Taihoro Nukurangi

Foveaux Strait dredge oyster strategic research plan for the period 2000–2004

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Final Research Report for
Ministry of Fisheries Research Project OYS1999/01
Objective 4 (*revised objective*)

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Final Research Report

Report Title	Foveaux Strait dredge oyster strategic research plan for the period 2000–2004
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7. Objective

This report covers work required under the revised objective, Objective 4 of Project OYS1999/01 "To provide an overview of a strategic research plan for the management of Foveaux Strait dredge oysters".

8. Introduction

8.1 Overview

The Bluff oyster (*Tiostrea chilensis*) has been commercially fished in Foveaux Strait for 130 years. Annual landings of oysters, fishing effort and the size of the area exploited by fishers steadily increased until 1986 (Cranfield *et al.* 1999a). A *Bonamia* sp. epizootic between 1986 and 1992 devastated the Foveaux Strait oyster population (Doonan *et al.* 1994). From the initial focus of infection in the western oyster beds, the disease spread through the population to reach the periphery of oyster distribution in 1992. In 1992, the size of the oyster population in the area surveyed in 1975–1976 was less than 10 % of that present in 1975–1976 and recruitment was considered to be at risk (Doonan *et al.* 1994). The Minister of Fisheries partially closed Foveaux Strait to oyster fishing in 1992 and fully closed it in 1993, to allow the population to rebuild. By 1995, the prevalence and intensity of infection by *Bonamia* sp. had decreased and the population had reached a size large enough to sustain

some commercial fishing (Cranfield *et al.* 1996). The Minister of Fisheries reopened the fishery in 1996.

The reappearance of *Bonamia* sp. in 2000 (Dunn *et al.* 2000b) forced a reassessment of the information required to manage the Foveaux Strait Oyster Fishery. It is now apparent that Bonamiasis is a recurrent feature of oyster population dynamics and their interaction with the fishery. It is therefore necessary to focus research effort in the short-term on better understanding the biology and epidemiology of *Bonamia* sp., with medium to long-term research focusing on improved management of the fishery.

Of particular importance is an understanding of the mechanics of disease transmission through oyster populations and its relationship with the fishery. Without this information management will be reactive and incapable of maximising the yield that can safely be taken from the fishery. In this plan we outline strategic research in four broad areas:

- *Bonamia* sp. investigations
- Effects of fishing on the environment
- Short term management of the fishery (determination of sustainable yields)
- Improved management (determination of sustainable yields in the long term)

An overview of the research plan is shown as Figure 1, and approximate times given in Table 1.

8.2 Biological overview

8.2.1 Population biology of oysters

Jeffs & Hickman (2000) found that *Ostrea chilensis* in 1970–71 in Foveaux Strait were protandric, maturing as males at about 20 mm in height (1+ old oysters). At about 50 mm (2–3+ old oysters) most oysters that were producing sperm began developing ovocytes around the periphery of these predominantly male follicles. Fully female follicles with mature ova were found in a very small proportion of the population during the spring. The smallest oyster found brooding larvae was 60 mm in height (dorso-ventral axis). They found that follicles with mature sperm were common from late July, increased to a peak through September, October and November, started to fall off in December and reached a minimum in March. Mean gamete loss (largely sperm as 90% of individual gonads were male), rose from September to reach a peak in March. Mean phagocytosis increased from October onwards to reach a peak in March. Brooding oysters were found between July and January, peaking in December, and only 7–10% of the population sampled during the year was brooding larvae; 90% spawned as males. The gonads of females that spawned were largely filled with ova and few follicles showed any development of sperm. On the other hand, most of the oysters that spawned as males, apart from having mature sperm in every follicle, had developing oogonia, oocytes and ova around the periphery of follicles later in summer.

These ova continued to increase in number and grow in size through the latter part of the summer but few reached full maturity and the female cycle seldom dominated the gonad. Oogonia, ovocytes and immature and mature ova were mainly phagotycosed and reabsorbed between February and April, however phagocytosis was observed in about 10% of oyster gonads all the year around. Jeffs (1998) found gonads of *Ostrea chilensis* in Auckland Harbours were functionally hermaphoditic, oysters continued to spawn all the year around and there was no phagocytic cycle like that found in Foveaux Strait. If the multiplicative

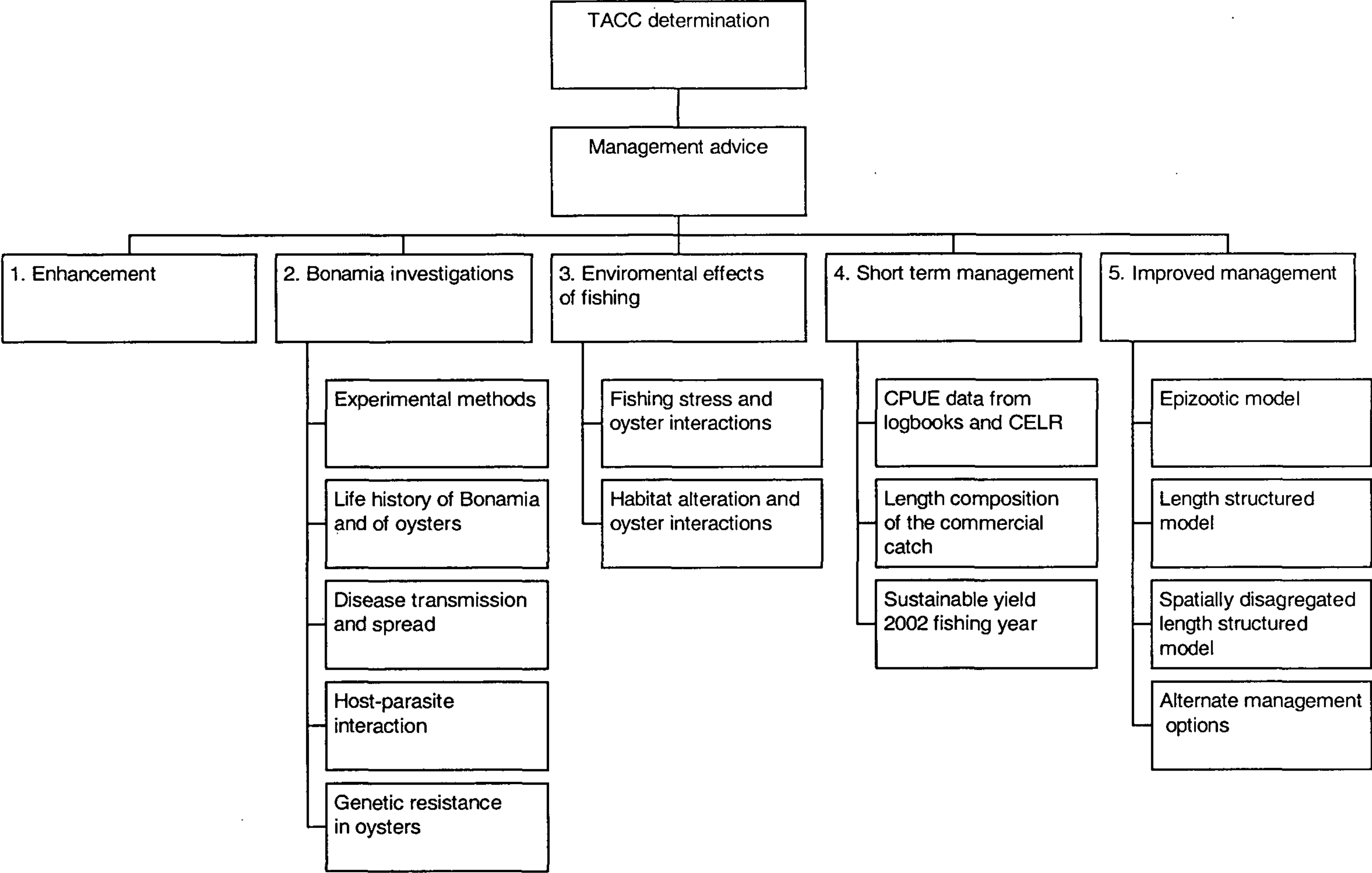

Figure 1: Outline of research as inputs into management advice and subsequent TACC.

