held in NIWA library, Wellington.)

Cranfield, H.J., Doonan, I.J., & Michael, K.P. 1993: Foveaux Strait oyster (*Tiostrea chilensis*) assessment, 1993. N.Z. Fisheries Assessment Research Document, 93/21. 14 p. (Unpublished report held in NIWA library, Wellington.)

Cranfield, H.J., Doonan, I.J., & Michael, K.P. 1999a: Changes in the distribution of epifaunal reefs and oysters during 130 years of dredging for oysters in Foveaux Strait, southern New Zealand. *Aquatic Conservation: Marine and Freshwater Ecosystems 9*: 461–483.

Cranfield, H.J., Michael, K.P., & Doonan, I.J. 1996: Foveaux Strait oyster (*Tiostrea chilensis*) assessment, 1995. N.Z. Fisheries Assessment Research Document, 96/19. 25 p. (Unpublished report held in NIWA library, Wellington.)

Cranfield, H.J., Michael, K.P., & Doonan, I.J. 1999b: Foveaux Strait oyster (*Tiostrea chilensis*) assessment, 1997. N.Z. Fisheries Assessment Research Document, 99/11. 31 p. (Unpublished report held in NIWA library, Wellington.)

Cranfield, H.J., Michael, K.P., Wesney, B., & Doonan, I.J. 1995: Distribution of Foveaux Strait oysters (*Tiostrea chilensis*) and prevalence of infection by *Bonamia* sp. in March 1995. N.Z. Fisheries Assessment Research Document, 95/25. 18 p. (Unpublished report held in NIWA library, Wellington.)

Cressie, N.A.C. 1991: Statistics for spatial data. 1st edition. John Wiley & Sons, New York. 900 p.

Culloty, S.C. & Mulcahy, M.F. 1996: Season-, age-, and sex-related variation in the prevalence of bonamiasis in flat oysters (*Ostrea edulis* L.) on the south coast of Ireland. *Aquaculture 144*: 53–63.

Dinamani, P., Hine, P.M., & Jones, J.B. 1987: Occurrence and characteristics of the haemocyte parasite *Bonamia* sp. in the New Zealand dredge oyster *Tiostrea lutaria*. *Diseases of Aquatic Organisms 3*: 37–44.

Doonan, I.J. & Cranfield, H.J. 1992: Foveaux Strait oyster (*Tiostrea chilensis*) assessment, 1992. N.Z. Fisheries Assessment Research Document, 92/11. 29 p. (Unpublished report held in NIWA library, Wellington.)

Doonan, I.J., Cranfield, H.J., & Michael, K.P. 1994: Catastrophic reduction of the oyster, *Tiostrea chilensis* (Bivalvia: Ostreidae), in Foveaux Strait, New Zealand, due to infestation by the protistan *Bonamia* sp. *New Zealand Journal of Marine and Freshwater Research 28*: 335–344.

Flores, B.S., Siddall, M.E., & Burreson, E.M. 1996: Phylogeny of the Haplosporidia (Eukaryota: Alveolata) based on small subunit ribosomal RNA gene sequence. *Journal of Parasitology 82*: 616–623.

Grizel, H., Mialhe, E., Chagot, D., Boulo, V., & Bachère, E. 1988: Bonamiasis: a model study of diseases in marine molluscs. *Special Publication of the American Fisheries Society 18*: 1–4.

Hervio, D., Bachère, E., Boulo, V., Cochennec, N., Vuillemin, V., Le Coguic, Y., Cailletaux, G., Mazurie, J., & Mialhe, E. 1995: Establishment of an experimental infection protocol for the flat oyster *Ostrea edulis* with the intrahaemocytic protozoan parasite *Bonamia ostreae*: application in the selection of parasite resistant oysters. *Aquaculture 132*: 183–194.

Hine, P.M. 1991a: The annual pattern of infection by *Bonamia* sp. in New Zealand flat oysters, *Tiostrea chilensis*. *Aquaculture 93*: 241–251.

Hine, P.M. 1991b: Ultrastructural observations on the annual infection pattern of *Bonamia* sp. in flat oysters *Tiostrea chilensis*. *Diseases of Aquatic Organisms 11*: 163–171.

Hine, P.M. 1992: Ultrastructural and ultracytochemical observations on *Bonamia* sp. in oysters (*Tiostrea chilensis*), with a consideration of organelle function. *Aquaculture 107*: 175–183.

Hine, P.M. 1996: The ecology of *Bonamia* and decline of bivalve molluscs. *New Zealand Journal of Ecology 20*: 109–116.

Hine, P.M. & Jones, J.B. 1994: *Bonamia* and other aquatic parasites of importance to New Zealand. *New Zealand Journal of Zoology 21*: 49–56.

Hine, P.M. & Wesney, B. 1992: Interrelationships of cytoplasmic structures in *Bonamia* sp. (Haplosporidia) infecting oysters *Tiostrea chilensis* : An interpretation. *Diseases of Aquatic Organisms 14*: 59–68.

Hine, P.M. & Wesney, B. 1994a: The functional cytology of *Bonamia* sp. (Haplosporidia) infecting oysters *Tiostrea chilensis*: An ultracytochemical study. *Diseases of Aquatic Organisms 20*: 207–217.

Hine, P.M. & Wesney, B. 1994b: Interaction of phagocytosed *Bonamia* sp. (Haplosporidia) with haemocytes of oysters (*Tiostrea chilensis*). *Diseases of Aquatic Organisms 20*: 219–229.

Howard, A.E. 1994: The possibility of long distance transmission of *Bonamia* by fouling on boat hulls. *Bulletin of the European Association of Fish Pathologists 14*: 211–212.

Montes, J. 1991: Lag time for the infestation of flat oyster (*Ostrea edulis* L.) by *Bonamia ostreae* in estuaries of Galicia (N.W. Spain). *Aquaculture 93*: 235–239.

