ROCK LOBSTER ENHANCEMENT AND AQUACULTURE SUB-PROGRAM: HEALTH ASSURANCE OF SOUTHERN ROCK LOBSTERS

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NON TECHNICAL SUMMARY

2001/094 Rock Lobster Enhancement and Aquaculture Subprogram: Health Assurance Of Southern Rock Lobsters

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OBJECTIVES:

1. To undertake a health survey of representative groups of wild juvenile southern rock lobsters.
2. To undertake similar examination of statistically relevant numbers of cultured rock lobsters of similar age and to compare the prevalence of diseases with those found in wild stocks.
3. To use the information acquired from 1 and 2 in a risk analysis to determine the probability of adverse health consequences as a consequence of the release of cultured rock lobsters.
4. To define protocols for health testing of juvenile rock lobsters before release.

NON TECHNICAL SUMMARY:

Novel options for enhancing catch in the Tasmanian rock lobster fishery have been proposed, including the harvest and ongrowing of puerulus, and the translocation of under-size lobsters from slow growth to fast-growth regions. Both of these proposals involve translocating lobsters between locations, which has led to concerns about the risk of disease transfer between regions. The issue of disease transfer is even broader than these novel management measures because rock lobsters are extensively moved around the State through normal fishing operations. In response to these concerns, a health survey was initiated to determine what diseases are present in Tasmanian rock lobster populations, and the distribution of those diseases.

The survey assessed the health of 374 juvenile southern rock lobsters, and compared the health of the 234 wild animals from six of the seven recognised bioregions around Tasmania and the 140 animals from four cultured populations that had been ongrown since collection as pueruli. (The seventh Tasmanian bioregion contains few animals and was not included in the sampling.) The cultured groups comprised two populations raised in consecutive years from one on-shore flow through facility, and two populations raised in sea-cages. The number of ongrown samples was less than anticipated due to slower than expected development of the ongrowing sector, but represented samples from all the available populations.

Examinations consisted of gross and histological examination of a range of tissues from all animals, plus culture and haematology counts from selected animals. No clinical, bacteriological or histopathological evidence of significant infectious diseases was detected in any groups, though mild lesions associated with ubiquitous organisms and occasional individual animals with more marked pathology were seen.

An assessment of the risk posed by release of these ongrown animals into wild areas was carried out, and was completed with no potential hazards identified from the survey. A contraction of further planned releases from ongrown juveniles prevented development of a tailored health testing protocol for this sector. Nevertheless a precautionary approach to future translocations, and a proactive approach to continued general health surveillance for the wild as well as ongrowing sector is recommended.

Findings were further analysed to increase understanding of disease processes and reactions in this species, to aid diagnosis and health testing in the future, to compare the health implications of the two culture systems, and to allow comparisons with populations sampled in the future. A summary of major findings from these analyses is as follows:

- The only conditions consistently more marked in ongrown than wild stock were those of gill fouling and minor integument erosion, which were probably related. There were more differences between the batches within each type of culture system than between the systems, with length of culture period (and its corollary, size) appearing to be the major determinant of the severity.

- The level of bacterial carriage was low in all groups, despite other evidence of capture and transport stress. The aim for bacterial culture was to sample at least 10% of animals, including any ill animals. The actual number of haemolymph cultured was 87 (23%), with very few ill animals detected. Although eight animals showed carriage of low levels of bacteria, bacteria were not isolated in sufficient number from any animal to indicate active infection prior to sampling, and there was no histological evidence of bacterial disease in these animals. The bacteria identified from haemolymph cultures were low numbers of *Vibrio splendidus* I on seven occasions, from two ongrown animals (one on-shore, one sea-caged), and five wild animals from two growing areas, and one isolate of *Vibrio navarrensis*. While *V. splendidus* I has been implicated as a pathogen from some species it is more commonly seen as an opportunistic invader, *V. navarrensis* has not been reported as a pathogen in aquatic animals. Both are regarded as opportunistic infections of animals stressed by the collection process.

- Histological examination only occasionally showed bacteria within the tissues of other animals apart from surface fouling. Bacteria were detected in one wild animal with severe and apparently long-standing hepatopancreas damage resulting in almost total destruction of this organ. Similar cases have been reported elsewhere in this and other decapod species, generally as sporadic cases or related to inadequate manufactured diets.

- Several external parasites were associated with a mild host reaction though the overall effect on lobster health appeared slight. Parasites were almost entirely restricted to ectoparasites of the gill and (of less significance) the skin. These included protozoan fouling agents, turbellarians, goose barnacles, and other unidentified small crustacea. Turbellarians appear not to have been previously reported from lobsters, though they are common ectoparasites of molluscs.

- As well as the recognisable mild pathology associated with these pathogens, a number of tissue changes of uncertain origin were seen. One change, a zonal degeneration of the antennal glands, was regarded as of sufficient severity to be of concern with regard to long-term health of these individuals, though the number of affected animals was low and virtually all were in wild caught lobsters. The appearance generally was that of a recent change, though it was not established whether any of these lesions were likely to have been present prior to capture. Electron microscopic examination, undertaken as a precautionary measure only, excluded viruses as a cause of this condition but did not establish the nature of the cell changes. This change has been reported previously in Australia in one tropical lobster, and similar but not identical changes have occasionally described from other species. Though the cause or causes have not been identified for any of these, they do not appear to be infectious.

- Acute muscle damage was very common (almost 100% in one wild group), and was suspected to be an indication of capture or post-collection stress. The most severely affected group also showed a number of animals with mild turgid lobster syndrome.

- The reactions and cell changes associated with this and other pathology were examined for possible correlations which may warrant follow up under more controlled conditions, or by comparison of more sample sets over time. The detailed record of findings from this survey will facilitate subsequent examinations.

OUTCOMES ACHIEVED

The project provided confirmation that:

- the overall disease levels in juvenile southern rock lobsters *Jasus edwardsii* in Tasmania are low;
- that there was no evidence of major diseases, and;
- that juvenile rock lobsters could be ongrown under conditions which did not enhance diseases in a manner posing a risk to wild populations, if released.

As well as providing background data on health status of Tasmania's rock lobsters, this project provided knowledge on interpretation of pathological changes and tissue variations in this species.

KEY WORDS: lobster, health survey, Tasmania, aquaculture, *Jasus edwardsii*.

Acknowledgments

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1 BACKGROUND

1.1 General Background

At the time this project was proposed, Tasmania was embarking on what was anticipated as a major new aquaculture initiative to culture southern rock lobster, commencing with ongrowing of puerulus harvested from the wild. This formed part of a national program of FRDC, supported through its Rock Lobster Enhancement and Aquaculture Subprogram. While a major driver for this initiative was diversification of the aquaculture base, another major imperative in this instance is to avoid impact on the wild industry through puerulus harvest. Because wild harvest was already limited to maintain sustainability, there was concern that puerulus collection could impact on wild stock numbers. To prevent this, it was proposed to return a proportion of these animals to the wild after ongrowing, as a method of effectively neutralising the effects on wild stock numbers (Gardner et al., in press). The ability to use the ongrowing technology to enhance the wild fishery was also yet to be explored. Subsequent and parallel work has helped to determine the survival rates of puerulus from different microenvironments and of ongrown juveniles returned to the wild (Mills et al., 2005; Oliver et al., 2005; Mills et al., in press). However the health status of returned juveniles, in absolute terms and relative to those growing under natural conditions, is a critical determinant of both the survival of ongrown animals and of their risk to wild lobsters in the area of release. This work was to define and compare the health status of wild and ongrown juvenile rock lobsters, and to assess such risks.

The initial collections of puerulus for ongrowing were delayed with less collecting gear deployed than anticipated. This slowed the momentum of the ongrowing program and the course of the project, and reducing the emphasis on the release of ongrown juveniles. This, plus the lack of overt infectious disease risks detected, resulted in some redirection of the project towards an increased emphasis on the long-term understanding and assessment of factors affecting the general health of lobsters in culture.

1.2 Project development

Operators from the wild fishery had voiced deep concern about the possibility of ongrown lobsters transferring infectious diseases to the wild and thereby impacting the fishery, possibly on an on-going basis. There was also a broad community concern recognised. There was general recognition that addressing these concerns was inhibited by the lack of sound knowledge of diseases of southern rock lobsters as the health of lobsters in general, and Tasmanian rock lobsters in particular, had been little studied.

Comprehensive health surveys had been proposed previously but had been judged premature until the feasibility of culture was established but a pilot study of disease conditions in potential aquaculture species had been was undertaken in conjunction with culture projects. Results from that study (FRDC funded project 98/304 Evans et al, 2004a, Handlinger et al, 2004) provided some background information on diseases encountered in culture, but were regarded as only peripheral to this issue as this had included very few juvenile examined directly from the wild.

Consultation with industry and other stakeholders to develop a project to address this specific need was extensive.

In July 1997, FRDC sponsored a workshop in Hobart on rock lobster aquaculture where health monitoring was identified as a major issue for future research. Wild harvest and aquaculture industry representatives at the Tasmanian Rock Lobster Aquaculture Working Group (RLAWG) identified the need for research specifically on the health of reseeded lobsters in 1998. These concerns were reiterated at the 3rd International Rock Lobster Congress, in Adelaide, September 1999; the 1998/99/00 annual general meetings of the Tasmanian Rock Lobster Fishermen's' Association (TRLFA); and in industry articles in the magazine "Fishing Today". In October 1999, the Tasmanian Crustacean Fishery Advisory Committee to the minister (CFAC) noted the need for disease monitoring capability for rock lobster aquaculture. The project was developed under an anticipated acceleration of the culture program in this state, with the support of the Scientific and Steering committees of RLEAS, the Tasmanian Crustacean Research Advisory Group (CRAG), which comprises research representatives from several organisations as well as community and industry stakeholders; representatives of the Tasmanian Government, of the wild fish industry and Aquaculturists involved with the lobster culture program; the Tasmanian Fish Health Advisory Group (TFHAG); and the national Fish Health Management Committee (FHMC). FHMC noted that this represents an initiative consistent with AQUAPLAN Program 6 (Research and Development), in particular with its new Project 6.2.4 "Health R&D for infant industries and new aquaculture species". The need for baseline data is recognised as a key research area for fish health by SCFA (Subprogram B for Environmental Management, National Research and Development Plan). AQUAPLAN also recognises the need for adequate surveillance and for health studies for new aquaculture industries, and FHMC has noted that this project represents an initiative consistent with these AQUAPLAN objectives.