Table 1: Research plan GANTT chart

Timeline columns (months): 2000: J A S O N D; 2001: J F M A M J J A S O N D; 2002: J F M A M J J A S O N D; 2003: J F M A M J J A S O N D; 2004: J F M A M J

Investigation		Study
1 Bonamia investigations	Experimental methods	1 Amplify infection
		2 Identification
		3 Purification
	Life history	4 Life history
		5 Size relationship
		6 Seasonal variation
		7 Extra-host survival
		8 Shedding rates
		9 Gametogenic cycle
	Disease transmission	10 Transmission distances
	Host-parasite interaction	11 Mathematical models
	Genetic resistance	12 Role of oyster resistance
2 Environmental effects	Stress	Altered habitat
		Burial
		Dredging
	Habitat	Habitat complexity
3 Short-term management	CPUE	13 Logbook & CELR
	Catch composition	14 Length composition
	Sustainable yield	15 2002
4 Improved management	Epizootic model	Causes of epizootic
		Epidemiology
		Disease spread
		Density dependence
		Time dependence
		Epizootic model
	Length structured model	Growth rates
		Mortality
		Recruitment
		Length-at-age
		Exploitation
		Population length structure
	Spatially disagregated model	Spatial distribution
		Spatial exploitation
		Habitat
	Alternate management options	Alternative management

stage of *Bonamia* sp. is dependent on phagocytosis of ova, then northern populations of oyster could be *Bonamia*-free. Furthermore, growing oysters at higher temperatures could trip oyster physiology into the northern mode, and might be used to combat *Bonamia* sp. in culture.

Cranfield & Allen (1977), found that incubation of larvae in one population commenced in September, peaked in November and December and ceased after March. They found that 6–12% of the population sampled in the summer of 1967–1968, were brooding larvae (very similar to the percentage Jeffs & Hickman (2000) found in data from 1970–1971). Cranfield & Allen (1977) found the smallest oyster incubating larvae was 51 mm in height. Cranfield (1968a, 1968b) found that settlement of larvae released by the incubators started in October peaked in December and ceased after March. These data confirm that successful spawning as females is confined to the early summer period. Because so few oysters spawn as females in any one season, their time of spawning cannot be demonstrated by loss of gametes, loss of oyster condition or other indirect measures. These data also confirm that the late summer development of ova in mature male gonads did not result in any late summer or autumn spawning.

The gametogenic cycle, incubation and settlement are shown in Figure 2.

Because *Bonamia* sp. has a lipid-based metabolism it is able to proliferate rapidly in haemocytes that are phagocytosing lipid rich ova. In Foveaux Strait some 90% of the oyster population functions as males and in February and March these oysters are phagocytosing the numerous ova that have been developing secondarily around the follicle walls of their gonad. The life cycle of oyster and parasite come together perfectly for the parasite at this time and must play an important part in epizootics.

Hine (1991a) investigated the relationship between *Bonamia* sp. infection and the gonad cycle in 10 samples of oysters between September 1986 and May 1989. There were no samples between January and April in 1987, or November 1987 and April 1988, or December 1988 and May 1989, the period in which Jeffs & Hickman (2000) found the female phase developing in male gonads and then later being reabsorbed by phagocytes. Nevertheless, even from the samples at the beginning and end of the phagocytic cycle Hine (1991a) was able to propose the importance for the proliferation phase of *Bonamia* sp. of the phagocytic cycle and re-absorption of the lipid rich ova at this time.

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

Bonamia spp. are protozoa belonging to 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 closer 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 only known from New Zealand (Doonan *et al.* 1994, Hine & Jones 1994, Hine 1996). *Bonamia* sp. occurs in *Ostrea* (= *Tiostrea*) *chilensis* in New Zealand (and possibly Chile), and *Ostrea angasi* in Australia. 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 has also accidentally infected *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*. *Bonamia* sp. does not infect Pacific oysters (*Crassostrea gigas*).

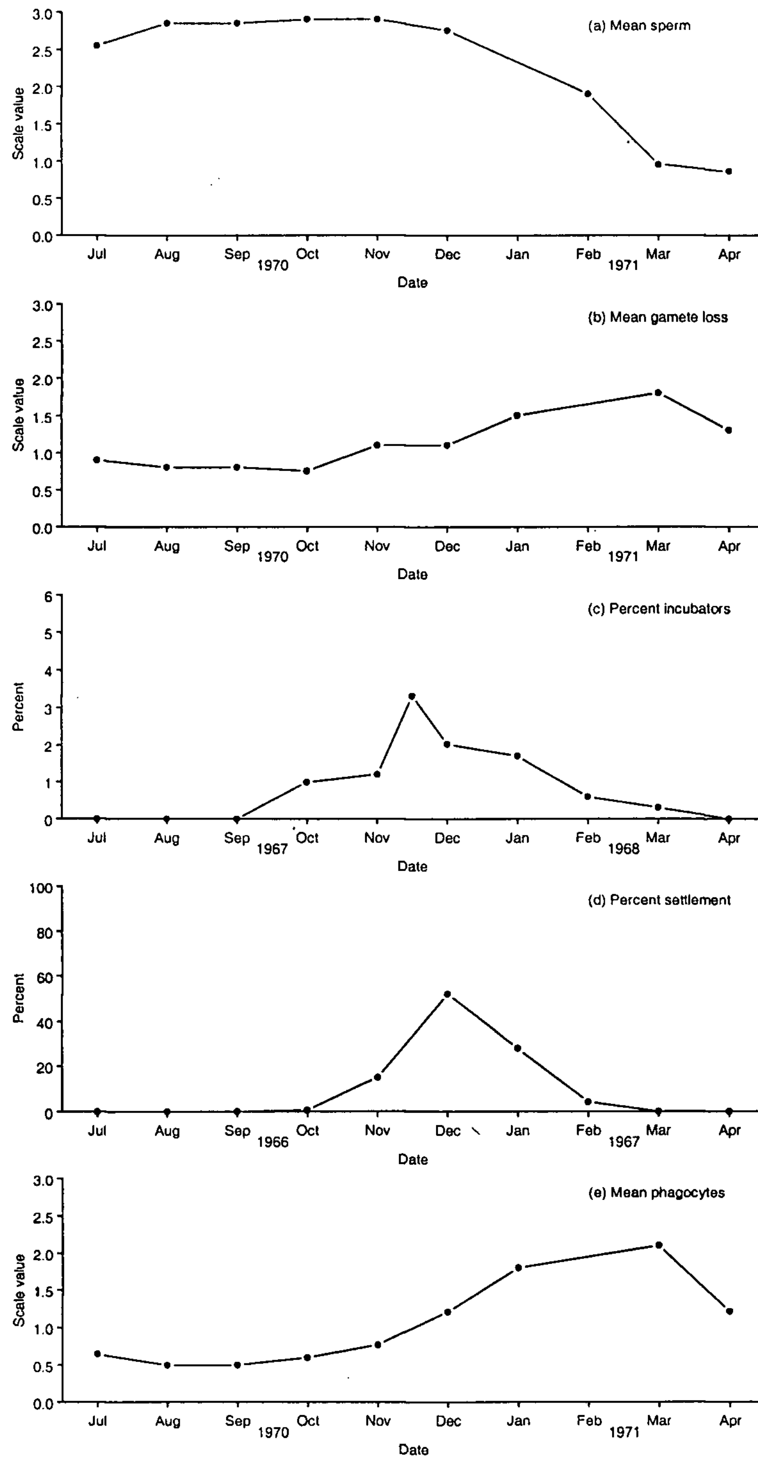

Figure 2: The Foveaux Strait dredge oyster gametogenic cycle.

In New Zealand flat 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 it closely resembles *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 more than 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 the gonad and in so doing provides nutrition for the parasite. Eventually the oyster dies of exhaustion.