Naciri-Graven, Y., Martin, A.G., Baud, J.P., Renault, T., & Gérard, A. 1998: Selecting the flat oyster *Ostrea edulis* (L.) for survival when infected with the parasite *Bonamia ostreae*. *Journal of Experimental Marine Biology and Ecology 224*: 91–107.

Pascual, M., Martin, A.G., Zampatti, E., Coatanea, D., Defossez, J., & Robert, R. 1991: Testing of the Argentina oyster, *Ostrea puelchana*, in several French oyster farming sites. ICES Council Meeting Papers. ICES CM 1991/K:30 (ICESCM1991K30), Copenhagen, Denmark. 17 p.

Ripley, B.D. 1981: Spatial Statistics. John Wiley & Sons, New York. 252 p.

Stead, D.H. 1971: Observations on the biology and ecology of the Foveaux Strait dredge oyster (*Ostrea lutaria*, Hutton). *Fisheries Technical Report 68*. 49 p.

van Banning, P. 1991: Observations on bonamiasis in the stock of the European flat oyster, *Ostrea edulis*, in the Netherlands, with special reference to the recent developments in Lake Gravelingen. *Aquaculture 93*: 205–211.

Wand, M.P. & Jones, M.C. 1995: Kernel smoothing. Chapman and Hall, London. 224 p.

Appendix A: Figures of *Bonamia* sp. infection in oysters and estimated surfaces

Figure A1: Oysters with increasing levels of *Bonamia* sp. infection; (from left to right) healthy, lightly diseased, and heavily diseased, March 2000 survey.

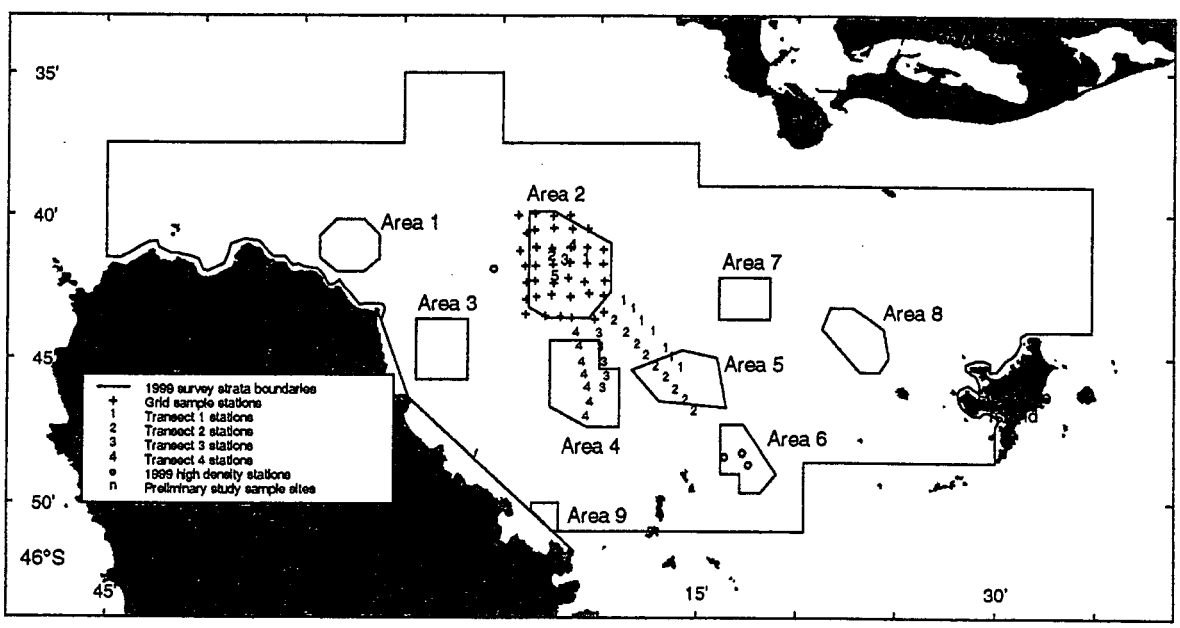

Figure A2: The October 1999 survey strata boundaries, the location of the five tows for preliminary study, and the location of the sample tow midpoints, March 2000 survey.

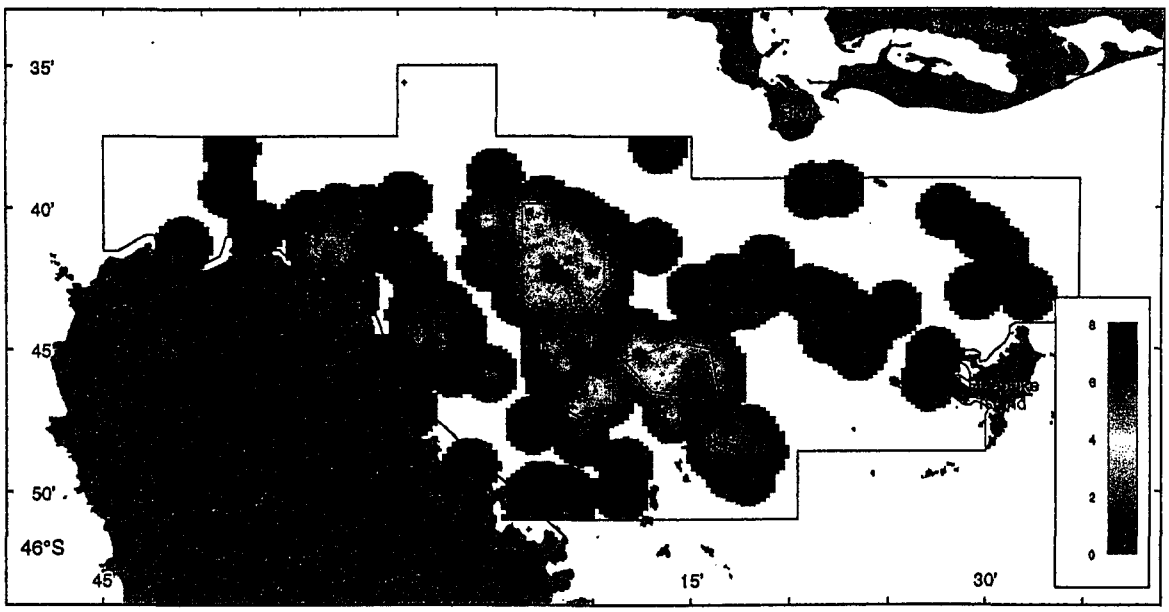

Figure A3: Estimated (kriged) density (oysters m^{-2}) of live recruited oysters from the October 1999 survey. Sample locations are shown as crosses.