1.3 General Introduction

Diseases hitherto encountered in Australian spiny lobsters from a previous FRDC project have been recently documented in Evans, 2004a (those of adult lobsters by Stephens *et al*, 2004, those of larvae and juveniles by Diggles and Handlinger, 2004). Diseases which have been recognised in Australia include vibriosis (with several *Vibrio* species of bacteria implicated), other bacterial diseases and fungal infections. A number of parasites have been observed (in various species) including nemertean worms, microsporidiosis, free living nematode worms, ciliates and dinoflagellates which may result in external fouling of exoskeleton and/or clinical disease. Turgid lobster syndrome is a non-infectious condition, which has been reported in Australian post harvest lobsters. Diseases of larvae and juveniles are similar (Diggles and Handlinger, 2004), and include external shell and gill fouling bacteria, protozoa and fungi, which may progress to shell disease, internal fungal infection or localised internal bacterial infections, plus a range of internal *Vibrio* infections, either as a generalised infection (especially in larvae) or in juveniles as an enteritis, with or without suspected flagellate involvement.

Overall the infectious diseases in Australian spiny lobsters were assessed as similar to those occurring in clawed lobsters from the northern hemisphere with the exceptions of the absence of the major northern hemisphere diseases gaffkemia, "bumper car disease" due to the ciliated protozoan *Anophryoides haemophila*, and the more recently described herpes viral disease of the Caribbean spiny lobster, *Panulirus argus* (Shields and Behringer, 2004).

Internal parasites appear more likely to have a limited distribution. For example, *Haematodinium* dinoflagellates have been reported as a disease of the Norway lobster, especially on the West Coast of Scotland (Bower, 2000). This disease is apparently absent from Australian lobsters, though similar species of dinoflagellates have been found in

Queensland crabs (Hudson and Lester, 1994, Hudson and Shields, 1994). Paramoebiasis also appears to be absent from Australian lobsters, though a high level of paramoeba gill infection is encountered in salmonids farmed in the study area (albeit with a different paramoeba species). In contrast, *Ameson* sp. microsporea have been reported only from the Australian region, causing "white tail disease" in *Panuliris cygnus* and *Panulirus ornatus,* but have not been reported in *Jasus edwardsii*, the subject of this study (Denis and Munday, 1994).

Diseases of Australian freshwater crayfish, which need to be considered as potential cross-infections in other decapods have been summarised by Edgerton, 2004.

In general the internal protozoan or protozoan like diseases and internal bacterial infections, plus one virus, have been shown to have the potential to become overwhelming in individual animals, but the prevalence and the severity in affected individuals will vary with stress levels. While ongrowing of puerulus in culture within the same region would not in itself provide a means of introduction of new disease agents (other than through additional external inputs) the crowding and water quality in culture systems could lead to significant stress. This is likely to be especially so during the development phase while optimum conditions are being determined. This may have exacerbated overall levels of endemic diseases, potentially resulting in release of animals with either a low survival rate or an active source of infection of diseases that otherwise occur naturally only at low prevalence. This could increase the exposure rate of animals within an area to these diseases. Comparing the overall health of animals collected from the wild and held in culture is therefore essential for an assessment of health risks associated with release (reseeding) of juveniles.

2 NEED

While population neutrality as the result of rock lobster culture based on puerulus collection remained a priority for the rock lobster wild fishery, this could only be assured if both survival and impact of the juveniles returned after ongrowing was satisfactory. Operators from the wild fishery have voiced concern about the possibility of cultured fish transferring infectious diseases to the wild and thereby impacting on the fishery, possibly on an ongoing basis. It would be relatively simple to address this concern if there were a sound knowledge of diseases of southern rock lobsters and a database on their occurrence in different marine bioregions. Unfortunately, practically nothing was known about diseases of wild, Tasmanian rock lobsters prior to this study. Monitoring health of wild caught puerulus has provided preliminary data under limited circumstances (Handlinger et al, 1999), and limited data from other populations and other species had been collected and recently collated (Evans, 2004a), but baseline data was clearly inadequate.

The second requirement for successful return of ongrown puerulus to the wild is healthy and competent cultured stock likely to survive in the new environment. This project also aimed to fill the need for criteria to assess general health and fitness in culture systems. It was carried out in parallel with research to assess the predatory risks to healthy cultured lobsters newly returned to a wild environment (Gardner et al., 2004). Such knowledge of general health assessment, and the use of this to evaluate the effect of different culture systems on lobster health, are also needed for efficient ongrowing of stock, regardless of the source and destination.

3 OBJECTIVES

1. To undertake a health survey of representative groups of wild juvenile southern rock lobsters.
2. To undertake similar examination of statistically-relevant numbers of cultured rock lobsters of similar age and to compare the prevalences of diseases with those found in wild stocks.
3. To use the information acquired from (a) and (b) in a risk analysis to determine the probability of adverse health consequences as a consequence of the release of cultured rock lobsters.
4. To define protocols for health testing of juvenile rock lobsters before release

4 METHODS

4.1 Experimental design

The project was designed to examine and compare the health of wild and captive juvenile populations in a statistically relevant manner that would permit an informed decision on whether the latter are likely to pose a health hazard to the former. One round of sampling over one season was anticipated, covering each of the designated bioregions and every population considered for release in the initial year, enabling a decision of the release of animals from the actual populations sampled.

The number of animals examined in any one population was set at a level adequate to detect a 10% prevalence of disease with a 90% confidence level (Simon and Schill, 1984). At this prevalence diseases with a serious outcome could be expected to have an effect at the population. In 95% of such cases, infection within the population would be detectable in a single random sample of 30 animals, provided there is a high test efficiency (Cannon and Roe, 1982), or with adequate correction for sensitivity of the method (Langdon, 1991). For the size of the populations in consideration this level was 40 animals/population, presuming a sensitivity of 75% for the analytical techniques.

For the wild populations 40 animals were to be collected from the six designated Tasmanian bioregions with significant lobster stocks, giving a total of 240 animals. It was anticipated that there would be about seven growers of the rock lobster pueruli, which would give a total of 280 cultured animals for examination. However the number and composition of grower and growout systems changed, initially with amalgamation of permit holders and the holding of combined growout stock at one facility, and later with reduction of the number of active permit holders. This resulted in fewer culture facilities, requiring an extension of the project to provide samples from multiple cohorts over an extended period to provide an appropriate comparison. The project design was also complicated by the division of culture systems into on-shore flow-through systems and sea-caged systems, which provided another source of variation.

4.2 Sample Collection

Designated bioregions are provided in Figure 1. Wild samples were collected by diving from six of these regions, in conjunction with stock assessment work where possible, but requiring a dedicated collecting trip for the Flinders Island area, the cost of which prevented re-sampling when bad weather prevented collection of the required number of samples. A similar

situation prevented a full sample being collected from the southern Davey region. The Boags region was not targeted as this has very few lobsters and contributes virtually nothing to the fishery.

Ongrown stock were to be sampled after at least six months in the culture system, though the length of the culture period varied from close to that period to a few animals held for approximately 3 years. The ability for true random sampling varied between cultured stocks due to variations in the culture systems, but all samples approximated random samples within the sampled cages where multiple cages were present.

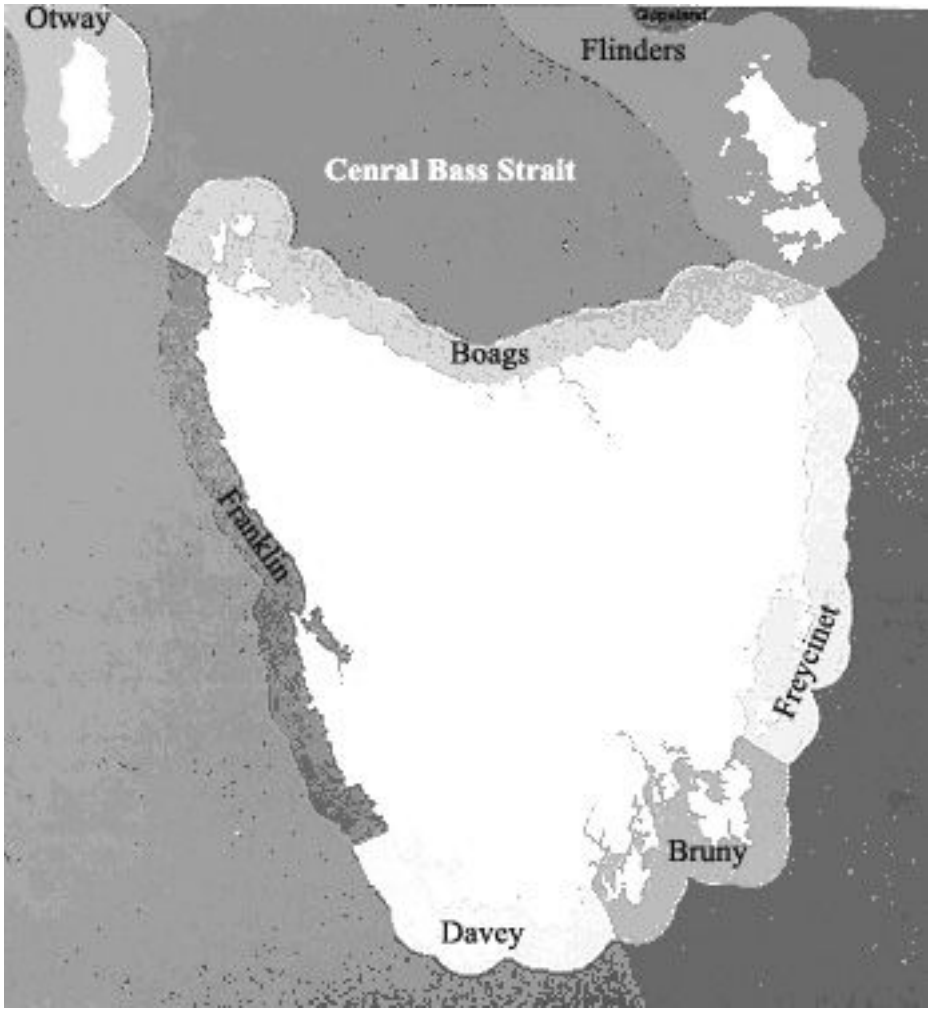


Figure 1: Tasmanian southern rock lobster bioregions

4.3 Examination procedure

The examination consisted of a naked-eye external examination to detect and document such conditions as shell-disease, leg loss and the presence of parasites, and collection of primary size and condition data, followed by dissection and internal gross examination and fixation of sections from selected organs for histological examination.

After gross assessment for activity, animals were sedated with AQUI-S anaesthesia, according to product instructions, examined for the presence and location of any lesions, blisters, leg or antennae damage; the sex and maturity of the animal; and carapace length and (in most cases)

total weight. From up to 10% of the animals, including any considered in ill-health, approximately 1 ml of haemolymph was then collected aseptically, usually from ventral sinus on the base of the fifth walking leg, or the pericardial sinus in smaller animals. A haemocytometer chamber was immediately filled for total haemocyte counts and the remainder used for bacterial culture. At this stage appropriate additional samples were taken for bacteriological culture from significant gross external lesions.