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 but many fail to spawn all, or possibly any, of their ova, 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 the intensity and prevalence of infection in one year, the density of oysters, and the probability of an outbreak the following year 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 using heart smears or histopathology) 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 10^1–10^6 *Bonamia ostreae*, is about 80 000 per oyster for 3 year-old oysters (Hervio *et al.* 1995). Oysters differ in their susceptibility to *Bonamia ostreae* (Naciri-Graven *et al.* 1998) with this difference in susceptibility probably 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 indicative evidence that oyster boats may spread the infection (van Banning 1991, Howard 1994).

Bonamia sp. may have spread along southern Australia from west to east on the currents of the Southern Ocean and from there to New Zealand. Subsequently it may have spread from New Zealand to Chile in oysters rafting across the Pacific (Foighil *et al.* 1999). The annual

pattern of infection suggests that a large proportion of oysters in Foveaux Strait may have the parasite at low levels throughout the year.

The life cycle of *Bonamia* sp. is summarised in Figure 3.

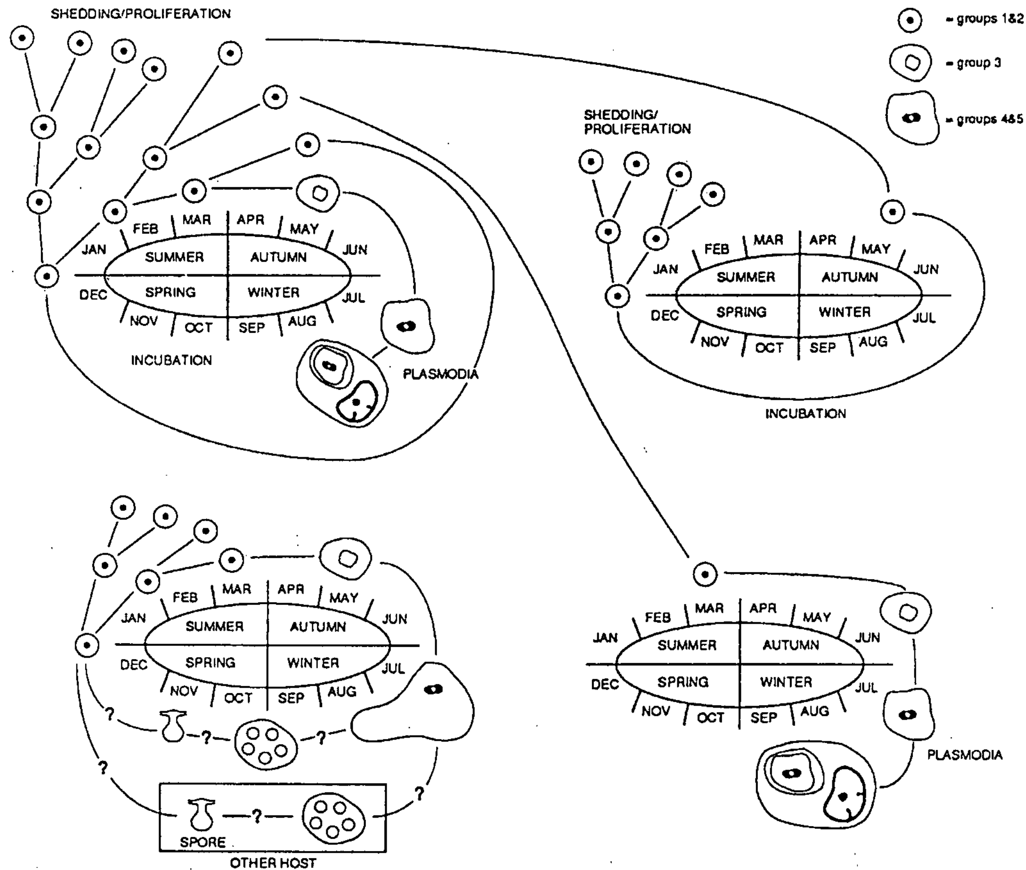

Figure 3: Relationship between the course of infection of *Bonamia* sp. to time of infection. The annual cycle, described in detail in Hine (1991b), is shown at the top left. Bottom left shows the cycle if sporogony occurs elsewhere in the oyster or in an alternative host. If dense forms enter the oyster late in the proliferation phase (top right) they may incubate to proliferate the following December. If they infect earlier (bottom right) in the proliferation phase, they may develop on to the plasmodial phase. (Figure courtesy of Hine 1991b).

8.2.3 Understanding how *Bonamia* sp. epizootics start

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). But the causes for this outbreak are unknown (Dunn *et al.* 2000b).

Prevalence of infection between epizootics is low and borders on the undetectable, nevertheless, infection is widespread at these low levels throughout the oyster population. Thus, although there is no evidence of shedding of infective particles at these low incidences and intensities of infection, some particles must be shed to account for the widespread nature of infection. For an epizootic to commence, some stimulus must trigger the proliferation of disease within enough individual oysters locally for the intensity of infection to increase and

cascade effects of infection spread to come into play and increase the prevalence of infection within the population.

9. *Bonamia* sp. investigations

9.1 Introduction

Current knowledge of the biology of *Bonamia* sp. includes the annual pattern of infection (Hine 1991a, 1991b), the functional cytology of the parasite (Hine 1992, Hine & Wesney 1992, 1994a), and the relationship of the parasite with the host (Hine & Wesney 1994b). Even when our knowledge of *Bonamia ostreae* is included, we still do not know the necessary key information in order to manage the fishery around the problem. The key information includes:

Developing experimental methods and developing an understanding of how *Bonamia* sp. epizootics start;

1. Determining the best protocol to use to amplify infection levels in wild *Bonamia* sp. infected oysters by the application of stress.
2. Determining the optimum conditions using an *in situ* hybridisation (ISH) technique, to identify very lightly infected oysters.
3. Determining the best method of purifying viable *Bonamia* sp. from infected oysters, by adapting published techniques on *Bonamia ostreae*.

Determining the time course of infection from initial infection to death of infected oysters;

4. Understanding the life history of *Bonamia* sp. and its relationship to oysters
5. Determining the size at which oysters become a) infected with *Bonamia* sp., and b) diseased with *Bonamia* sp.
6. Determining the extent to which the parasite population is depleted over winter
7. Determining how long the parasite can survive outside the host
8. Determining the rate of shedding of *Bonamia* sp. by live, moribund and dead oysters
9. Determining the relationships between the gametogenic cycle of oysters and life cycle of *Bonamia* sp., and between *Bonamia* sp. and concurrent infections.

Understanding the mechanism of spread of *Bonamia* sp. epizootic;

10. Determining the distance over which the parasite can be transmitted between oysters.

Understanding the host-parasite interaction between the oyster population and *Bonamia* sp.;

11. Mathematically model the host-parasite interactions between oysters and *Bonamia* sp.

Understanding the role of genetic resistance in oysters to *Bonamia* sp.

12. Determining the role of oyster resistance in the epidemiology of *Bonamia* sp.

In order to acquire this information it is necessary to conduct studies in the laboratory and, where possible, validate the results in field trials. The experiments can be viewed in two steps, first to develop experimental methods and the experimental protocol to assure availability of infected material and identify uninfected or lightly infected stocks (1–4 above). Secondly, to carry out experiments that will provide the information needed to develop a model (5–12 above). In the following sections, we detail the studies required to collect this information.

9.2 Developing experimental methods and developing an understanding of how *Bonamia* sp. epizootics start

9.2.1 Study 1

Objective

To determine the best protocol to use to amplify infection levels in wild *Bonamia* sp. infected oysters by the application of stress.

Comment

This objective is placed first because of the need for heavily infected material outlined in this research plan. The April 2000 *Bonamia* sp. survey showed that heavily infected individuals were mainly near the focus of mortality, and that in other beds, when heavily *Bonamia* sp.-infected oysters were observed, they usually comprised less than 10% of the oysters sampled. For the work detailed below to be carried out, it is necessary to have an ample supply of *Bonamia* sp.-infected oysters, and to achieve this it will be necessary to amplify the levels available in field samples.

Experimental design

Step 1: 480 oysters will be collected from near the site of the 1999 epizootic in Foveaux Strait and divided into 4 groups of 60 oysters, and two groups of 120 oysters. Both groups of 120 oysters will be subdivided into 2 groups of 60 oysters,. These groups will be subjected to the following stresses.