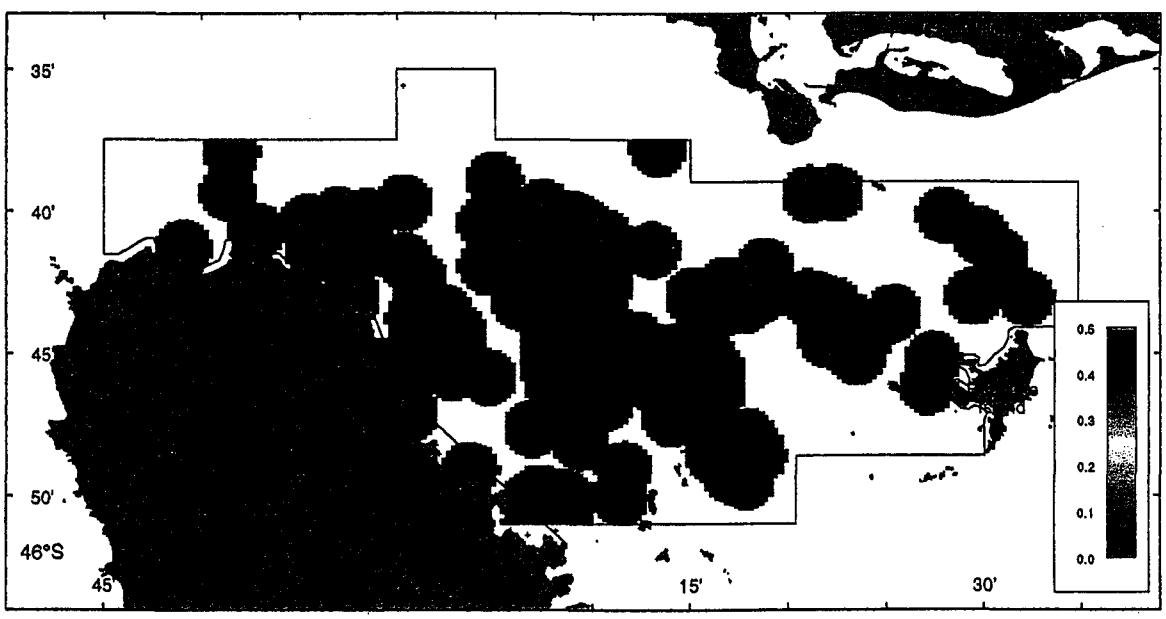

Figure A4: Estimated (kriged) new clocks as a proportion of the number of live recruits plus new clocks from the October 1999 survey. Sample locations are shown as crosses.

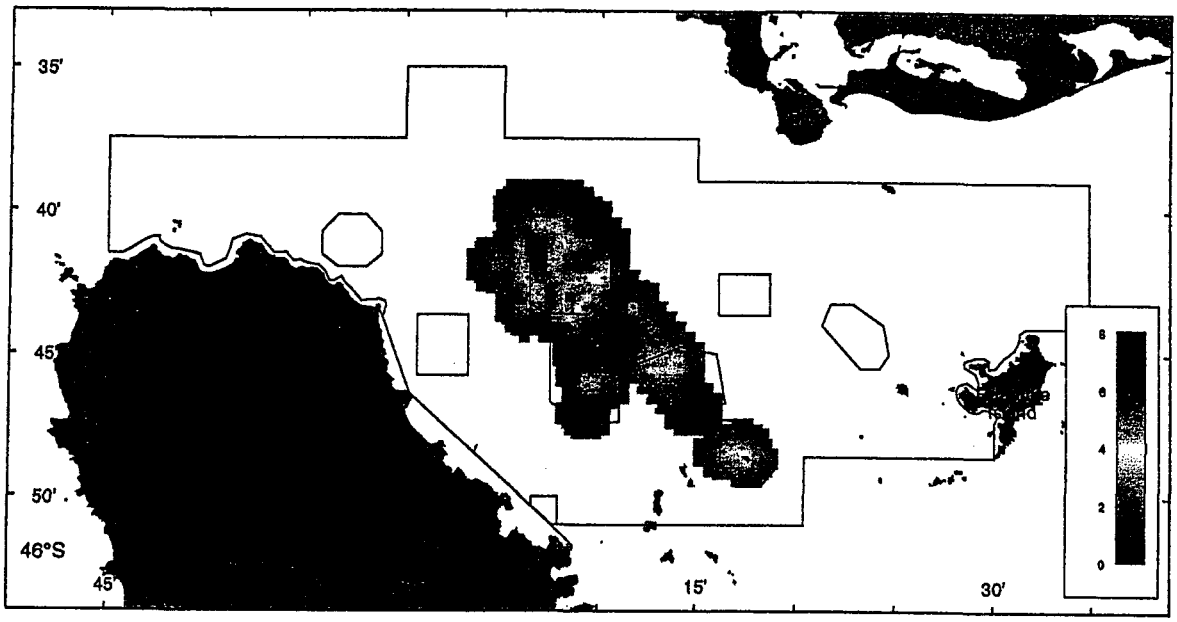

Figure A5: Estimated (kriged) density (oysters m^{-2}) of live recruited oysters, March 2000 survey. Sample locations are shown as crosses.