Lobsters were then euthanased and dissected according to the preliminary FRDC/RLEAS Rock Lobster Autopsy Manual (project 1999/202), as only the preliminary form of the manual was available until recently (Evans, 2004b). Gills were examined and any parasites collected and placed in preservative, and tissues for histopathology were collected and fixed from all lobsters.

4.3.1 Haemocyte counts

As haemolymph was required for both bacteriology and haemocyte counts, no cell preserving diluents were used for collection, and haemocyte counts were carried out immediately after collection on undiluted haemolymph using a standard Improved Neubauer haemocytometer. Previously, it had been confirmed that this technique was satisfactory and practical for routine diagnostic use, given the slower clotting time for this species, compared to other Australian lobsters (Evans et al, 2004). Satisfactory counts depended on speed and on avoiding tissue damage during collection as this accelerates the cell clotting process. Any samples showing cell clotting were noted but rejected.

4.3.2 Bacteriological methods

Bacterial sampling was primarily directed to detectable lesions and / or lobsters showing ill-health, though in general at least 10% of lobsters were sampled to provide background data on the level and nature of non-detectable bacterial carriage as well as bacterial assessment of grossly detectable lesions. For culture of external lesions or blisters, swabs from the site were cultured on plates of selective Shieh's culture medium (CRC for Aquaculture proprietary formulation) for marine flavobacteria. Haemolymph or samples from internal lesions were cultured on blood agar with 2% NaCl and TCBS and Johnson's marine agar (JMA) for heterotrophic bacteria (Johnson, 1968[1])or ZoBell's marine agar 2216E (ZMA) (ZoBell, 1946[2]) culture plates. Cultures were incubated at 25°C for 48 hours. Smears were prepared from all primary material cultured and Gram stained. Primary cultures were assessed on the basis of purity and abundance and predominant bacterial forms were subcultured and purified on JMA or ZMA. Nearly all isolated bacteria were determined to be Vibrionaceae and these were identified initially using MicroSys® V48 and latterly V36 identification system for *Vibrio* and related species (Dept. Primary Industries, Water & Environment, Launceston). MicroSys is a miniaturised identification system using biochemical tests. Identification was undertaken by matching the profile of an unknown against a database of reactions for known species. Matching is undertaken by probabilistic methods using the software package PIBWin (Bryant 2004[3]) and the VibEx6 database for MicroSys V36. An identification was accepted if the Willcox probability score ≥0.99.

[1] Johnson, P. T. (1968) A new medium for maintenance of marine bacteria. Journal of Invertebrate Pathology 11:144

[2] ZoBell CE (1946) Marine Microbiology. Chronica Botanica Company, Waltham, MA.

[3] Johnson, P. T. (1968) A new medium for maintenance of marine bacteria. Journal of Invertebrate Pathology 11:144

4.3.3 Histological preparation

For routine analysis, 10% seawater formalin fixation was used, after initial comparison of fixatives (Diggles and Handlinger, 2004) confirmed overall morphology retention with seawater formalin were comparable to other fixatives, provided that tissues were rapidly and sufficient exposed for fixation. This fixative was preferred as samples retained moderate suitability for electron microscopy where required. Organs from most animals were dissected for fixation, as appropriate, but some smaller animals were fixed after mid-line or tangential section.

After decalcification of keratinised tissues in Fastcal*, fixed tissues were processed by standard means and examined after standard haematoxylin and eosin staining, or other stains such as Geimsa, Zeihl-Neelsen's acid fast stain or Gram stain as required for follow up work.

Electron microscopy was carried out as required on residual formalin fixed tissue or by re-fixation and re-embedding from the routine paraffin blocks, according to the following procedure:

The following steps for reprocessing from were performed on an Automatic Mixer:
1. Tissue for EM impregnated with wax is placed into glass vial containing xylene overnight. Xylene removed and replaced with fresh xylene (24 hours total);
2. Xylene removed and absolute ethanol added to vial overnight. Following morning, fresh absolute ethanol x 2 changes (18 hours total);
3. Remove absolute ethanol. Rehydrate with ethanol to distilled water via 1 hour of each 95%, 70%, 50%, 30% distilled water. Finish with 1 hour of cacodylate buffer;
4. Dissect into 0.5mm^2 blocks;
5. Fix in 2.5% glutaraldehyde in 0.1M NaCaCO buffer;
6. Rinse with buffer;
7. Post- fix and process as per routine electron microscopy samples as follows: Post fixation in 1% osmium tetroxide for 1 hour at room temperature; rinse with distilled water then add uranyl acetate for 30 minutes and rinse again. Dehydrate tissue to 100% ethanol, clear in 100% propylene oxide; infuse with 50:50 mix resin/propylene oxide on rotator for 2-3 hours, embed in resin. Thin sections were routinely prepared and stained and examine in Hitachi H300 electron microscope.

4.4 Histological examination

For a comparison of significant diseases between wild and farmed groups, it was first necessary to define what diseases were present in Tasmanian rock lobsters. Parallel and integral with this was an assessment of the apparent clinical significance of these in individual animals, before undertaking a formal assessment of their likely significance in populations exposed by re-release of juvenile lobsters from culture systems.

As both normal tissue variations and the pathology of lobsters is poorly defined, tissue changes were examined in detail and tabulated, using a scoring system of 0 to 3 (absent, minimal, moderate, severe or abundant). This provided a basis for sequential assessment of

[3] ZoBell CE (1946) Marine Microbiology. Chronica Botanica Company, Waltham, MA.
[3] Bryant, T., 2004. PIBWin – Software for probabilistic identification. Journal of Applied Microbiology 97, 1326–1327

the disease agents and pathology present, the overall health of the groups examined, a comparison of the health of wild and farmed animals, and a background database for health assessments of lobsters examined in the future regardless of whether current interpretation of these changes is valid. During this process, each lobster was assessed for significant pathology, or an overall pattern of ill-health or unusual presentation.

Tissues examined were the integument, gills, heart, antennal gland, anterior gut, hepatopancreas and digestive gland, ventral nerve cord, caudal or hind-gut, gonad, the skeletal muscle from the first body segment plus any white muscle lesions, and any other tissues showing signs of abnormalities. The smaller mid-gut and ventral nerve cord were infrequently recovered and examined.

After an overview for significant whole body pathology, each of these tissues was examined for the presence of disease agents, evidence of degeneration, degenerative processes and evidence of inflammation. The disease agents detected were classified as invasive bacteria, crustacean parasites, protozoan and metazoan parasites (which were further subdivided on follow-up examinations) and general biofouling. The degenerative processes were divided into autolysis, degeneration, necrosis, coagulation of the haemolymph, oedema, disruption of the tissues, intracellular storage components and intracellular inclusions such as protein aggregations. The inflammatory changes were divided into evidence of haemocyte aggregation without visible cell adhesion, the presence of eosinophilic granular haemocytes, melanisation and granulomas. The results were then analysed for the presence of distinct disease entities, both infectious and non-infectious, and the prevalence and severity of these compared between the different groups.

The process of overall assessment of the variability, cause and significance of these findings was undertaken as a two-step process. Initially, group total and means scores for each change were compared for each change, in each organ, as well as evaluation of individual animals showing a significant overall pattern of pathology. This step was undertaken primarily to gain an understanding of the overall significance of the changes, and the underlying physiological basis for these. Compiled data were assessed for any apparent overall correlations, to enable a better interpretation of tissue changes and understanding of lobster pathology. Assessment was descriptive rather than statistical as collection times, size, and submission conditions between groups were not controlled and did differ, and hence possible correlations may well spurious. An understanding of the biology of possible links was regarded as a sounder basis for further verification. Findings were then assessed in detail for each bioregion and culture group, to describe the health status of that region or group. This step was undertaken primarily to determine any differences between farmed and wild caught animals, and between farming systems.

4.5 Risk Assessment

These results were then included in a formal risk analysis undertaken to determine the probability of adverse health consequences resulting from the release of cultured rock lobsters. This was undertaken with particular reference to the cultured batches that were samples and to be released in the course of this project, and with general reference to on-going risk management for subsequent releases. The risk analysis was to be carried out according to the AQIS Import Risk Analysis Handbook (AQIS, 1998 as revised in 2003), which incorporates international standards of the World Trade Organisation (WTO) sanitary and phytosanitary (SPS) Agreement and OIE International Aquatic Animal Health Code. To meet the consistency obligations of the SPS agreement, there was a need for the assessment to

be consistent with Tasmania's policies on other animal movements and ALOP (allowable level of protection), as previously defined (Tasmanian import risk analysis document, Anon, 2000). The steps outlined for a risk assessment process under each of these guidelines are the identification of hazards, followed by the assessment of risk, including consequences assessment, and if necessary a consideration of risk management.

For the purpose of defining the range of lobster diseases in Tasmania and parameters for general health and fitness of lobsters, this project also utilised results from DPIWE passive health surveillance of cultured lobsters and other University of Tasmania lobster research.

5 RESULTS AND DISCUSSION

5.1 Samples examined.

Samples examined are presented in Table 1. There were a total of 374 animals examined of which 234 were wild and 140 farmed animals. Wild animals were sampled from six of the seven recognised bioregions as shown in Figure 2. The total number of 40 samples was generally collected as two smaller batches and incomplete samples were obtained from two locations. Weather conditions during planned sampling periods and the unbudgeted high cost of re-sampling prevented a second sampling from the Flinders' and Davey regions, (though an additional half-sample was obtained from close to the margin of the Davey and Bruny regions). As indicated in the project proposal, no samples were collected from the Boags bioregion as this has very few lobsters and contributes very little to the fishery.