Group 1 (60 oysters): These will be lifted from the water, but kept cool, for 12 hours each day, for 14 days, and 30 examined 3 months later, and 30 examined 4 months later by routine histopathology.

Group 2 (60 oysters): These will be shaken vigorously 4 times a day each day for 14 days, and 30 examined 3 months later, and 30 examined 4 months later by routine histopathology.

Group 3a (60 oysters): These will be flushed with warm (25°C) water each day for 1 hour, for 14 days.

Group 3b (60 oysters): These will be flushed with cold (5°C) water each day for 1 hour, for 14 days. Thirty oysters of both groups will be examined 3 months later, and 30 of each group examined 4 months later, by routine histopathology.

Group 4a (60 oysters): These will be kept in hyposaline (15‰) water for 14 days.

Group 4b (60 oysters): These will be kept in hypersaline (35‰) water for 14 days. Thirty oysters of both groups will be examined 3 months later, and 30 of each group examined 4 months later, by routine histopathology.

Group 5 (60 oysters): These will be kept in 0.22 µm-filtered seawater for 14 days, and will be examined 3 months later by routine histopathology.

Group 6 (60 oysters): These will be kept under ambient tankroom temperatures for 4 months, and will then be examined by routine histopathology.

The oysters will be inspected twice daily and all gapers and dead oysters will be removed. Gapers and, where possible freshly dead oysters will be fixed for histopathology to determine the cause of death.

Step 2: Large numbers of oysters from the known most infected area in Foveaux Strait, will be subjected to those protocols in Step 1 that were best at elevating *Bonamia* sp. infection levels.

Timeframe

December 2000 to March 2001.

9.2.2 Study 2

Objective

Determine the optimum conditions using an *in situ* hybridisation (ISH) technique, to identify very lightly infected oysters.

Comment

Currently two diagnostic methods are generally available; examination of heart smears, and histology. Histology is slightly more sensitive than heart smear examination, but it is more time consuming and expensive. Neither technique is sensitive enough to be sure that oysters are uninfected, and thus a more sensitive technique is needed to insure that the control or uninfected oysters in the experiments below, truly are uninfected. The French government marine research agency IFREMER have developed molecular probes specific for the genus *Bonamia*, and for both the *Bonamia* spp. This permits detection of as few as 20 *Bonamia* in one oyster by a technique called *in situ* hybridisation (ISH), compared with as few as an estimated 200 *Bonamia* in one oyster that can be detected by histology after examination of 5 sections for two hours. However, this does not definitively identify uninfected oysters and requires killing of the oyster to carry out ISH. Therefore the intention is to use the technique to identify beds elsewhere in the country that can be used as a source of uninfected oysters.

NIWA will pay for the IFREMER scientist who developed the probe (Nathalie Cochennec) to optimise the technique in New Zealand, on fresh and fixed material.

Experimental design

Three groups of oysters will be used.

Group 1: Oysters sampled in April 2000 were used to prepare heart smears, but some of them were also fixed in Davidson's fixative for 24 hrs, and then in 70% ethanol, to preserve DNA. Wax blocks will be prepared of those oysters, which varied from heavily infected to apparently uninfected, on the basis of the heart smears. Two sections will be cut from each of 20 blocks, one stained for histology, the other to be used for ISH.

Group 2: Oysters taken over the winters of 2000 and 2001, will be fixed and processed using the same protocol.

Group 3: These oysters (n=500) will be freshly shucked and processed for heart smears, histology and ISH. They will be used to optimise the use of the DNA probe to detect *Bonamia.* Once this has been done, the probe will be used for ISH on Group 1 and Group 2 oyster sections. Thus at the end of the study the relative sensitivities of the 3 techniques, and the depletion of *Bonamia* sp. over winter will have been determined.

Timeframe

January to March 2001.

9.2.3 Study 3

Objective

To determine the best method of purifying viable *Bonamia* sp. from infected oysters, by adapting published techniques on *Bonamia ostreae.*

Comment

In order to carry out studies on survival of *Bonamia* sp. outside the host, transmission in relation to distance/density, the course of infection, it is necessary to have quantified viable purified *Bonamia* sp. Purification techniques have been developed for *Bonamia ostreae* (Mialhe *et al.* 1988), and a similar sized pathogen of oysters, *Mikrocytos roughleyi* (Hervio *et al.* 1996), using sucrose density gradient centrifugation. Purification of *Bonamia ostreae* showed the yield to be between 10^7 and 5 x 10^7 (Mialhe *et al.* 1988). New Zealand *Bonamia* sp. is slightly larger and therefore these techniques will have to be modified.

Experimental design

Foveaux Strait oysters (n=180) from areas of recent mortality will be divided into 6 groups of 30 oysters). Each group will be shucked 10 at a time, heart imprints prepared and stained, and the imprints examined microscopically for the presence of *Bonamia* sp. Heavily infected oysters will be kept chilled and, after a small sample of digestive gland has been fixed for histology, the remainder will be weighed and then homogenised, and layered over sucrose and Percoll density gradients. After centrifugation, the cells at each interface will be removed, half the volume removed will be quantified using a haemocytometer, and the other half will be stained with acridine orange (Morvan *et al.* 1997), and examined under a fluorescence microscope, to assess viability. The purity of each interface will be assessed, the levels of *Bonamia* sp. retrieved will be compared with the levels of infection determined by histopathological examination of the fixed tissue, and viability assessed in relation to temperature and centrifugation speed.

Timeframe

January to March 2001.

9.3 Understanding the life history of *Bonamia* sp. and its relationship to oysters

9.3.1 Study 4

Objective

To determine the time course of infection from initial infection to death of infected oysters.

Comment

This information is required for direct application to the model, and in order to keep up a supply of heavily infected oysters. As in 2.4, the results will be affected by temperature, and the experiments must therefore be carried out at 8°C and 15°C.

Experimental design

Groups of 20 live uninfected oysters will be held in small tanks (40 x 20 x 20 cm), and inoculated into the digestive gland with 10^2, 10^4, 10^5 and 10^6 purified viable *Bonamia* sp. in 0.22 µm filtered seawater (FSW), and one group with FSW only (control) and held at 8°C and 15°C. This requires 200 oysters. At 2, 3, 4, and 5 months, 5 oysters from each group will be fixed and examined using routine histology. Should the results prove inconclusive, the experiment will be repeated and possibly modified.

Timeframe

March to June 2001, and March to June 2002

9.3.2 Study 5

Objective

To determine the size at which oysters become (i) infected with *Bonamia* sp., and (ii) diseased with *Bonamia* sp.

Comment

Each year only 10–12% of Foveaux Strait dredge oysters spawn as females, but 70–90% have male gametes (Jeffs & Creese 1996). Observations on the distribution and abundance within oysters between 1986 and 1988 showed that *Bonamia* sp. was most abundant in female oysters that were absorbing their eggs, rather than spawning them (Hine 1991a, 1991b). The absorption of ova by oysters is a marked feature of their reproductive cycle, particularly from January to May (Jeffs & Creese 1996, Cranfield pers. comm.), and may be related to temperature as the proportion of females spawning their ova is higher in the north of New Zealand (Jeffs & Hickman 2000). As the absorption of ova appears to be such an important feature in the annual pattern of infection, it would seem likely that in small oysters, before the first female cycle, the parasite may be present but not cause disease, while during their first female cycle disease may develop during ova absorption. Unfortunately, there is a dearth of information on this, and what little is known is conflicting. In European *Ostrea edulis,* parasite development is not clearly tied to reproductive cycle (Culloty & Mulcahy 1996), and infection levels may range from 4% as spat, to 39% at 18 months, 66% at 2 years, and more than 77% at over 3 years (Tigé *et al.* 1982). Foveaux Strait dredge oysters first enter their male cycle at 20 mm, and their female cycle at 50 mm (Jeffs & Hickman 2000), but during a survey of Foveaux Strait oysters in June–July 1990 of oysters over 30 mm, 17% (n=12) of oysters 31–40 mm were infected, with all oysters falling between 16% (41–50 mm; n=87) and 25% (61–70 mm; n=685). Therefore there appeared to be little difference in infection levels in the different size groups, but the survey only included 12 oysters in the 31–40 mm size group, and none of the size of first maturity, 20 mm. Whether an individual oyster becomes infected is likely to be determined by a combination of size and temperature, small oysters and low temperatures resulting in the ingestion of less food, and therefore less infective particles, than larger oysters at higher temperatures. The experiment will therefore be run at 8°C and 15°C.