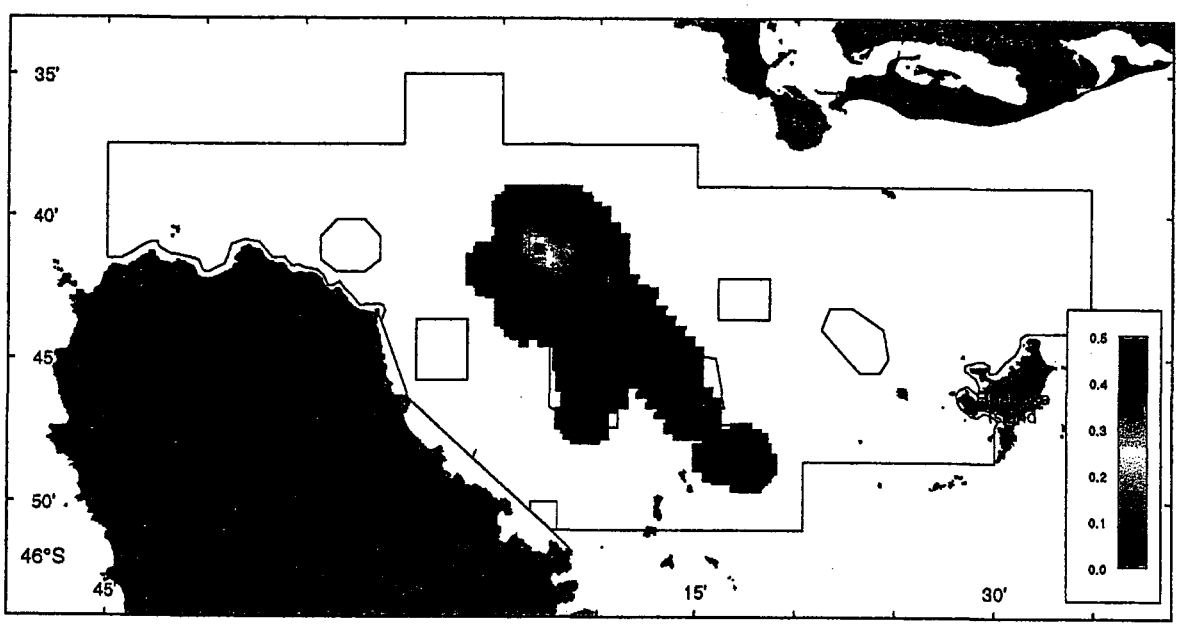

Figure A6: Estimated (kriged) new clocks as a proportion of the number of live recruits plus new clocks, March 2000 survey. Sample locations are shown as crosses.

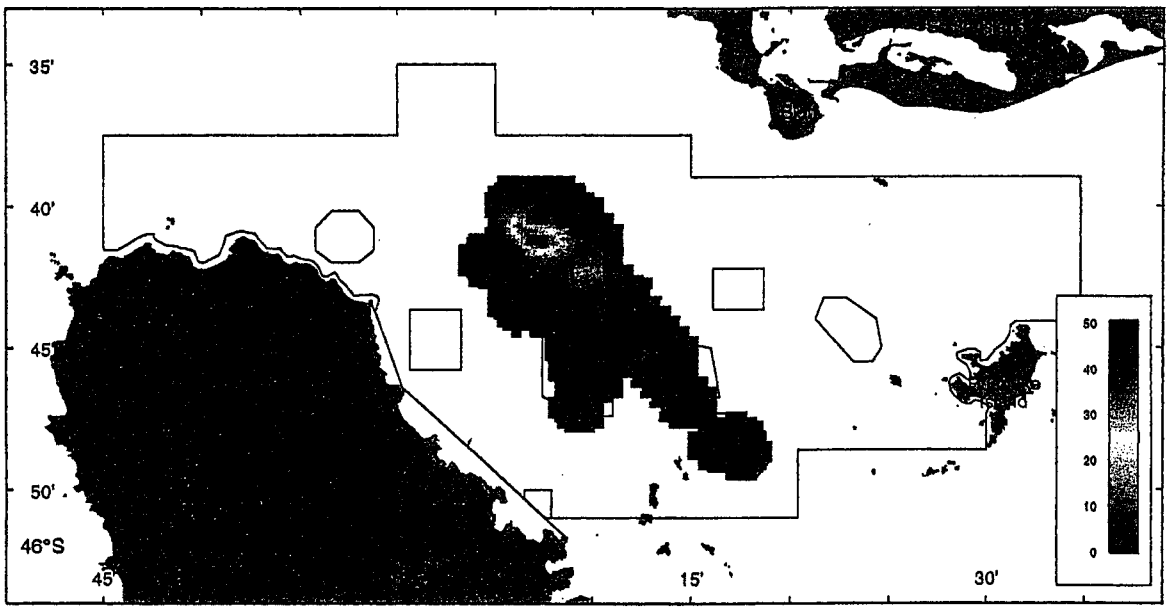

Figure A7: Estimated (kriged) prevalence of *Bonamia* sp., March 2000 survey. Sample locations are shown as crosses.