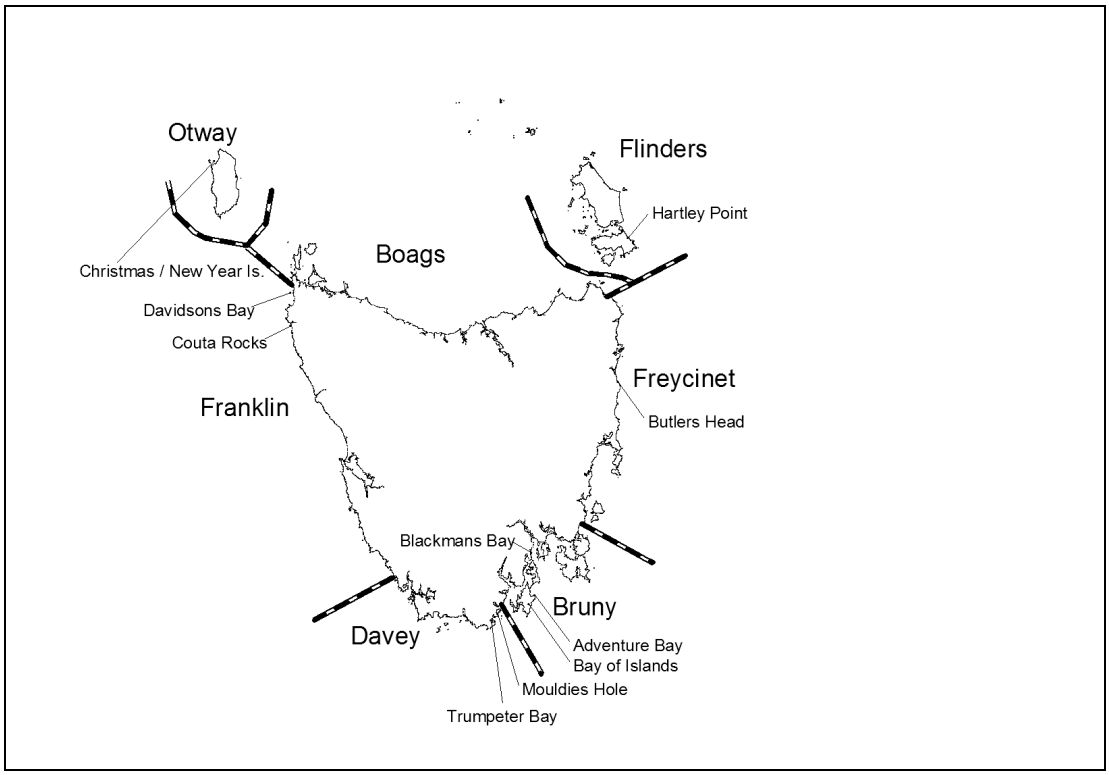


Figure 2. Sampling sites in relation to rock lobster bioregions.

Table 1. Samples received.

Source	wild	wild	wild	wild	wild	wild	wild	wild	wild	wild	wild	Farmed	Farmed	Farmed	Farmed
Region	King Is / Otway Area	King Is / Otway Area	East Coast / Freycinet	East Coast / Freycinet	Flinders Is	Bruny Area	South Coast / Davey	South Coast / Davey	West Coast / Franklin Region	Bruny Area	West Coast / Franklin Region	Bruny Area	Bruny Area	Bruny Area	Bruny Area
Site	Christmas / New Year	Christmas / New Year	Butler's Head	Butler's Head	Hartley Point	Adventure Bay & Blackman's Bay	Mouldies Hole	Trumpeter Bay	Davidsons Bay, Woolnorth	Bay of Islands	Couta Rocks	On-shore 1 (2 year classes)	On-shore 2	Sea-cage 1	Sea-cage 2 (3 aged / diets)
Date	01/16/02	01/18/02	02/19/02	02/28/02	03/22/02	04/22/02	04/29/02	05/29/02	02/11/02	01/22/03	02/05/02	09/20/02	05/01/03	12/15/03	04/09/04
Accn no.	020145	020171	020485	020607	020848	021116	021169	021450	023217	030150	030244	022476	03/0845	032783	040571
No of lobsters	27	15	21	20	24	42	22	5	25	18	15	40	42	20	38
Transport	boat / bus	boat / bus	boat / bus	boat / bus/ holding 2-5 d	boat / air	boat / bus	boat / bus	boat / held briefly at MRL / bus	boat / bus	boat / bus	boat / bus	? bus	Bus	Car, 2 days holding	Car (3 diets)
Av length mm	65.00	85.60	82.20	82.70	79.90	70.80	80.90	60.30	74.70	81.40	80.60	39.50	27.90	28.20	82.20
L range	(33-96)	(75-102)	(59-109)	(22.8-104)	(60-93.5 & 1 "small")	(35-108)	(50-98)	(47-75)	(47-105)	(49-104)	(51-102)	24-70	22.2-35	21-36	46.7-107

The farmed samples were taken from four main batches, two raised on shore in flow through systems and two raised in sea cages. The on-shore batches were collected on behalf of permit holders by the Marine Research Laboratory (MRL) and Tassal, and held on-shore at MRL facilities during on-culture. There were age sub-divisions within two of these samples. All culture sites were within the Bruny bioregion. Puerulus collection sites and proposed release sites for the on-shore cultured groups are shown in Figures 3 and 4.

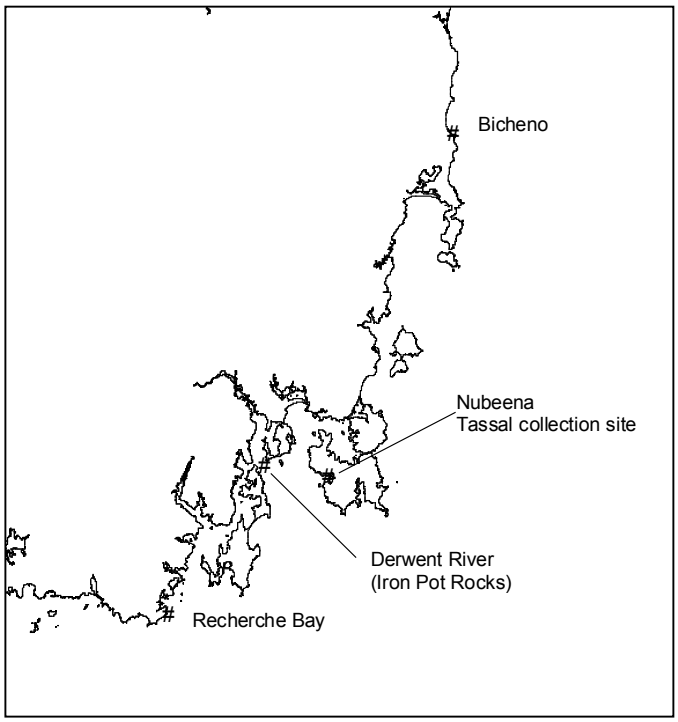


Figure 3. MRL and Tassal puerulus collection sites

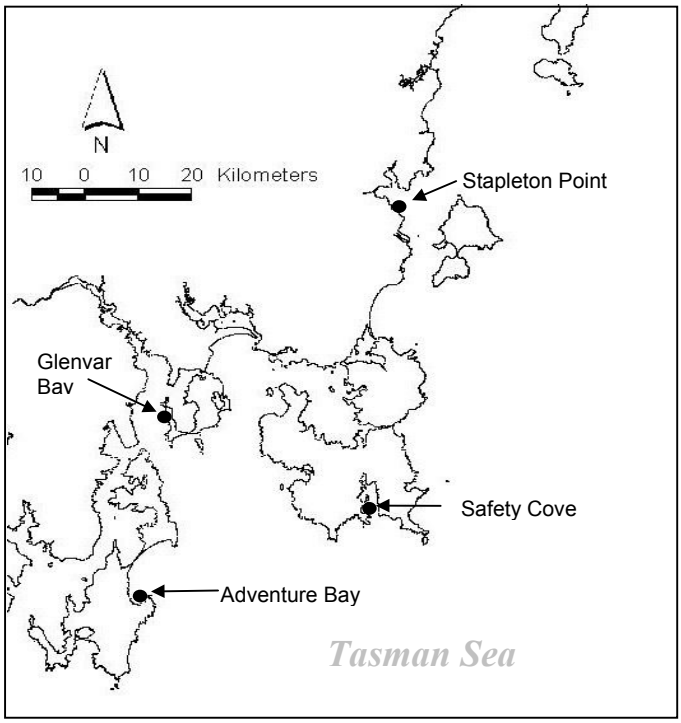


Figure 4. Lobster release sites

5.2 Overview of disease survey findings

"The study of things caused must precede the study of the cause of things" – John Francis.

5.2.1 Gross findings

Limb loss pattern, blisters and erosions are summarised in Table 2, and are considered below together with histological findings from each area.

5.2.2 Bacteriology results

Haemolymph was cultured from 87 lobsters. This included any lobsters judged to be moribund or showing evidence of poor health, though the majority were randomly selected with the exclusion of animals judged too small for routine haemolymph collection. Results are presented in Table 3.

Vibrio s*plendidus* I was the only bacteria isolated from haemolymph, apart from one isolate of *Vibrio navarrensis. V. splendidus* I was isolated on seven occasions (three from Bruny area, two from the West or Franklin area, and one each from sea-cage and on-shore systems), but on each occasion was present at low or very low levels (+/- or in one case +), and in one case as part of a mixed *Vibrio* infection where not all colonies could be speciated.

There was no histological evidence of bacterial disease in the animals from which positive cultures were obtained.

Grossly, these animals showed no tail blisters, four showed some evidence of tail integument erosions, three with lost limbs. Limb losses were moderate only (one or two limbs lost), but on the basis of closure and melanisation, all were classed as old lesions, though it was uncertain if the time-frame extended beyond the collection period of up to several days for some animals between initial collection and autopsy.

The most consistent and significant change seen in these animals was muscle degeneration. Six of these animals showed muscle lesions (four of these severe, three with inflammatory infiltrates), including one lobster with pale muscle streaking grossly though muscle lesions were not seen in the sections examined. One also showed orange discoloured haemolymph. No significant findings were seen in the one small sea-caged animal.

There was no apparent correlation with other tissue changes: total reserve inclusion cell count varied from 0 to high (7) in culture positive animals; and the degree of haemolymph coagulation also varied. Most showed minimal levels of eosinophilic granulocytes in tissues, though total body granulocyte levels were high in the animal with orange haemolymph, and moderate but mainly associated with integument surface in another.

Total haemocyte counts were done on haemolymph from most of these animals. The small sea-caged lobster showed a high haemocyte count (28,450 / mm^3). No haemocyte count was carried out on the on-shore cultured animal with a mixed infection. Haemocyte counts in two wild lobsters from the Bruny region ranged from very low (1100 / mm^3), to medium (9700 and 19800 / mm^3), with a similar level (18350 / mm^3), in a lobster from the West or Franklin region.

Table 2. External gross findings from Tasmanian juvenile rock lobsters *Jasus edwardsii* from all areas.