Experimental design

Spat and juveniles of wild *Ostrea chilensis,* 10–70 mm in height, will be collected and divided into 10–20, 21–40 and 41–70 mm size groups. To determine whether they are already infected with *Bonamia* sp., 25 of each size group will be fixed using the fixation routine giving optimal results for *in situ* hybridisation (ISH), and the prevalence and intensity of infection with *Bonamia* sp., if any, will be determined. The two combinations of dose and time giving optimum infections, determined from the results of study 10., will be used to expose the three size groups at 25 oysters/tank measuring 40 x 20 x 20 mm. After exposure, the oysters will be held in clean seawater for the time needed to develop patent infections, determined previously. Another group of 25 oysters, held in a tank 40 x 20 x 20 mm, will be held in clean seawater for the same times as for experimental groups and will act as a control group. The exposures and subsequent holding of oysters will be run at 8°C and 15°C. After 4 months, or at the deaths of oysters, heart smears will be prepared, and tissues will be fixed for histology and ISH

Timeframe

February 2002 to June 2002

9.3.3 Study 6

Objective

To determine the extent to which the parasite population is depleted over winter.

Comment

After May each year the parasite population shows intracellular changes suggestive of abnormal lipid metabolism and senescence (Hine 1992, Hine & Wesney 1992, 1994b), which precedes an apparent crash in the parasite population (Hine 1991a, 1991b). However, the degree to which the parasite population crashes is unknown as it has been difficult to obtain samples over winter, and thus current knowledge is based on few data. It is important to discover the extent to which the population crashes, because if the crash is severe, the annual pattern of infection can be treated as an annual event not influenced by the events of the previous year. Alternatively, if the crash is partial, the annual event will be influenced by the size of the parasite population in the preceding year. Although senescence of the parasite population may account for the sudden decline of parasites, it may be that tissue levels are also influenced by the rate of uptake of *Bonamia* sp., and thus decline in parasites may be due to reduced feeding at the cold temperatures of winter. This may be investigated in studies 4 and 5 above.

Experimental design

Fifty wild oysters will be dredged monthly from 4 stations around the area of Foveaux Strait that experienced high mortality in April 2000, from August to October 2000 and 2001 and fixed using a protocol giving good results with ISH for *Bonamia ostreae* in Europe (2000 samples), and the protocol giving optimum results using ISH (2001 samples). Heart imprints will also be prepared from each oyster, and infection levels using heart imprints, routine histology and ISH for each oyster, will be compared. The distribution of the parasite within each oyster will be recorded in order to try to determine whether those parasites detected are remnants of the previous year's infection, or newly occur at likely portals of entry.

Timeframe

August to October 2000 and 2001.

9.3.4 Study 7

Objective

To determine how long the parasite can survive outside the host.

Comment

There are two facets to survival outside the host; survival in the water column during transmission between hosts, and survival on boats and fishing gear when the parasite will be exposed to partial desiccation and ultraviolet light. *Bonamia* sp. has no known spore stage resistant to desiccation, and the stage that exists outside the host, the dense form of the parasite, is simply a small (~2 μm) protoplasmic stage. It therefore appears unlikely that it can survive for long outside the oyster, particularly in only semi-moist conditions, or exposed to UV light. The host, *Ostrea chilensis,* is known to be able to survive at a wide range of temperatures (6–23°C) and salinities (8–33‰) (Buroker *et al.* 1983).

Experimental design

There are no published studies on the survival or dynamics of haplosporidians outside their hosts, but studies have been published on *Perkinsus marinus* (the cause of Dermo disease in oysters, *Crassostrea virginica*, in the eastern U.S.). In the latter case, studies have utilised

immunoassays based on polyclonal and monoclonal antibodies (Dungan & Roberson 1993, Roberson *et al.* 1996, Dungan 1997, Dungan & Hamilton 1997), and the polymerase chain reaction (PCR) (Wright *et al.* 1997, Yarnall *et al.* 1997). While these give very accurate results, the development of similar techniques for *Bonamia* sp., are costly, time-consuming, and outside the scope of this study. Other techniques will have to be developed to undertake this work, but basically one of two relatively cheap experimental approaches are envisaged. Both involve holding purified viable *Bonamia* sp. in FSW of various salinities, for different periods of time and a range of temperatures, in plastic multiwell trays. Before being placed in the wells, the *Bonamia* sp. will be tested for viability using acridine orange, and will be quantified. At the end of the experiment, the water will be removed and either gently spun down in a centrifuge tube, or the water passed through a 0.22 μm nitrocellulose filter. The viability of the parasite will be assessed using acridine orange, and a simple procedure developed to quantify the remaining parasites.

Stage 1 Survival in water: The environmental parameters tested will be salinity (i.e., 5, 15, 25‰), temperature (i.e., 0, 4, 25°C), and time (i.e., 1, 2, 3, 4, 6, 8, 12, 24, 48 hours).

Stage 2 Survival on surfaces: These experiments will be carried out at 16–18°C using FSW of ~32‰ salinity. The effects of desiccation alone, sunlight alone, and sunlight and desiccation, will be investigated. In all cases, nitrocellulose filters will be used to simulate surfaces, and it will be necessary initially to determine the best method of placing *Bonamia* sp. on the filters without significantly affecting parasite viability. The best method may be to filter small volumes of seawater containing purified *Bonamia* sp. onto filters in chambers attached to syringes. Once this has been achieved, desiccation will be investigated by placing uncontaminated filters in small plastic wells, covered with a drop of FSW, and determining how long the filters desiccate away from sunlight, at room temperature. Filters with viable *Bonamia* sp. will then be placed in small plastic wells and covered with a drop of FSW. Half the filters will be allowed to desiccate, while the other (control) half will be irrigated as necessary to retain a drop of FSW. Duplicate filters of both desiccating and moist (control) filters will be taken at one fifth of the time needed for complete desiccation, and parasite viability will be determined using acridine orange on one half of each filter, and the other half fixed for transmission electron microscopy (TEM). A parallel experiment will be carried out in direct sunlight; i.e., desiccated filters exposed to sunlight over the period of desiccation compared with moist filters exposed to sunlight over the same period. Each combination of desiccation/exposure will be carried out twice.

Timeframe

April to June 2001. This study is timed to be carried out during a period when infected material will be available.

9.3.5 Study 8

Objective

To determine the rate of shedding of *Bonamia* sp. by live, moribund and dead oysters.

Comment

It cannot be assumed that infected oysters shed *Bonamia* sp. at the same rate throughout the course of infection. The stage of infection, and degree of infection, will influence the rate of shedding. Oysters in the early stages of infection, or with light infections may not shed *Bonamia* sp., while conversely, those that are heavily infected or In reply to: the late stage of infection may shed many particles. Shedding will also be affected by temperature, because temperature directly affects the physiology of the host, and it is therefore necessary that rate

of shedding is studied at the temperature extremes of the bottom of Foveaux Strait, 8°C and 15°C (Cranfield 1968a, 1968b).

Experimental design

Initially two methods, filtration and centrifugation will be evaluated, modified from the techniques of Pernin *et al.* (1998). Initially, small tanks (100 x 100 x 150 mm) will be constructed that have a funnelled outflow leading to a circular grid of ~20mm diameter, on which a nitrocellulose filter can be placed. A slow flow of water through the tank will be seeded with a known number of purified *Bonamia* sp., and the outflow either filtered through a nitrocellulose filter, or collected for centrifugation. The filter will then be removed, cleared and stained with Hemacolor or another stain, and the number of *Bonamia* sp. will be counted, and an aliquot of the centrifuged water will be filtered, the filter cleared, and treated as for the filtered sample. This initial experiment will be used to determine which of filtration alone, or centrifugation of an aliquot followed by filtration, gives the best retrieval, the percentage of *Bonamia* sp. retrieved compared with numbers seeded, and the best way of detecting filtered *Bonamia* sp.