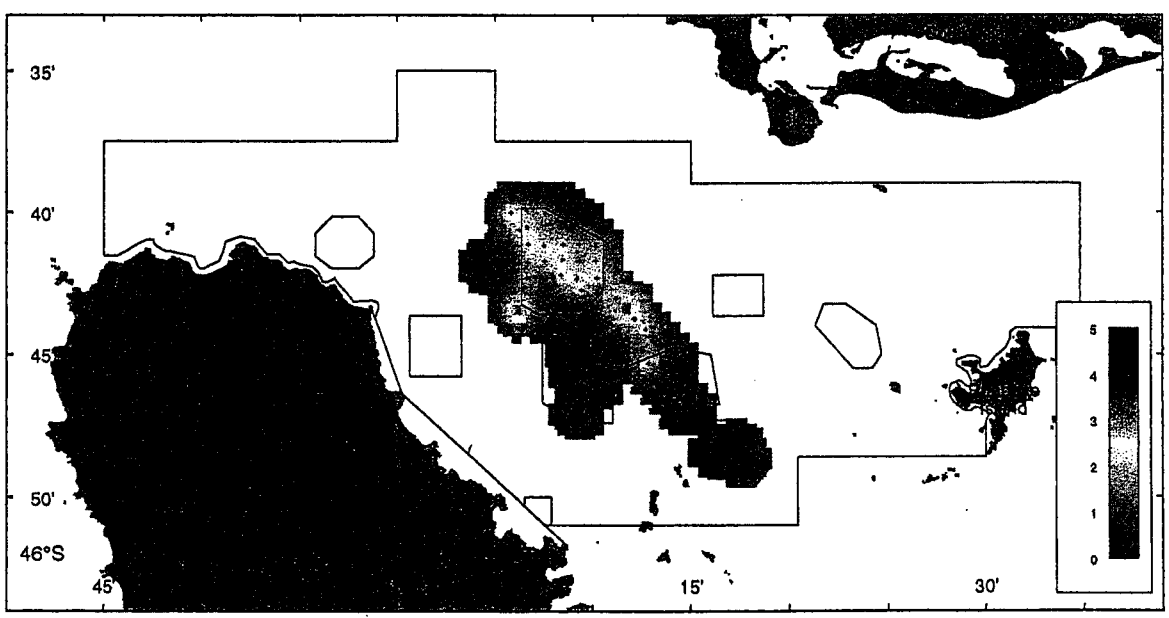

Figure A8: Estimated (kriged) mean intensity of *Bonamia* sp., March 2000 survey. Sample locations are shown as crosses.

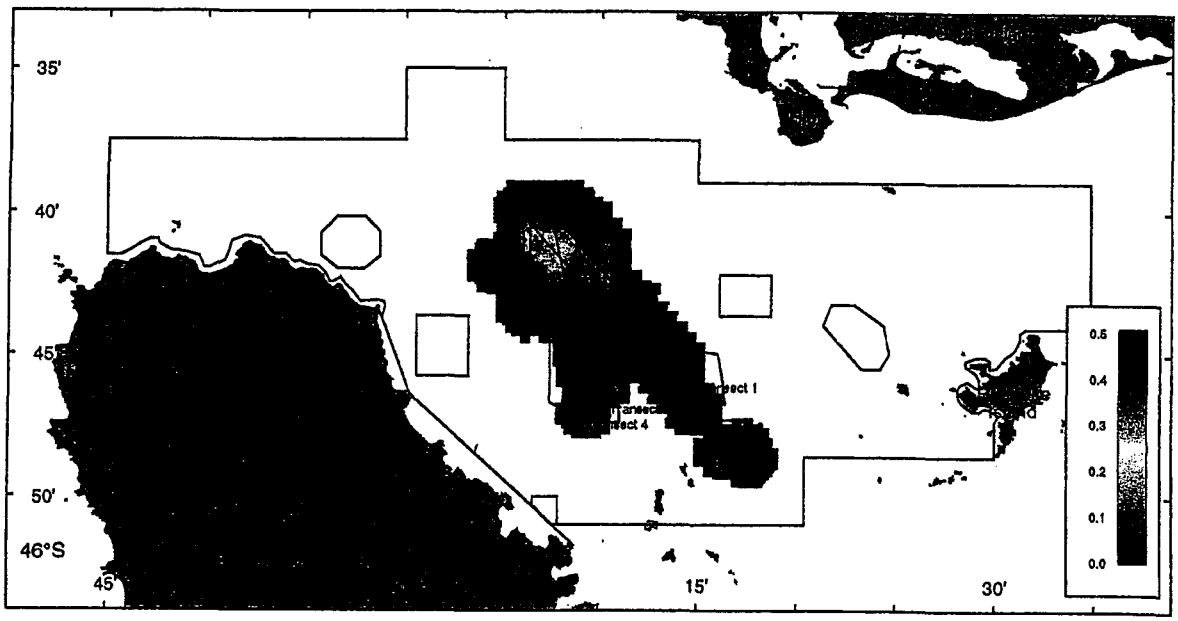

Figure A9: Location of the four transect lines, overlaid on the estimated (kriged) new clocks as a proportion of the number of live recruits plus new clocks, March 2000 survey. Sample locations are shown as crosses.