Region	King Is / Otway Area	King Is / Otway Area	East Coast / Freycinet	East Coast / Freycinet	Flinders Is	Bruny Area	South Coast / Davey	South Coast / Davey	West Coast / Franklin Region	Bruny Area	West Coast / Franklin Region				
Site	Christmas / New Year Is	Christmas / New Year Is	Butler's Head	Butler's Head	Hartley Point	Adventure Bay & Blackman's Bay	Mouldies Hole	Trumpeter Bay	Davidsons Bay, Woolnorth	Bay of Islands	Couta Rocks	On-shore 1 (2 year classes)	On-shore 2	Sea-cage 1	Sea-cage 2 (3 aged / diets)
Date	01/16/02	01/18/02	02/19/02	02/28/02	03/22/02	04/22/02	04/29/02	05/29/02	02/11/02	01/22/03	02/05/02	09/20/02	05/01/03	12/15/03	04/09/04
No of lobsters	27	15	21	20	24	42	22	5	25	18	15	40	42	20	38
Av length mm	65.00	85.60	82.20	82.70	79.90	70.80	80.90	60.30	74.70	81.40	80.60	39.50	27.90	28.20	82.20
Tail blisters	1	0	4	0	0	0	0	1	8	2	4	6	0	0	13
Tail erosion	0	1	5	7	0	5	0	4	15	7	6	39	13	2	21
% erosions	0%	7%	24%	35%	0%	12%	0%	80%	60%	39%	40%	98%	31%	10%	55%
App. Loss (No affected.)	15	7	17	16	16	23	12	3	17	16	7	17	2	38	12
Total limbs lost	32	11	40	41	30	40	31	8	28	29	12	24	2	11	18
Mean no lost	0.47	0.64	0.43	0.39	0.53	0.58	0.39	0.38	0.61	0.55	0.58	0.71	1.00	3.45	0.67
Max limbs lost	5.00	3.00	7.00	5.00	4.00	4.00	3.00	5.00	5.00	5.00	3.00	3.00	1.00	10.00	3.00
Severity	9 old, 19 recent, 5 v recent	10 old, 1 recent	(not classified)	all old	2 old	36 old	17 old, 11 recent, 3 Initial	all old	16 old	5 old, most Recent	6 old - 3 animals	18 old. 4 recent, 2 imm	1 old, 1 recent	lots in dead, most others recent	11 old

Table 3. Bacterial culture results from Tasmanian juvenile rock lobsters *Jasus edwardsii* from all areas.

Disease agent **Bacteria**	West / Franklin	King Is / Otway	East / Freycinet	Flinders Is	Bruny	South Coast / Davey	Farmed On-shore 1	Farmed On-shore 2	Farmed Sea-cage 1	Farmed Sea-cage2
Haemolymphs cultured	14	1	5	5	16	5	12	10	10	9
Bacteria isolated from haemolymph: Level (no animals isolated)										
Vibrio splendidus I	+/- (2)				+/- (3)		+ mixed (1)		+ (1)	
Vibrio navarrensis					+ (x 1)					
Bacteria isolated from external lesions (occasional culture only)										
Flavobacteria					+++ (1, external lesion)					

The only other isolate from haemolymph was one isolate of *Vibrio navarrensis*. This species has not been reported to be a pathogen of aquatic animals. Isolation from a single animal suggests that this may simply represent opportunistic infection in a single animal failure. This animal also showed large areas of muscle necrosis, globules in the antennal gland cells, and a moderate haemocyte count of 10,750 / mm^3.

Histological examination only occasionally showed bacteria within the tissues of other animals not sampled for bacteriology (as distinct from surface fouling). Bacteria were rarely seen in the numerous small muscle lesions, and when present were generally seen as focal invasions associated with integument damage or occasionally as a post mortem change in the few animals which died between collection and sampling. Bacteria were detected in one animal with severe and apparently long-standing hepatopancreas damage resulting in almost total destruction of this organ, which will be discussed further below.

5.2.3 Haematology

Direct total haemocyte counts were carried out on a total of 145 animals, with mean data for each area by accession summarised in Table 3 and Figure 5. Overall the counts were slightly higher in the farmed groups, but this was most obvious in the smaller animals. Plots of total haemocyte counts against size (Figure 6) showed a slight trend to decreasing counts with increasing size in the wild but not farmed groups, over the size ranges tested, with considerable scatter in both groups.

Thus it is uncertain whether apparent slight differences between farmed and wild animals reflect true differences in background haemocytes levels induced by the culture systems, or are spurious results due to size or transport differences, the proportion of animals showing overall ill-health, moult or excessive stress levels. While an increase in haemocyte count was seen in both culture systems, it was most marked in the first on-shore groups, but not apparent in the smaller animals from the second submission from this system. The most notable differences between the two on-shore groups were age / size (and therefore time in culture), the level of gill parasites (which is likely to be a reflection of this), and a higher level of gill fouling in the initial group. Indeed gill fouling overall was higher in farmed than wild groups.

Differences could also be due to diet. Three different diets were used for one of the farmed submissions, allowing a comparison of diet and haemocyte count (albeit with small numbers). Haemocyte counts from these three groups are submission are summarised in Table 5, and suggest that the higher haemocyte counts are not related to size (which is similar for both mussel fed groups, though the ages varied), but are predominantly a feature of the mussel-only diet group.

Table 4. Haematology results (cells / mm^3) from Tasmanian juvenile rock lobsters *Jasus Edwardsii* from all areas.

	Wild											Farmed			
Area	**King Is / Otway Area**		**East Coast / Freycinet**		**Flinders Is**	**Bruny Area**		**South Coast / Davey**		**West Coast / Franklin Region**		**On-shore 1**	**On-shore 2**	**Sea-cage 1**	**Sea-cage 2**
Accn no	020145	020171	020485	020607	020848	021116	030150	021169	021450	023217	030244	022476	03/0845	032783	040571
Date	1/16/2002	1/18/2002	2/19/2002	2/28/2002	3/22/2002	4/22/2002	1/22/2003	4/29/2002	5/29/2002	12/11/2002	2/5/2003	9/20/2002	5/1/2003	12/15/2003	4/9/2004
Mean	16938	13136	13908	11954	12362	9281	15345	10845	19268	12207	13893	19580	12805	17100	15416
Min	9925	5350	12625	9200	1050	2925	1100	6775	14275	6300	9400	11300	2100	8950	5500
Max	18000	24100	15750	15350	16,600	14250	28150	16,325	23025	18350	20050	27300	25350	29550	21950
No counted	3	7	3	6	4	4	10	5	4	7	7	10	10	9	16
SD	1503	6291	1909	2735	7549.88	4735	7974	3858	3979.76	4615	3921	5167	8732	8221.3	4108.2
Area AMean	13575		12572		12362	13613		14589		13050		19580	12805	17100	15415.6
Area SD	5547.95		2500.45		7549.88	7568		5752.8		4205.81		5167.32	8732.71	8221.31	4108.15
No / area	10		9		4	14		9		14		40	19	9	17

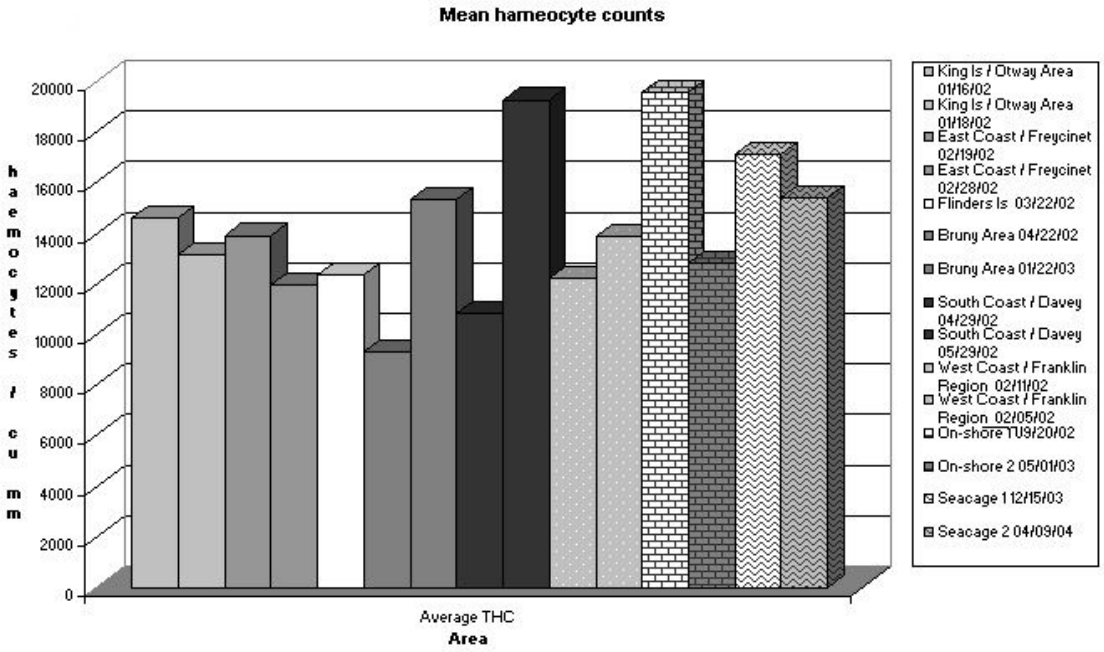


Figure 5. Group mean total haemocyte counts.

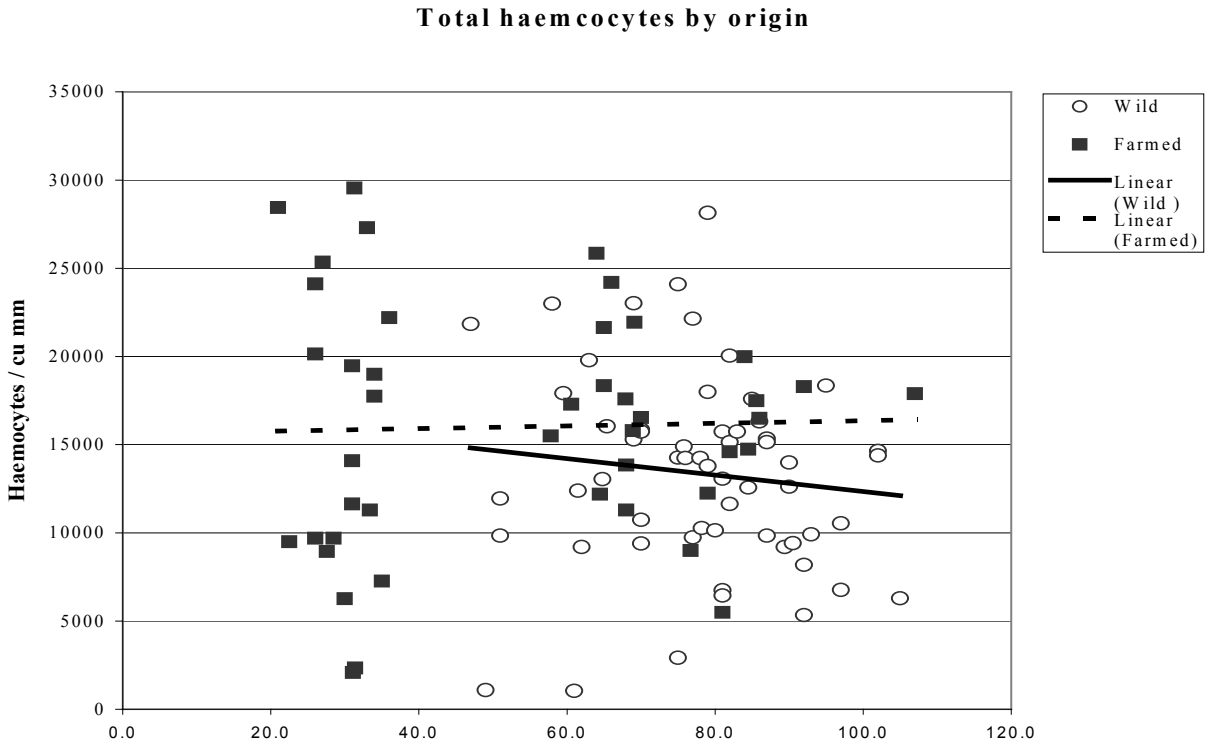


Figure 6. Relationship of haemocyte counts to size and origin.