At each temperature, 20 individual adult uninfected oysters will be inoculated with *Bonamia* sp. at a dosage that will lead on to disease and death, using the information gathered from experiment 4 (above). They will be held in the small tanks at 8°C and 15°C for 3–4 months or until the experiment ends, and twice a week the water that flows through each tank in one hour will be examined, and the number of *Bonamia* sp. retrieved will be determined. From this the number shed over 24 hours can be determined. Should oysters be seen to gape and be moribund, samples will be taken more frequently. Four individual oysters will be sham injected, but otherwise treated as the experimental oysters, and serve as controls. Surviving oysters will be fixed and examined histologically to determine the status of infection.

Timeframe

January to April 2002.

9.3.6 Study 9

Objective

To determine the relationships between the gametogenic cycle of oysters and life cycle of *Bonamia* sp., and between *Bonamia* sp. and concurrent infections.

Comment

During 1986 and 1987, detailed information was collected on each oyster (reproductive state, distributions of lesions), other parasites (apicomplexans, microsporidians, Rickettsias, larvae of bucephalid trematodes) and their intensity and tissue distributions, and neoplastic conditions. These data have yet to be analysed, but represents a considerable resource. These data will be analysed to better describe the biology of *Bonamia* sp. and oysters.

Timeframe

Winters of 2000 and 2001.

9.4 Understanding the mechanism of spread of *Bonamia* sp. epizootic

9.4.1 Study 10

Objective

To determine the distance over which the parasite can be transmitted between oysters.

Comment

This experiment will require oysters that are likely to be *Bonamia* sp.-infected, and oysters that are not infected. The former may be obtained as a result of the results obtained from study 1 (above). The latter can only be obtained with certainty when we have access to a sensitive molecular probe for New Zealand *Bonamia* developed by IFREMER, which will be available after a visit by an IFREMER scientist in January–February 2001. With regard to transmission, two parameters are likely to be important, oyster density, and the distance between oysters. It is known from circumstantial evidence that the parasite readily transmits between oysters lying close to each other, but the critical distances that are involved are unknown. This experiment will be carried out early in the programme because transmission by close cohabitation between heavily infected oysters (obtained from study 1) and uninfected oysters, may be the most effective way of obtaining heavily infected oysters for subsequent studies.

The setting up experiments in which uninfected oysters of varying density are exposed to infected oysters at different distances would entail considerable cost and the need for considerable space. However, it is possible to take an alternative approach using different concentrations of viable purified *Bonamia* sp. as a proxy for distance. Thus the dilution of infective particles with distance from an oyster would be simulated by exposing oysters to different parasite concentrations. For this quantitative approach to be applicable to a model, it would be necessary to determine the degree of dilution with distance from the infected source, and the approximate numbers of *Bonamia* sp. shed by living, moribund, and dead infected oysters (see study 8). The experimental work must be carried out at temperatures simulating the bottom of Foveaux Strait, 8°C–16°C (Cranfield 1968). Temperature affects the rate at which oysters feed, and therefore the rates at which they ingest particles. For an infection to become established, a certain number of infectious particles have to be ingested within a short time (Hervio *et al.* 1995). If feeding rates slow with declining temperature, infectious particles are ingested more slowly, and destroyed by the host (Hine & Wesney 1994b). Therefore, the distance over which infection can be successfully established is affected by temperature, and would be different in summer and winter.

Experimental design

Initially it will be necessary to find a source of uninfected oysters. It is known that *Bonamia* sp. occurs in flat oysters in Dusky Sound, in Foveaux Strait and around Stewart Island, Golden Bay, Tasman Bay, the Marlborough Sounds, Port Underwood, and Wellington Harbour. North Island stocks are known to be genetically different from South Island stocks (Foighil *et al.* 1999), and a survey of North Island sites in 1986 showed them to be uninfected. However, North Island stocks are scarce, possibly the two best sites being around Kawau Island and in Manukau Harbour. Therefore, an exploratory survey will have to be carried out of the Catlins, Port Chalmers, possibly other east and west coast sites, and other North Island sites.

Assuming we examine 150 oysters each from 6 possibly uninfected sites, using the *Bonamia* sp. probe, 900 oysters will have to be fixed for histology/ISH. Histological examination will be carried out on each, and any showing haemocytosis, or any other pathology suggestive of *Bonamia* sp., will be sectioned again and examined by ISH.

This work will essentially follow the experimental approach of Hervio *et al.* (1995). The experiment will be carried out in at least two stages.

Stage 1. Groups of 10 live uninfected oysters will be held in small tanks (40 x 20 x 20 cm), and exposed to 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 purified viable *Bonamia* sp. (as there are 10 oysters in each exposed group, they will be exposed to 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 purified viable *Bonamia* sp.) in non-circulating seawater for 1, 2, 3, 6, 12, and 24 h at 15°C. The water will then be replaced by clean seawater, and the oysters reared for 3–4 months at 15°C. Another group of 10 oysters, held in non-circulating clean seawater for 12 h and then in clean circulating seawater for 3–4 months at 15°C, will act as controls. This involves 6 groups held for 6 time periods, plus controls (37 variables; 370 oysters). Those gaping or dying during the 3 months will be fixed and examined by routine histology. All surviving oysters will be fixed and examined by routine histology after 3–4 months.

Stage 2. Will be a repeat of stage 1, but at 8°C.

Timeframe

December 2000 to April 2001, and December 2001 to March 2002.

9.5 Understanding the host-parasite interaction between the oyster population and *Bonamia* sp.

9.5.1 Study 11

Objective

To model the host-parasite interactions between oysters and *Bonamia* sp.

Comment

Bonamia sp. outbreaks in Foveaux Strait dredge oysters have an important influence on the dynamics of the population. Mathematical models of the host-parasite or predator-prey interaction between *Bonamia* sp. and oysters can provide an insight into the effect of *Bonamia* sp. epizootics on population, fishery management, and estimates of sustainable yield. Such models have not been developed for *Bonamia* sp. in oysters, although models for related pathogens have recently been developed for some oyster populations (Powell *et al.* 1994, Hofmann *et al.* 1995, Powell *et al.* 1996, Powell *et al.* 1998, Ford *et al.* 1999, Paraso *et al.* 1999, Powell *et al.* 1999). These results suggest that environmental effects, such as temperature and salinity, can have a major role in regulating the epizootic events (Ford *et al.* 1999, Paraso *et al.* 1999). In addition, such mathematical models allow quantitative assessment of the relative impact of varying factors on disease epidemiology.

Experimental design

Develop mathematical models of the host-parasite interaction between *Bonamia* sp. and oysters in Foveaux Strait, and validate such models using simulation with the historical data (Doonan *et al.* 1994, Doonan *et al.* 1999, Dunn *et al.* 2000b) and experimental data from Section 2 above.

Timeframe

July 2001 to June 2002

9.6 Understanding the role of genetic resistance in oysters to *Bonamia* sp.

9.6.1 Study 12

Objective

To determine the role of oyster resistance in the epidemiology of *Bonamia* sp.

Comment

In the 1986–1992 epizootic, the pattern of infection observed was consistent with a transmission cascade in which most oysters in a population had low to moderate *Bonamia* sp. infections. These were thought to rapidly built up to heavy infections by recruitment into the population of infective particles from up-current mortality. This led to the death of heavily infected oysters and release of more infective particles causing mortality downstream. Thus the epizootic was seen as a cascade (Hine 1996). It is well known that oysters vary in their resistance to many diseases, such as *Haplosporidium nelsoni* (MSX), *Perkinsus marinus,* and juvenile oyster disease (JOD) (Sunila *et al.* 1999), nocardiosis (Friedman *et al.* 1999) and *Bonamia ostreae* (Naciri-Graven *et al.* 1998, Naciri-Graven *et al.* 1999). Such resistance, when it is lasting, may be genetically determined (Faisal *et al.* 1998), but short term differences in survival may be due to local differences in environmental factors (temperature, salinity, food availability, turbidity) affecting growth and feeding rates (Gaffney & Bushek 1996).