Appendix B: Station record form

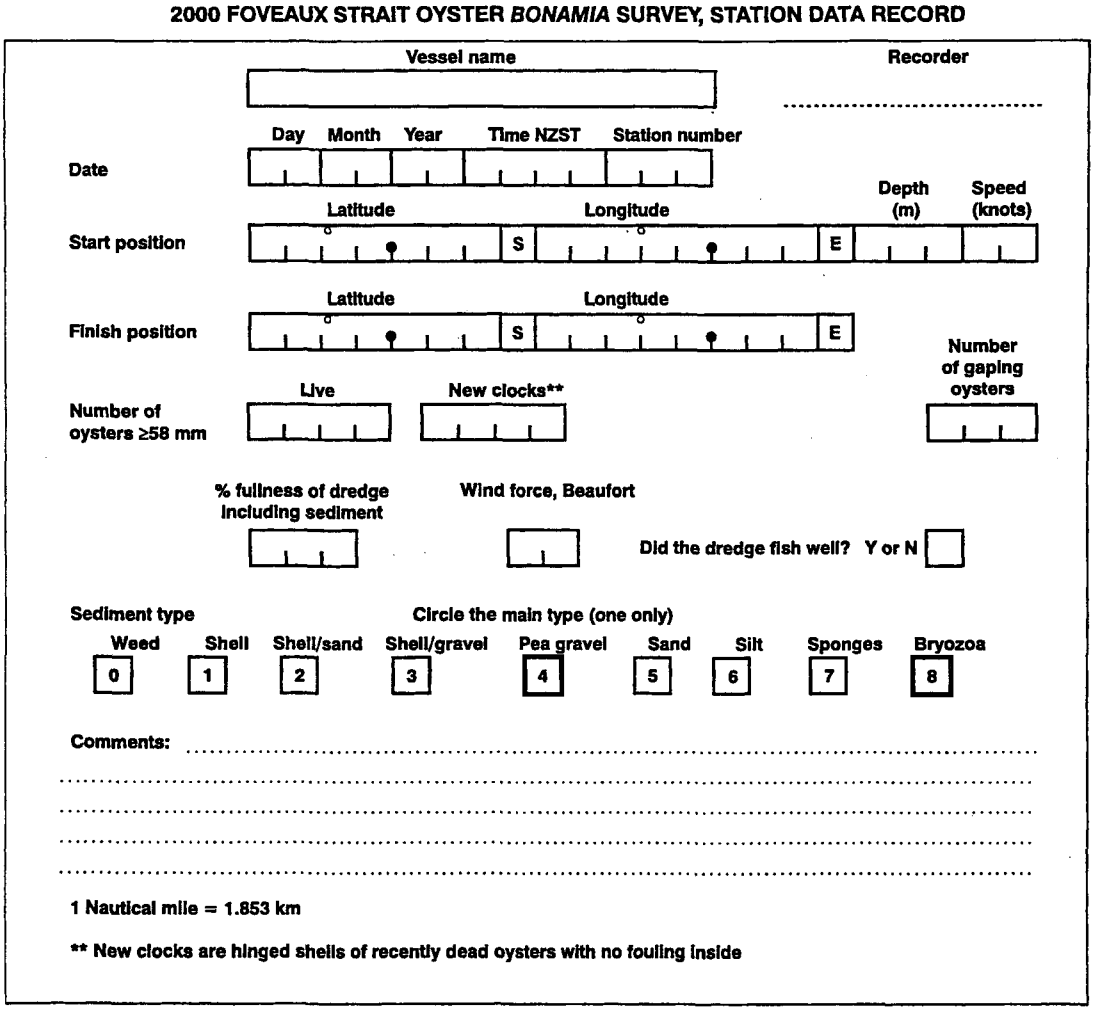

Figure B1: Station Data Record Form, March 2000 survey.

Appendix C: Data recorded by location and heart imprint data

Table C1: Sample location, live recruits, new clocks (recruit size), gapers (recruit size), and heart imprint data by station

Station no.	Longitude	Latitude	Live recruits	New clocks	Gapers	Proportion new clocks (95% CI)	Prevalence by stage 1	2	3	4	5	Total	Prevalence (95% CI)	Mean intensity
58	168°11.170' S	46°43.043' E	81	4	0	0.05 (0.01–0.12)	0	1	0	1	0	50	4 (0–14)	3.0
59	168°11.670' S	46°43.322' E	540	10	0	0.02 (0.01–0.03)	0	0	0	1	0	50	2 (0–11)	4.0
60	168°12.106' S	46°43.750' E	247	6	0	0.02 (0.01–0.05)	1	0	0	1	0	50	4 (0–14)	2.5
61	168°12.684' S	46°44.121' E	292	10	2	0.03 (0.02–0.06)	0	1	1	0	0	50	4 (0–14)	2.5
62	168°13.322' S	46°44.696' E	220	6	0	0.03 (0.01–0.06)	0	0	0	0	0	50	0 (0–7)	–
63	168°13.626' S	46°45.017' E	430	7	1	0.02 (0.01–0.03)	0	0	0	0	0	50	0 (0–7)	–
64	168°14.126' S	46°45.363' E	562	6	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
69	168°10.692' S	46°43.725' E	175	5	1	0.03 (0.01–0.06)	0	0	0	0	0	50	0 (0–7)	–
70	168°11.318' S	46°44.146' E	122	9	1	0.07 (0.03–0.13)	0	0	0	0	0	50	0 (0–7)	–
71	168°11.863' S	46°44.564' E	207	0	0	0.00 (0.00–0.02)	1	0	0	0	0	50	2 (0–11)	1.0
72	168°12.309' S	46°44.926' E	272	7	1	0.03 (0.01–0.05)	0	0	0	0	0	50	0 (0–7)	–
73	168°12.822' S	46°45.314' E	760	24	2	0.03 (0.02–0.05)	0	2	0	1	0	50	6 (1–17)	2.7
74	168°13.355' S	46°45.703' E	385	2	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
75	168°13.817' S	46°46.120' E	225	2	0	0.01 (0.00–0.03)	0	0	0	0	0	50	0 (0–7)	–
76	168°14.364' S	46°46.489' E	172	4	0	0.02 (0.01–0.06)	1	0	0	0	0	50	2 (0–11)	1.0
77	168°14.789' S	46°46.859' E	35	0	0	0.00 (0.00–0.10)	0	0	0	0	0	50	0 (0–7)	–
78	168°09.939' S	46°44.168' E	115	2	0	0.02 (0.00–0.06)	0	0	0	0	0	50	0 (0–7)	–
79	168°10.002' S	46°44.646' E	487	4	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
80 [1]	168°10.213' S	46°45.187' E	21	0	0	0.00 (0.00–0.16)	0	0	0	0	0	36	0 (0–10)	–
81	168°10.325' S	46°45.671' E	314	4	0	0.01 (0.00–0.03)	0	0	0	0	0	50	0 (0–7)	–
82	168°10.124' S	46°46.070' E	310	2	0	0.01 (0.00–0.02)	1	0	0	0	0	50	2 (0–11)	1.0
85	168°08.756' S	46°44.144' E	313	0	0	0.00 (0.00–0.01)	0	0	0	0	0	50	0 (0–7)	–
86	168°08.928' S	46°44.631' E	65	0	0	0.00 (0.00–0.06)	0	0	0	0	0	50	0 (0–7)	–
87 [1]	168°09.058' S	46°45.188' E	7	0	0	0.00 (0.00–0.41)	0	0	0	0	0	17	0 (0–20)	–
88	168°09.187' S	46°45.604' E	314	2	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
89	168°09.352' S	46°46.063' E	668	4	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
90	168°09.463' S	46°46.565' E	153	4	0	0.03 (0.01–0.06)	1	0	0	0	0	50	2 (0–11)	1.0
91 [1]	168°09.285' S	46°47.067' E	42	1	1	0.02 (0.00–0.12)	0	0	0	0	0	50	0 (0–7)	–
92	168°04.592' S	46°41.914' E	308	5	0	0.02 (0.01–0.04)	0	0	0	0	0	50	0 (0–7)	–
108	168°16.381' S	46°48.425' E	450	3	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
109	168°17.295' S	46°48.290' E	416	4	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
110	168°17.588' S	46°48.695' E	531	1	0	0.00 (0.00–0.01)	0	0	0	0	0	50	0 (0–7)	–
111	168°06.192' S	46°43.485' E	391	0	0	0.00 (0.00–0.01)	0	0	0	1	0	50	2 (0–11)	4.0
112	168°07.177' S	46°43.536' E	155	4	0	0.03 (0.01–0.06)	0	0	0	0	0	50	0 (0–7)	–
113	168°07.969' S	46°43.569' E	417	1	1	0.00 (0.00–0.01)	0	0	0	0	0	50	0 (0–7)	–
114	168°08.562' S	46°43.605' E	539	4	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
115	168°09.721' S	46°43.657' E	197	0	0	0.00 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
116	168°10.177' S	46°43.412' E	183	0	0	0.00 (0.00–0.02)	1	0	0	0	0	50	2 (0–11)	1.0
117	168°06.225' S	46°42.963' E	276	0	0	0.00 (0.00–0.01)	0	0	0	0	0	50	0 (0–7)	–
118	168°06.738' S	46°42.877' E	666	7	2	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
119	168°07.512' S	46°42.798' E	160	7	0	0.04 (0.02–0.08)	0	0	0	0	0	50	0 (0–7)	–
120	168°08.486' S	46°42.879' E	810	7	1	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–
121	168°09.442' S	46°42.761' E	517	12	0	0.02 (0.01–0.04)	1	1	2	0	0	50	8 (2–19)	2.3
122	168°10.186' S	46°42.838' E	246	12	0	0.05 (0.02–0.08)	1	0	0	0	0	50	2 (0–11)	1.0
123	168°06.219' S	46°42.373' E	492	4	0	0.01 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–