Table 5. Carapace length (mm) and haematology results (cells / mm^3) from Tasmanian juvenile rock lobsters *Jasus edwardsii* from Sea-cage 2 fed different diets.

Diet	Mussel & Mackerel		Mussel only		Mackerel only	
Gp	L	Hcyts	L	Hcyts	L	Hcyts
A Average	**93.3**	**13580.0**	**80.0**	**18358.3**	**65.3**	**13720.0**
A StdDev	*10.1590*	*5060.95*	*79.3*	*2287.88*	*62.5*	*13500.0*
A Min	79	5500	67.9	15800	46.7	9000
A Max	107	17900	92	21950	82	17300
A Count	14	5	17	6	7	5

5.2.4 Parasitology findings

No parasites with obvious pathogenic potential at either an individual or population level were detected. However a number of parasites / commensals which could possibly have a lower effect on productivity were seen. Parasites and commensals were predominantly a feature of external surfaces, especially the gills where they were sometimes associated with host responses. External fouling of integument was neither uncommon nor unexpected in animals which had not moulted for some time, and was therefore regarded as normal at low levels and was generally recorded only when the levels or maturity of fouling agents suggested a prolonged period since the previous moult. The parasites detected and their distributions are summarised in Table 6. The pathology associated with parasites from each area and farm group are discussed further below (section 5.4).

Protozoan fouling agents, generally attached sessile peritrich protozoa as seen in Figure 7A, were relatively common, especially but not always in association with significant levels of gill bacterial fouling. They did not appear to elicit tissue damage or a host response, which is as expected for a commensal whose main interaction with the host is surface attachment.

The most common parasites detected were small apparently free-living crustaceans (total score for the survey of 91, representing 87 lobsters as four lobsters had a score of 2). It was uncertain in many cases whether these were resident on the gill, and liable to cause irritation and a host response, or transient incidental commensals, especially as in many cases only fragments were seen in section. A typical crustacean is shown in Figure 7B.

While the smaller parasites were generally not seen grossly and could not be identified from section, the largest crustacean parasites, identified as goose barnacles, were readily seen grossly (Figure 7C) and distinguished histologically (Figure 7D)**.** No major pathology was associated with these parasites, other than the mild gill changes associated generally with parasites in the gill.

Another gill parasite with wide distribution in was a rhabdocoel turbellarian, a ciliated flatworm. This was rarely seen grossly (Figure 8A), though parasites with a similar appearance were more often seen histologically (Figure 8B) usually on the surface at the base of the gill lamellae (Figure 8C) and in some cases embedded at this location.

Table 6. Parasites, putative parasites and commensals of juvenile southern rock lobster *Jasus edwardsii* from all areas of Tasmania.

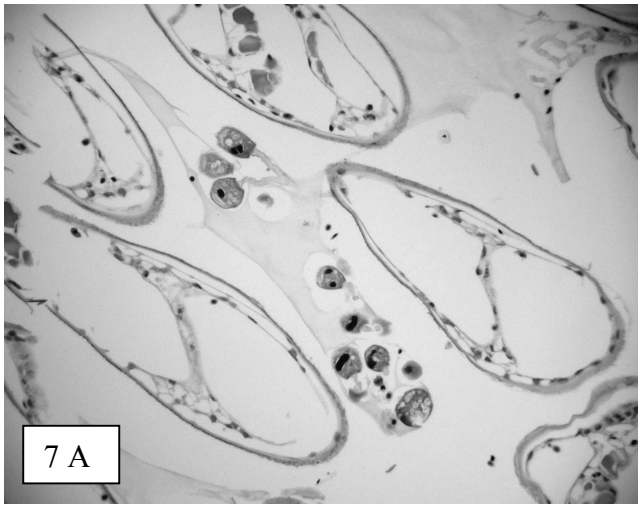
Disease agent **Parasite**	West / Franklin	King Is / Otway	Flinders Is	East / Freycinet	Bruny	South Coast / Davey	Farmed on-shore 1	Farmed on-shore 2	Farmed Sea-cage 1	Farmed Sea-cage2
No of animals	41	41	24	41	60	26	40	41	19	38
Gill goose barnacles	0	0	+ (3)	+ (3, heavy)	+ (1)	0	0	0	0	+ (2 heavy)
Gill crustaceans (other)	+	+	+	+	+	+	+	+ (1)	+ (1)	+
***Mean** score / lobster*	*0.33*	*0.13*	*0.42*	*0.30*	*0.24*	*0.25*	*0.28*	*0.02*	*0.05*	*0.62*
Gill turbellarians (Total score)	0	0	+ (1)	+ (2)	+ (7)	+ (8)	+ (2)	0	0	+ (7)
Parasite egg cases (Total score)	+ (4)	+ (1)	+ (5)	0	+ (5)	+ (1)	+ (1)	0	0	+ (1)
Gill protozoa (mostly sessile ciliates)	+ (13)	+ (1)	+ (4)	0	+ (11)	+ (4)	++ (26)	+ (1)	0	+ (3)
Metazoa on gill, not otherwise specified (total score)	+ (2)	0	0	+ (2)	+ (4)	0	+ (2)	0	0	0
Metazoa, other external* surfaces, not otherwise specified									0	+ (1 mussel)
Spirobid fouling+							0		0	+ (13)
Metazoan parasites (Internal)	+ (1, HP)	0	0	0	0	0	+ (1, SkM)	0	0	0
Eosinophilic spherical bodies (suspected protozoa) in hepatopancreas	+ (8)	0	+ (2)	+ (1)	+ (5)	+ (3)	0	0	0	0
* entered only if specific examination note. Minor levels of external fouling agents may not all have been recorded										

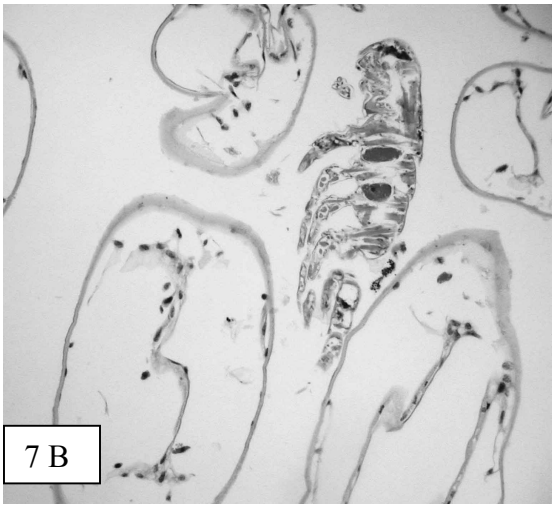
An apparent egg-stage was seen attached to gill filaments on 18 occasions (Figure 7E). Whether this was a stage of any of the above parasites is uncertain. The egg sacs were present in 11 sections where small crustacea were present, and in seven sections with where no crustacea were seen. These were present on one gill with goose barnacle, and on one gill with recognisable turbellarians. However these are not typical of turbellarian eggs (Marty Deveney, pers comm). Some evidence of crustacea were seen in one quarter of the sections (87 of the 373 gills), but inferences from these figures is likely to be influenced by the skew induced by the reduction of surface parasites at the time of moult, so spurious correlations of attached parasites is likely. Egg sacs were attached to the second gill filaments in particular and were present mainly in wild animals (two in the farmed animals and 17 in the wild animals). Higher numbers of these were present in the groups with the highest number of small unidentified crustacean sections.

Other commensals attached to external surfaces without evidence of an effect on the host included one large mussel on the ventral surface of a sea-caged animal, and spirobids, which were not uncommon but most obvious in larger animals of this submission.

Internal parasites were restricted to one metazoan worm like parasite in the hepatopancreas, a suspected parasite crustacean fragment in skeletal muscle, which is regarded as a likely intrusion with local damage, and suspected protozoan parasites of the hepatopancreas. The hepatopancreas metazoan was though to be a tapeworm (Figure 9). There was minimal host response. This animal, a wild caught lobster from the western Franklin area, had a large wound of the ventral distal tail muscle and associated degeneration, but was otherwise unremarkable. The suspected protozoan cells in the hepatopancreas (Figure 10) were seen more frequently, in all but one wild area, but not in the farmed animals. These have been seen previously at low levels in cultured animals during routine diagnostic examinations (Handlinger, unpublished), but were never in sufficient number for follow up work to determine their nature.

(Following page) **Figure 7. Gill crustacean and fouling parasites. A) Moderate level of sessile protozoa. B) A typical unidentified small crustacean commensal C) Goose barnacles on gills of a Sea-caged juvenile lobster. D) Histological appearance of goose barnacle, showing attachment of barnacle and lobster shells. E) Suspected egg case.**