The pattern of infection seen during the epizootic in Foveaux Strait in April 2000 did not show the widespread low levels of infection leading to higher levels in some individuals, and death in others leading to a cascade. Instead, around the site of the epizootic, most oysters had no detectable infection (although the technique used, heart smears, is not very sensitive), but one or two would have high infection levels, often without oysters being present with moderate infections. This seems to suggest that in an oyster bed there were many oysters that were resistant to (rather than tolerant of) infection, but a few that were highly susceptible. The epizootic may therefore have been due to large numbers of susceptible oysters at one site. If this interpretation of the infection pattern is correct (and as listed above, the epidemiology of many protozoan diseases of bivalves is partly determined by host genetics), then the genetics of resistance/susceptibility of Foveaux Strait oysters to *Bonamia* sp., may play an important role in the development of epizootics.

Timeframe

The studies detailed above will take about two years to complete. The role of resistance is seen as critical to the understanding of the impact of *Bonamia* sp. on the fishery, but studies on the genetics of resistance will take longer than two years and have not been costed.

10. Effects of fishing on the environment

10.1 Introduction

The investigations detailed in this section (10.1–10.3.1) will be carried out as part of the FRST contract CO1X0007 "Fishing: Ecosystem Effects and Resource Sustainability". The first group of experimental investigations will look at short-term effects: is mortality of oysters (specifically mortality from bonamiasis) related to effects of fishing on the seafloor habitat and/or is it directly related to stress caused by the fishing process? These experiments will complement the earlier experiments and are dependent on them for the development of experimental methods. The second group of investigations considers the long-term effects of changes in the seafloor habitat from fishing on growth and recruitment of oysters. These

studies will lead to the development of methods of avoiding mitigating and remedying the environmental effects of fishing. Key information includes:

Understanding the relationship between stress and *Bonamia* sp.;

1. Investigating the effects of stress from altered habitat.
2. Investigating the effects of stress from burial.
3. Investigating the effects of stress from dredging.

Understanding the role of habitat and habitat changes on recruitment, growth, and mortality of oysters and their long-term effect on the fishery;

4. Investigating the effects of habitat complexity on the occurrence and spread of *Bonamia* sp.

10.2 Does the occurrence and spread of disease result 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 present at a low level 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. The general strategy of fishers in focussing exploitation of individual oyster populations could cause disturbance that is localised enough to explain the focussed nature of the last two disease outbreaks. In the current outbreak it seems that the bed in which the disease occurred had been subject to only light fishing in the six months prior to the start of the fishing season. Whether the stress of this light fishing was a causal or contributing factor in the current outbreak is unknown.

Although oysters may be stressed by natural environmental events such as storms or food availability, these events tend to effect oysters over the entire range of distribution in Foveaux Strait and do not give rise to localised stress that could explain the onset of disease at focal points. Each previous epizootic has commenced at very localised sites and then radiated from that point. Stress that could trigger such a disease outbreak would therefore need to be equally localised. Algal distribution in the turbulent conditions of Foveaux Strait is uniform vertically and tends to be uniform over large horizontal scales, hence feeding conditions for oysters are unlikely to provide localised stress. Poor feeding conditions may stress the entire oyster population that in combination with further circumscribed stress could result in a local outbreak of disease. On the other hand, changes in the benthic habitat and sediment mobility as the result of circumscribed fishing (Cranfield *et al.* 1999a), could cause the sort of local stress consistent with the past focussed disease outbreaks.

10.2.1 Stress from altered habitat

Dredging not only captures oysters. It also captures other epifauna and changes the nature of the seafloor habitat. These changes remove other filter-feeding epifauna, may render the remaining oysters more vulnerable to infection.

(Cranfield *et al.* 1999b) noted that the epidemic of 1963 (attributed at the time to another disease but probably the result of bonamiasis Hine 1996) was largely restricted to the eastern area of Foveaux Strait where epibenthos was sparse, while the western areas abundant

epibenthos were little affected. In the epidemic between 1986 and 1992, bonamiasis commenced in the centre of western Foveaux Strait where commercial fishing had meantime reduced epibenthos. Infection radiated outwards from this focus and over six years, disease reduced oysters in previously fished areas to uneconomic densities. The epizootic ceased in 1992 in the periphery of Foveaux Strait where epibenthos was still abundant.

Areas with high mortality during the two earlier disease outbreaks corresponded with areas from which fishing had largely removed epibenthos. These data suggest that epibenthos may mitigate the spread of disease. Such an effect could be the result of greater numbers of filter feeders (biogenic reefs are dominated by very high densities of filter feeding mytilids, tunicates, encrusting bryozoa and sponges) filtering infective particles in the vicinity of oysters. Alternatively (or additionally) it could be the result of the release of specific biologically active compounds by these organisms. Many species of these groups are known to produce and release biologically active compounds. Such secretions could kill infective particles.

Experimental design

The experimental techniques being developed in the initial *Bonamia* sp. investigation will allow us to identify and count living and dead infective particles of *Bonamia* sp. in the water. The experimental methods will also allow us to deliver standard doses of infective particles for experiments. These methods can then be used in simple laboratory experiments that test the impact of epifauna on transmission of infection. The experiments would measure the capacity of different densities of filter feeders (Mytilids, tunicates, sponges and bryozoa), alone and in combination, to remove or kill infective particles in the water column of static tanks compared to control tanks.

10.2.2 Stress from burial

At one western site (that is commercially dredged) oysters have been abundant on one dive, but not present on the next five months later. In the same period sampling gear that had been put down and left in place when oysters were visible, had become covered by several mm of coarse shell sand when revisited. Living oysters on this gear survived the burial. As oysters are dredged at this site commercially, the oysters that could not be seen were probably also buried under the sand. (Fishers have always insisted that oysters can become covered over after a big storm). Such burial may not suffocate oysters (the coarse shell sand is porous) but will impede free flow of water and algae so buried oysters will undergo nutritive stress. This could readily provide a localised stress that could trigger rapid increase of infection in the buried oysters that originally had had almost undetectable levels of infection. It is notable that many moribund oysters landed on oyster vessels have had considerable accumulations of coarse shell grit in the mantle. The possible role of such stress in enhancing existing levels of infection could be readily tested in laboratory experiments.

Experimental design

A sample of oysters will be dredged from an apparently uninfected area of Foveaux Strait. Heart smears of 50 will be examined to determine the baseline prevalence of infection by *Bonamia* sp. Replicate samples of 50 (or smaller number if aquarium space dictates it) randomly chosen oysters will be placed in single aquarium with water overflow running to waste and buried under 5 cms of coarse shell sand from Foveaux Strait. Oysters in control aquarium will not be buried. The oysters in one experimental and one control aquarium will be harvested from each aquarium at two-week, four-week, six-week and eight-week intervals and heart smears examined to determine the prevalence of infection in experimental and control groups.

10.2.3 Stress from dredging

Dredging only captures about 20% of the oysters on the seafloor and the remaining 80% are moved around by the dredge. Some are buried as they pass under the dredge others are damaged when they encounter the dredge. With the concentration of fishing on localised dense patches of oysters this disturbance may be repeated 15–40 times in a season. In addition a significant portion of the 20% oysters caught are undersized and are either washed out when the dredge is washed at the surface or are sorted out on the cultching benches of the vessel. These oysters undergo further stress before being returned to the seafloor. Such stress on oysters could increase their susceptibility to infection and could be readily tested in laboratory experiments with differing levels of handling and damage and validated against small-scale field experiments in the wild.

Experimental design

The methods (Group 1 and Group 2 disturbance) for providing a source of heavily infected material should throw some light on how important disturbance from fishing is likely to be in elevating levels of infection in oysters. Should the results of these experiments show it to be important, experiments that mimic the disturbance of dredging more precisely will be developed to investigate how much dredging disturbance is required to produce elevated incidence of infection.

10.3 Does alteration of the seafloor habitat affect growth and recruitment of oysters?

10.3.1 Studies of habitat complexity

Experiments investigating directly the linkage between recruitment and growth and mortality of the first two year classes of oysters on habitats of different complexity have found no effects on growth but significantly higher recruitment in complex environments. In the coming year this investigation will be extended in an indirect study covering a much wider range of habitats and oyster density.