Table C1 (continued): Sample location, live recruits, new clocks (recruit size), gapers (recruit size), and heart imprint data by station

Station no.	Longitude	Latitude	Live recruits	New clocks	Gapers	Proportion new clocks (95% CI)	Prevalence by stage 1	2	3	4	5	Total	Prevalence (95% CI)	Mean intensity
124 [2]	168°06.666' S	46°42.322' E	194	4	0	0.02 (0.01–0.05)	0	1	0	0	0	49	2 (0–11)	2.0
125	168°07.593' S	46°42.391' E	358	65	1	0.15 (0.12–0.19)	1	0	0	0	0	50	2 (0–11)	1.0
126	168°08.375' S	46°42.218' E	269	16	0	0.06 (0.03–0.09)	1	0	0	1	0	50	4 (0–14)	2.5
127	168°09.213' S	46°42.350' E	446	70	0	0.14 (0.11–0.17)	2	2	3	5	0	50	24 (13–38)	2.9
128	168°10.226' S	46°42.324' E	518	118	2	0.19 (0.16–0.22)	2	3	2	1	1	50	18 (9–31)	2.6
129	168°06.171' S	46°41.800' E	405	4	0	0.01 (0.00–0.02)	1	0	0	0	0	50	2 (0–11)	1.0
130	168°06.695' S	46°41.796' E	121	10	0	0.08 (0.04–0.14)	2	0	2	0	0	50	8 (2–19)	2.0
131	168°07.566' S	46°41.744' E	537	262	3	0.33 (0.30–0.36)	3	2	4	0	0	50	18 (9–31)	2.1
132	168°08.473' S	46°41.682' E	177	28	1	0.14 (0.09–0.19)	0	2	3	1	0	50	12 (5–24)	2.8
133	168°09.325' S	46°41.717' E	119	12	0	0.09 (0.05–0.15)	1	1	0	0	0	50	4 (0–14)	1.5
134	168°10.152' S	46°41.727' E	482	50	0	0.09 (0.07–0.12)	0	2	2	0	0	50	8 (2–19)	2.5
135	168°05.892' S	46°41.287' E	421	8	0	0.02 (0.01–0.04)	1	0	0	0	0	50	2 (0–11)	1.0
136	168°06.727' S	46°41.143' E	75	96	1	0.56 (0.48–0.64)	1	8	9	2	3	50	46 (32–61)	2.9
137 [3]	168°07.522' S	46°41.191' E	275	109	0	0.28 (0.24–0.33)	2	10	2	1	2	35	49 (31–66)	2.5
138	168°08.500' S	46°41.141' E	624	205	4	0.25 (0.22–0.28)	2	6	5	2	0	50	30 (18–45)	2.5
139	168°09.328' S	46°41.153' E	321	6	0	0.02 (0.01–0.04)	1	1	1	0	0	50	6 (1–17)	2.0
140 [1]	168°10.167' S	46°41.223' E	25	4	0	0.14 (0.04–0.32)	0	1	0	0	0	28	4 (0–18)	2.0
141	168°06.232' S	46°40.642' E	274	83	1	0.23 (0.19–0.28)	7	5	5	4	1	50	44 (30–59)	2.4
142 [2]	168°06.636' S	46°40.529' E	552	79	0	0.13 (0.10–0.15)	0	2	1	0	0	29	10 (2–27)	2.3
143	168°07.605' S	46°40.466' E	550	23	1	0.04 (0.03–0.06)	2	1	1	0	0	50	8 (2–19)	1.8
144	168°08.570' S	46°40.497' E	191	5	0	0.03 (0.01–0.06)	0	0	0	0	0	50	0 (0–7)	–
145 [1]	168°09.382' S	46°40.497' E	28	0	0	0.00 (0.00–0.12)	0	0	0	0	0	50	0 (0–7)	–
147	168°05.821' S	46°40.021' E	191	2	0	0.01 (0.00–0.04)	0	1	0	1	0	50	4 (0–14)	3.0
148	168°06.645' S	46°39.972' E	158	10	0	0.06 (0.03–0.11)	0	2	0	0	0	50	4 (0–14)	2.0
149	168°07.586' S	46°40.062' E	166	2	0	0.01 (0.00–0.04)	0	2	0	0	0	50	4 (0–14)	2.0
150	168°08.473' S	46°40.029' E	197	0	0	0.00 (0.00–0.02)	0	0	0	0	0	50	0 (0–7)	–

1. Stations where additional 50–57 mm sized oysters were added to the heart imprint and histology samples.
2. Some oysters were overlooked during the preparation of slides, and hence some data are unavailable.
3. The remaining 15 slides were unreadable due to poor preparation.