7 A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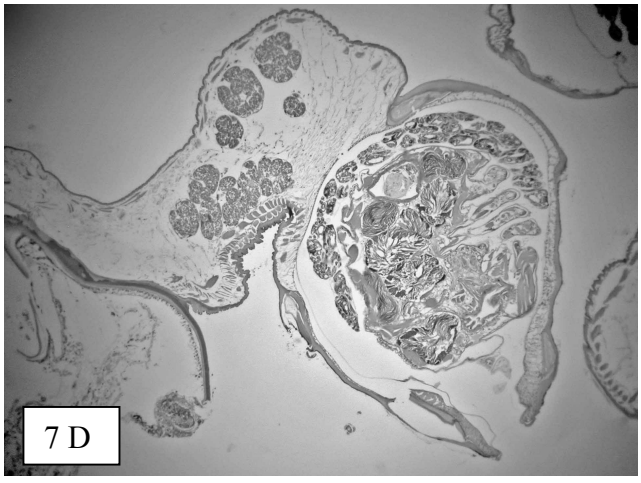
7 C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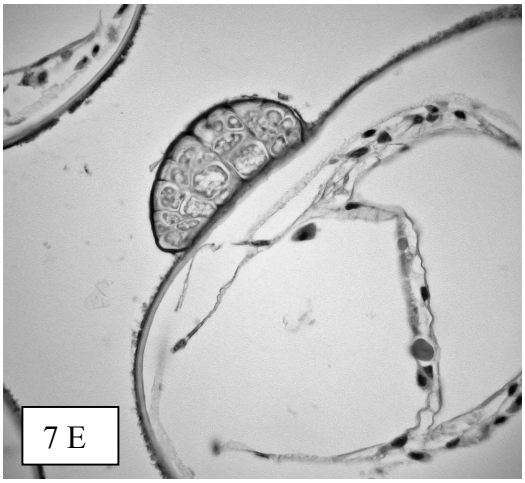
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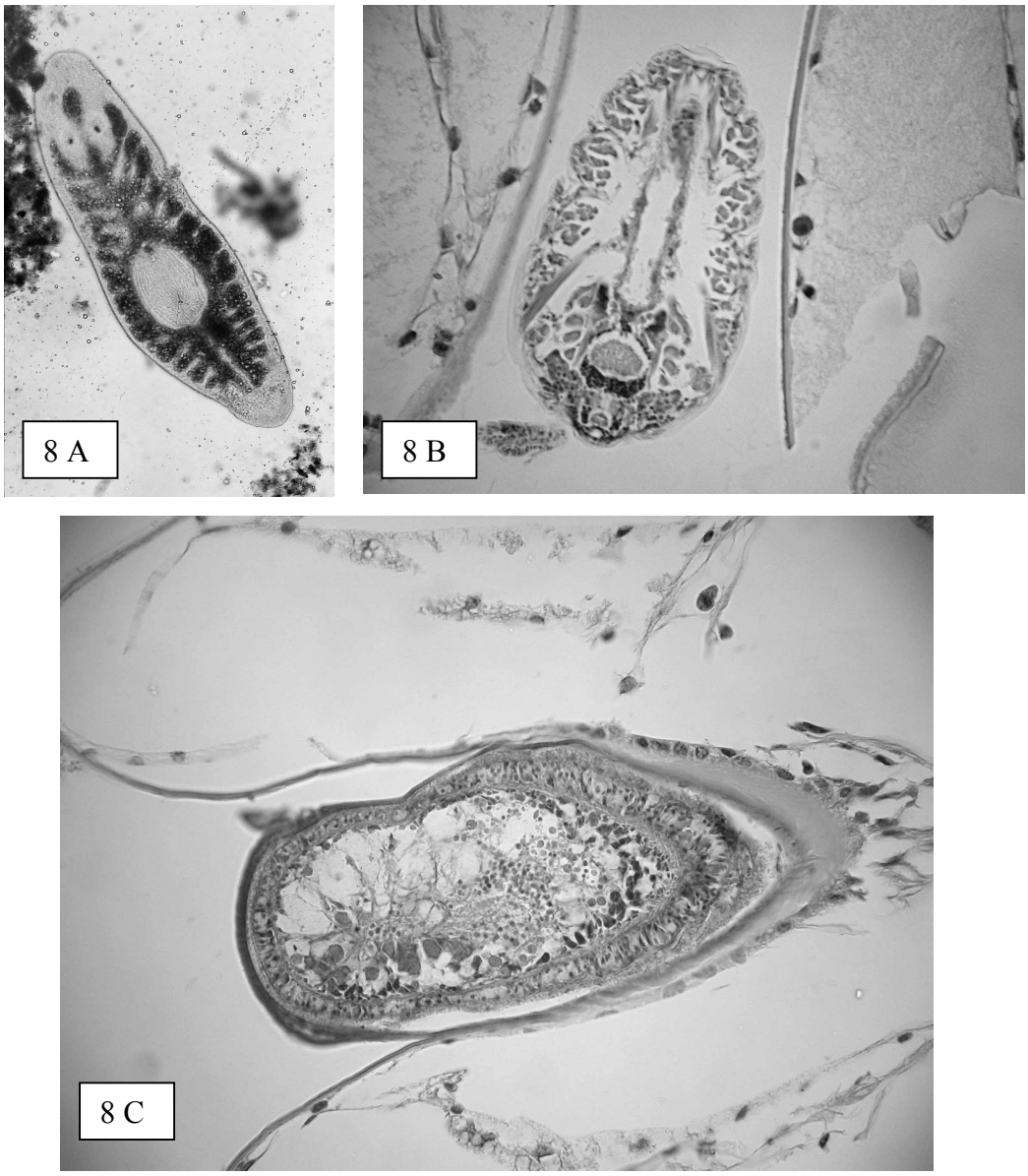
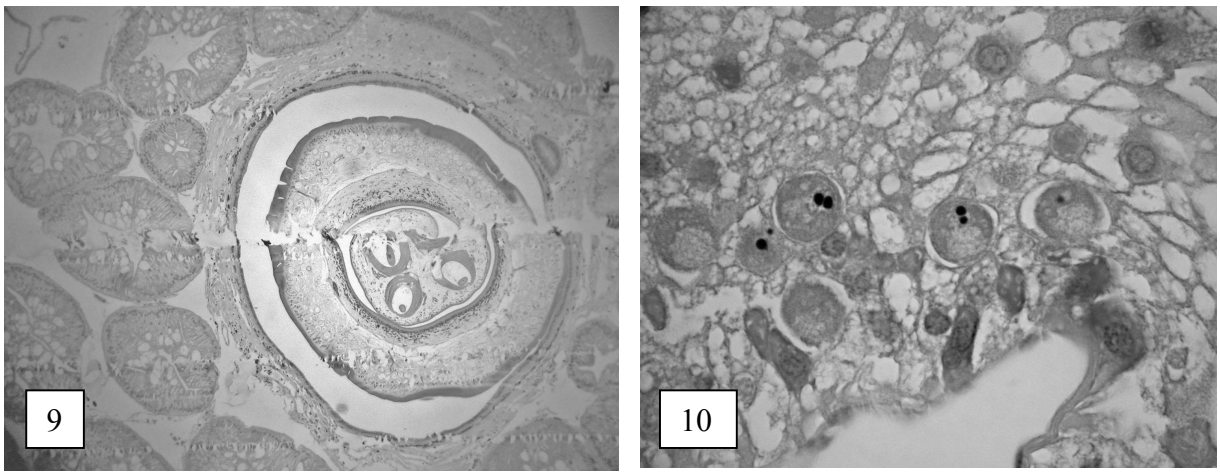


Figure 8. Gill turbellarians. A) Live (dark field) and histological appearance. B) Free living stage in section. C) Attached and partly embedded in the lamellar base.



(Above) **Figure 9. Suspected cestode parasite embedded in hepatopancreas of a wild juvenile *Jasus edwardsii.***

Figure 10. Several suspected protozoan parasites in the hepatopancreas of a wild juvenile *Jasus edwardsii.*

5.2.5 Pathology identified, by organ system

5.2.5.1 Gill:

The major changes present in the gill were biofouling (Figure 11A and B) with the presence of bacteria and to a lesser extent other biofouling organisms such as sessile protozoa and metazoan parasites, especially when a nidus of other substances such as ova or plant material were present. The total score for significant bacterial fouling was 117 in the farmed animals (with scores between 20 in the smallest group and 40 in all of the groups), and a total score of 49 in the wild animals, the highest score for a wild group being 17. Some groups showed no biofouling. This appeared to be one of the most significant findings between farmed and wild animals. If considering the scores for total biofouling from just the bacterial component, the differences were even more marked. Results for this are summarised in Table 7, presented as the mean score per animal. The farmed groups all showing scores per animal of between 0.74 and 1, and the wild animals showing mean scores per animal ranging between the groups from 0 to 0.8, but with only one group showing a score of greater than 0.5. The gill fouling was rarely associated directly with active inflammation, there being no inflammation obvious of the farmed animals and only 2 wild animals showing mild gill inflammation. However, there was occasionally evidence of past reactions with melanisation present in two groups, three animals in the farmed stock and in one wild animal. In individual animals, gill fouling sometimes appeared associated with increased levels of inflammation elsewhere, but this was not reflected at the population level.

In a few animals from one farmed (sea-cage) submission, the total bacterial fouling was increased in animals which were dead on arrival, but this was not seen in all dead animals and there was overall little autolysis in these animals, so it would appear more likely that lobsters with heavily infested gills were more susceptible to travel stress, rather than that this was a post-mortem change. Fouling overall was more common on smaller animals (hence there is a bias to farms), but many small animals (including those on farms) were not affected. There was no overall correlation with reserve inclusion cell numbers (which were considered in the context of a probable indicator of time since last moult), since while some highly fouled animals had high RC reserves, so to did unfouled animals. Overall this did appear to be a reflection of the culture systems.

In general, the level of gill associated parasites was similar between farmed and wild groups, although two of the farmed groups showed no obvious parasites. (The total score in the farmed group was 43 and that of the wild group was 77). This included a variety of protozoan and metazoan parasites, as above. Crustaceans in the gills were relatively common parasites, and the farmed animals appeared to be correlated with those groups with the highest level of general biofouling. This pattern was less obvious in the wild animals.

A number of general tissue changes were seen in the gills. Coagulated haemolymph was also common in the gills but was not universal (Figure 11C). In the farmed animals, higher levels were associated with those two groups with heavy biofouling but no such relationship was present in the wild animals where the average score for coagulated haemolymph varied from 0 to over 1.2 per animal, without any apparent correlation with fouling. This was considered a general change, probably reflecting activation of haemolymph components by a variety of insults, which may have differed between groups. Frank degeneration was quite rare in the gills, being present in only one wild animal. Eosinophilic granular haemocytes were only occasionally present in the gills in a scattered pattern with a total score of 4 in the farmed animals and 16 and the wild ones. Haemocyte aggregation and inflammation were also present at low levels in a scattered pattern (Figure 11D and 11E). Reserve inclusion cells were present in most groups; in farmed animals in levels between 0.12 and 0.68 per animal and in the wild animals between 0 and 0.67 per animal. Occasional granulomas were detected (Figure 11F), two in the farmed animals and one in the wild.

Table 7. Mean group scores for gill fouling relative to other common changes.