The direct study revealed that habitat complexity was redeveloping at some of the sites modified by fishing. The indirect study will extend the observations of redeveloping habitat and confirm that this represents a community succession. These data can be utilised in management strategies to avoid, mitigate and remedy adverse environmental effects of fishing on the environment. These strategies will have a long-term influence on production of oysters if the environment proves to be important in growth of larger size groups of oysters and their mortality as well as in their recruitment.

11. Short term management of the fishery (determination of sustainable yields)

11.1 Introduction

Estimates of sustainable yield for the Foveaux Strait dredge oyster fishery are presently made using Method 2 of Annala *et al.* (2000) in which sustainable yield (MCY) is defined, e.g.,

$$MCY = 0.5\, F_{0.1}\, B_{beg}$$

The reference fishing mortality $F_{0.1}$ was estimated with a Ricker (1975) YPR model (see Michael *et al.* 2000a for the most recent estimate). The biomass estimate used in the MCY calculations has been derived from density estimates from biannual surveys and of the entire population size in areas designated to be 'commercial'. Commercial fishers determined 'commercial' areas after examination of charts and consideration of analyses of the 1999 logbook data. TACC estimates in previous years have used the portion of the population estimated to be within patches with densities of greater than 400 oysters per standard tow (~1.95 oysters m^{-2}). It is widely acknowledged, however, that these estimates can be improved by the application of less simplistic models and, prior to the *Bonamia* outbreak in March 2000, planned research included modelling alternative options for managing the fishery. This research has been postponed until a greater understanding of *Bonamia* has been developed (Section 2). In the interim, however, the Fisheries Act (1996) requires the Minister of Fisheries to set a TACC for the fishery. In the next few years, and before impacts of *Bonamia* can be incorporated into the assessment process, it is likely that TACCs will have to be set on the basis of MCY analyses as described above. Below we outline the data necessary for modelling and improved methods for stock assessment — these data should be collected now, even though they will not be used in the short term. Key information includes:

TACC setting in the short term;

1. Continued implementation of a logbook to collect and analyse fine-scale catch and effort data from the commercial fishery in the 2001 fishing season.
2. Estimating the length composition of the commercial catch.
3. Estimating sustainable yield for commercial oyster fishing in Foveaux Strait for the 2002 oyster season.

11.2 CPUE assessments

11.2.1 Study 13

Objective

Continued implementation of a logbook to collect and analyse fine-scale catch and effort data from the commercial fishery in the 2001 fishing season.

Comment

Central to improved assessment and management of the Foveaux Strait dredge oyster fishery will be improved catch and effort statistics. CPUE data can provide some indication of stock status, although may be unreliable when used in isolation. These statistics need to be gathered in a logbook. The logbook jointly developed by NIWA and BOMC has been refined to provide information at the smallest spatial scale that is logistically possible.

Timeframe

March 2001 to October 2001.

11.3 Composition of the commercial catch

11.3.1 Study 14

Objective
Determine the length composition of the commercial catch.

Comment
Central to improved assessment and management of the Foveaux Strait dredge oyster fishery will be information on the length composition of the commercial catch. Length composition data can provide some indication of stock status, although may be unreliable when used in isolation. Shed or other forms of catch sampling are relatively easy and inexpensive to collect. Length structured information from catch sampling has proved to be crucial in assessments of Rock Lobster and Paua.

Timeframe
March to October in each fishing year.

11.4 TACC setting for the 2002 fishing year

11.4.1 Study 15

Objective
To estimate sustainable yield for commercial oyster fishing in Foveaux Strait for the 2002 oyster season.

Comment
We propose that the 2002 TACC be based on (i) an October 2001 survey of the grounds that repeats of the 1999 survey design (Michael *et al.* 2000a) and (ii) updated boundaries of 'commercial' areas after consultation with the BOMC and skippers.

Timeframe
October 2001 to March 2002.

12. Improved management of the fishery (determination of sustainable yields in the long term)

12.1 Improved management

The Foveaux Strait oyster fishery operates on a sessile species. Because of the retained larvae and short time between spawning and settlement, the system probably has highly localised recruitment dynamics. With the widespread usage of GPS in modern fishing operations, the fleet is able to operate in specific areas with a high degree of accuracy. These characteristics all suggest that management options, data collection, assessment and modelling should focus on the fishery as a collection of small, discrete spatial areas. Rotational fishing is used in the New Zealand fishery for Challenger scallops and for some sessile invertebrates overseas (e.g., geoduc clams in British Columbia (Campbell *et al.* 1998), and red sea urchins on the Pacific coast of North America (Botsford *et al.* 1993). Rotational fishing, or even fishing a mosaic of small discrete areas independently as part of a larger fishery, has the potential to:

- increase efficiency by raising the mean catch rate
- reduce handling mortality on sub-legal sized oysters
- reduce the effects of disturbance on oysters on the bottom
- increase egg production

A simulation model will be used to explore management options that could be applied to a set of small sub-stocks of oysters. The model will be developed by combining models of the dynamics of oysters, *Bonamia* sp. and the interaction between these dynamics and the fishery.

The oyster model will be length-based, following the trend in lobsters (Punt & Kennedy 1997, Starr *et al.* 2000) and abalone (Andrew *et al.* 2000, Breen *et al.* 2000, Chen *et al.* 2001), although exact methods of determining MSY will need some consideration (Francis 1999). In theory the model could be age-structured, but ageing of oysters has proved to be unreliable (Michael *et al.* 2000b); there are few catch-at-age data and the length-based model can easily be used to explore changes in minimum legal size. We have some estimates of the growth (Dunn *et al.* 1998b) and mortality (Dunn *et al.* 1998a, Dunn *et al.* 2000a) of oysters and a limited amount of information on recruitment and the size-structure of both the population and landed catch.

The epizootic model will be developed from the classic predator-prey models of Anderson & May (1992) and host-parasite models (Hofmann *et al.* 1992, Hofmann *et al.* 1994, Hofmann *et al.* 1995). The work described in Section 2 above will provide an understanding of how *Bonamia* sp. epizootics start; the life history of *Bonamia* sp. and its relationship to the life cycle and seasonal cycle of oysters; and the mechanism of spread of *Bonamia* sp. epizootic. This information will be used to model the impact of *Bonamia* sp. on oyster dynamics.

These two component models will be used to simulate the impact of fishing on oyster populations. For these simulations, patterns in the behaviour of the fleet (derived from the logbooks) will be used to assess the management implications of fishing in different ways (e.g., rotational fishing and fishing with different gear).

Key information includes:

Information needs of epizootic model;

1. Understanding how *Bonamia* sp. epizootics start.
2. Understanding the life history of *Bonamia* sp. and its relationship to the life cycle and seasonal cycle of oysters.
3. Understanding the mechanism of spread of *Bonamia* sp. epizootic (including how to manage fishing practices exacerbating this).
4. Knowledge of changes in distribution and density of oysters as *Bonamia* sp. sweeps through the oyster population.
5. Knowledge of how distribution and density of oysters changes after *Bonamia* sp. epizootics cease.
6. Develop a model of the start, spread and mortality of *Bonamia* sp. epizootic in the oyster population.

Information needs of a length-structured model;

7. Knowledge of growth rates of oysters and range and causes of variation.
8. Knowledge of the length specific mortality rates of oysters and range and source of variation.
9. Knowledge of variability in recruitment.

10. Knowledge of length-at-age for oysters.
11. Knowledge of length structured exploitation.
12. The length structure of the population.

Information needs of a spatially disaggregated model;

13. Knowledge of spatial distribution of oyster populations in Foveaux Strait.
14. Understanding patterns of exploitation.
15. Understanding how the sea floor environment effects oyster population processes.
16. Effect of present fishing strategy on spatially disaggregated populations.

Assessing alternative management options;

17. Determination of alternative management options.
18. Evaluation of risk.
19. Evaluation of habitat modification and associated effects of fishing.

Timeframe

Modelling management options will begin in 2003.

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