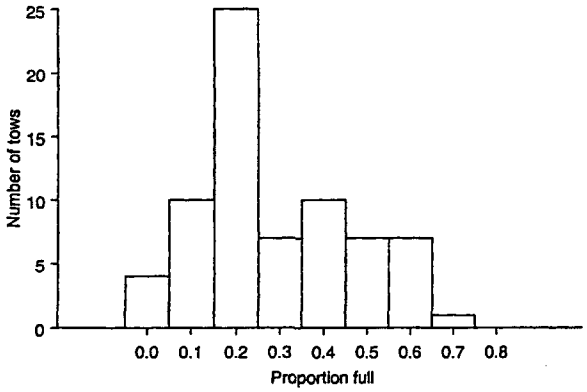

Figure C1: Percentage fullness of the dredge by the number of tows, March 2000 survey.

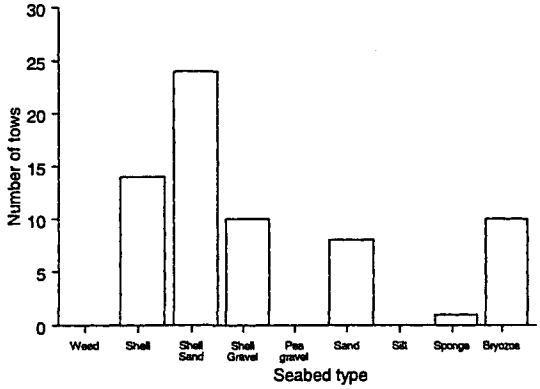

Figure C2: Recorded seabed type by the number of tows, March 2000 survey.

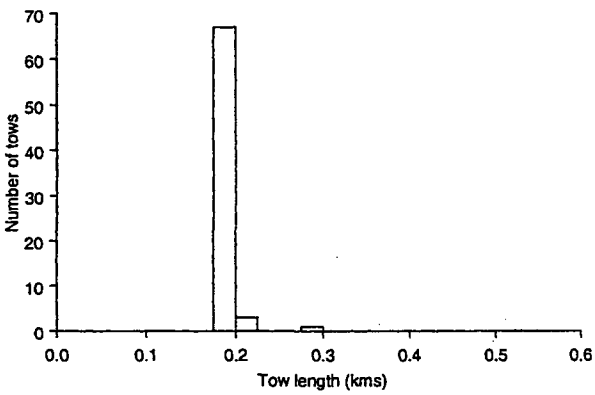

Figure C3: Calculated tow length by the number of tows, March 2000 survey.

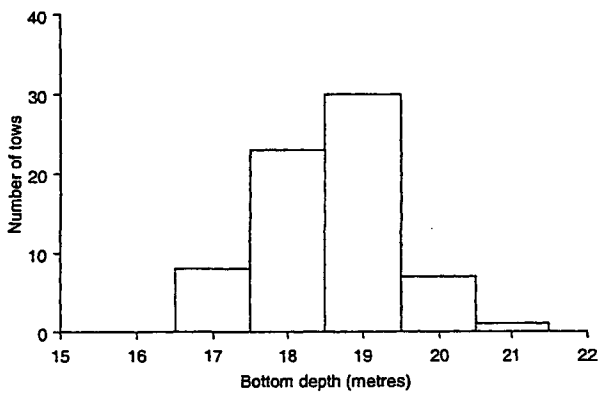

Figure C4: Recorded bottom depth by the number of tows, March 2000 survey.

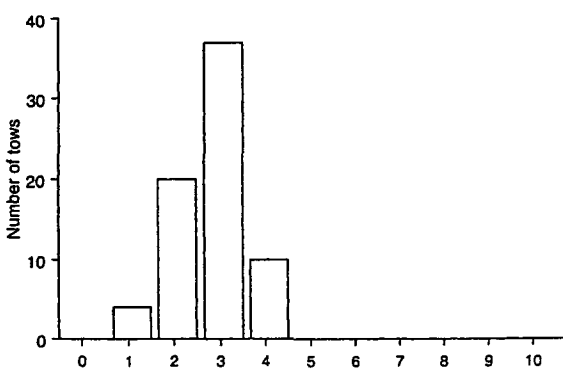

Figure C5: Recorded wind force (Beaufort scale) by the number of tows, March 2000 survey.

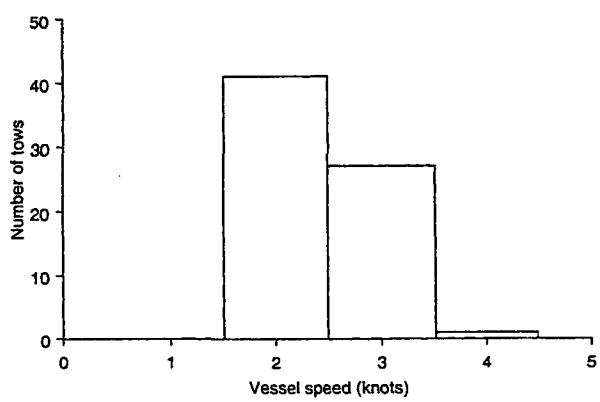

Figure C6: Recorded vessel speed by the number of tows, March 2000 survey.