		Mean Score			
Area	Date	Bacterial fouling	Crustaceans	Coagulated haemolymph	Reserve cells
On-shore 1	20/09/2002	0.95	0.28	0.13	0.28
On-shore 2	1/05/2003	0.69	0.00	0.02	0.12
Sea-cage 1	15/12/2003	1.00	0.05	0.00	0.37
Sea-cage 2	9/04/2004	0.94	0.62	0.44	0.68
Bruny	22/04/2002	0.43	0.15	0.33	0.55
Bruny	22/01/2003	0.06	0.44	0.33	0.67
East	19/02/2002	0.00	0.53	1.27	0.67
East	28/02/2002	0.05	0.11	0.32	0.47
Flinders	22/03/2002	0.28	0.40	0.36	0.52
King Is	18/01/2002	0.36	0.21	0.21	0.50
King Is	16/01/2002	0.00	0.06	0.59	0.00
South	29/04/2002	0.37	0.11	0.84	0.63
South	29/05/2002	0.80	0.20	0.00	0.00
West	11/02/2002	0.08	0.23	0.27	0.54
West	5/02/2002	0.36	0.29	0.21	0.43

5.2.5.2 Integument:

Overall, only low levels of integument damage were seen histologically, other than lesions and fouling detected grossly. This included low levels of bacteria, degeneration, necrosis, active inflammation, evidence of past inflammation seen as melanisation, and occasional parasites implicated in a local tissue reaction, including superficial melanisation and an active sub-cuticular response (Figure 12). Other changes such as coagulated haemolymph and eosinophilic granular haemocytes were seen in the integument at low levels but apparently reflected more general change throughout the body. Bacteria invasion of the integument was seen in only one farmed submission and one wild submission, both at low levels. Focal degeneration and necrosis was present in low levels in the majority of wild submissions, but only one of the farmed submissions, the initial on-shore submission which included animals held for up to 27 months. Impacts on individual animals were better reflected in the overall gross integument changes than on those seen histologically.

The pattern of inflammation scores closely followed that of the degeneration and necrosis as might be expected. There was a less marked correlation with melanisation that was occasionally present in animals not showing active inflammation, suggesting these were rarely a recent reaction. Occasional integument granulomas (score 2 / group) were seen in three of the farm groups, but not in the smaller animals of Sea-cage 1 or the wild animals.

Of the cell variations in the integument, eosinophilic granular haemocytes were an occasional finding in only one farmed submission and in four wild ones. Reserve inclusion cells were present in nearly all groups except for two wild submissions, and low levels in two other submissions, however the levels did vary quite wildly, with the group mean score per animal ranging between 0.21 and 1.21 with the farmed animals and 0 to 1 for the wild animals. (Table 8). Vacuolation of the reserve inclusion cells was present in two of the farmed groups and at a lower level in one of the wild groups. The significance of this change is not known at this stage. The significance of these cells and the variations of them in relation to the moult cycle will be discussed further below.

Table 8. Mean group scores for shell fouling relative to other common changes.

Integument		Mean Score / submission				
Area	Date	Biofouling	Reserve cells	Degeneration & necrosis	Inflammation	Melanisation
On-shore 1	20/09/2002	0.03	0.69	0.19	0.17	0.17
On-shore 2	1/05/2003	0.05	0.21	0.00	0.00	0.00
Sea-cage 1	15/12/2003	0.95	0.47	0.00	0.00	0.00
Sea-cage 2	9/04/2004	0.79	1.21	0.00	0.03	0.05
Bruny	22/04/2002	0.00	0.47	0.03	0.03	0.03
Bruny	22/01/2003	0.00	0.72	0.11	0.06	0.06
East	19/02/2002	0.25	0.25	0.75	0.75	0.25
East	28/02/2002	0.13	0.00	0.38	0.25	0.13
Flinders	22/03/2002	0.00	0.17	0.00	0.00	0.08
King Is	18/01/2002	0.00	0.20	0.00	0.00	0.00
King Is	16/01/2002	0.17	0.00	0.50	0.50	0.17
South	29/04/2002	0.00	0.63	0.00	0.00	0.00
South	29/05/2002	0.00	1.00	0.40	0.00	0.00
West	11/02/2002	0.00	0.65	0.00	0.00	0.00
West	5/02/2002	0.00	0.80	0.00	0.00	0.00

5.2.5.3 Heart

Changes in the heart were more limited. Bacteria were detected in one animal. Coagulated haemolymph was detected in the on-shore farmed groups, and in all but two of the wild groups although in differing numbers. Overall, the level was higher in the wild groups with a total mean average score of 0.23 per animal compared to an overall farmed score of 0.09. Degeneration and necrosis were occasionally seen in the wild animals, as were eosinophilic granular haemocytes. These were also at very low numbers. Small haemocyte aggregations were seen occasionally in three of the four farmed submissions and two of the wild submissions, but always at low numbers. Inflammation and melanisation were rare findings in the wild animals only. Reserve inclusion cells varied between groups in this organ also, although the overall mean score was similar for farmed and wild animals at 0.67 per animal for farmed and 0.61 per animal for wild animals. The variation was similar to the levels seen in the integument. Vacuolation of reserve cells was also seen scattered through most of these

groups occasionally. Several of these animals from two wild groups (two from the Southern area and 6 from the Bruny area), also showed apparent storage material in heavily vacuolated cells within the heart, though whether these were related to reserve cells was unclear. Granulomas were quite rare with a score of 1 for the farmed animals and 2 in the wild animals in one submission.

5.2.5.4 Skeletal tail muscle

Degeneration and necrosis was the major change seen in skeletal muscle ranging from disruption which is likely to have included sampling artefacts, through to definite degeneration with evidence of an inflammatory response. Some change was present in all but one wild group, although sometimes at low levels. The scores per group varied from 0.02 to 0.62 for the farmed animals, and 0 to 1.47 for the wild animals. The overall level of muscle degeneration was higher in the wild animals with a mean score of 0.64 compared to a mean score for farmed animals of 0.30. Muscle degeneration was seen with 125animals. All but one of the six animals with the highest levels (score 3) were in wild animals, the exception being 1 lobster from the on-shore-1 group. Of the 40 animals with a degeneration score of 2, only seven were farmed. There was no obvious association with appendage loss or external blisters and erosions. Geimsa, acid fast and Gram stains of several animals with a range of an active inflammatory reactions to degenerate muscle showed no evidence of bacteria, microsporea or other pathogens in these lesions.

An inflammatory response to the degenerate muscle was virtually restricted to the wild animals, being detected in only one farmed animal (on-shore 1), but present in all but one of the wild submissions. Eosinophilic granular haemocytes were an occasional finding in the muscle of wild animals only being present in four group, total score 10. Haemocyte aggregation without an obvious inflammatory focus was seen in two farmed animals and one wild lobster. These cells were only seen in muscles where degeneration was present. Muscle granulomas were also sporadic with a total score of 5 in two groups of farmed animals and one wild animal. More marked melanisation was seen as a sporadic finding in one animal.

All of the seven turgid lobsters showed some muscle degeneration. However as these were all from one submission (wild, West Coast), which also showed a high level of degeneration, the association could be spurious. Most, but not all, with discoloured (red / brown) haemolymph also showed muscle necrosis.

Occasional findings in the tail include bacteria with one report in farmed, one in wild animals, plus a small cluster from one of the farmed submissions possibly related to autolysis. There was one report of an embedded crustacean from a farmed animal on-shore. Reserve inclusion cells were only seen in one farmed animal at low levels.

5.2.5.5 Hepatopancreas

The hepatopancreas was examined from virtually all animals. Degeneration and necrosis was a rare event (total score of 5 from two submissions), however, in one Flinders Island animal this was a very severe change, with destruction and melanisation of virtually the entire digestive gland (Figure 13 A and 13B).

Autolysis was minimal except in one farm submission in animals which were dead on arrival, (total score 5). Nevertheless bacteria were occasionally present in the lumen in this and one other farmed submission, and in two wild submissions (Bruny and Flinders Island), with the

score per animal in these affected groups being similar between the wild and farmed groups. Bacterial aggregation such as seen in Figure 13C was not apparently an autolytic change. This was rare but the significance for individual animals is uncertain.

There was one report of low levels of eosinophilic granular haemocytes in a wild animal. Haemocyte aggregations were seen in only the animal with marked hepatopancreas degeneration and melanisation, and in one other animal from the Flinders Island group which otherwise showed only coagulated haemolymph and moderately abundant reserve inclusion cells in this organ. Inflammation was also absent except for the one severely affected animal. One suspected cestode parasite was seen (Figure 9, above).

Coagulated haemolymph was present in the majority of submissions, from both groups, although in the farmed group the majority of animals showing this were in one group, the first on-shore submission. Overall, the levels in the wild animals were approximately twice as high as the farmed animals, with a mean score of 0.12 per animal compared to 0.06 reflecting the patterns seen in other organs.

Reserve inclusion cells were similarly scattered amongst all accession, were present from all submissions, with high levels in the same groups showing these in other organs, and the overall level in the wild animals with a score of 0.65 per animal compared to the farmed animals of 0.34. Vacuolation was seen occasionally in both groups.

5.2.5.6 Mid-gut

Mid-gut was rarely examined. No significant findings were detected.

5.2.5.7 Hind-gut

This was examined from virtually all animals. One of the most significant findings in the hind-gut (which is a cuticle lined organ), was bacteria lining the wall of the gut (Figure 13D). This was present in moderately high levels in all animals of the farmed group with a mean score per animal overall of 0.24, and was occasionally seen, but quite rare in wild animals with an overall mean score of 0.04 per animal.

Coagulated haemolymph was present sporadically in both farmed and wild groups at similar levels, 0.04 and 0.05 score per animal respectively. Eosinophilic granular haemocytes in association with the hind-gut epithelium was seen in one farmed animal and in five wild groups, with a maximum level of 0.71 score per animal (Figure 14A). Two animals showed haemocyte aggregation not obviously involved in inflammation. More distinct inflammatory changes were occasionally seen in both groups, and were more common in one of the farmed submissions (Figure 14B). A mean score from this group was 0.64 per animal. Melanisation was seen in one farmed animal only, in association with the hind-gut. Two animals showed a granuloma associated with the hind-gut, both from the farmed group. Degeneration and necrotic changes were seen in one, wild, animal. Reserve inclusion cells were at low levels in some of the submissions in this organ, with an overall score of 0.06 in the wild animals and 0.02 in the farmed ones. There was one report of vacuolated reserve inclusion cells in a wild animal.

5.2.5.8 Antennal gland:

The most significant pathology was degeneration and necrosis that was detected in only some groups, but when present was generally present in several animals within these groups. Only two farmed animals, in the first on-shore group, showed antennal gland degenerative changes, which was seen as mild levels of early nuclear degeneration. In one animal it was uncertain whether this was a sampling induced artefact or a real change as early degeneration can be difficult to separate from artefact when no post reaction is evident. In the wild animals the changes were more variable as shown in Figures 15 (A-E), ranging from similar levels of early nuclear contraction to definite inflammatory reactions and more defined degenerative changes. In these animals the degeneration was focal and possibly segmental as large areas of affected organs were involved, with inflammatory changes being present only in the affected degenerate areas. The total score for degenerative changes in the wild animals was 19 and that in the farmed animals of 2 (mild, nuclear degeneration only). Mean scores / animal for affected groups being shown in Table 9. There was no obvious positive correlation with other pathology (such as muscle degeneration). There was a negative correlation with reserve cells, all except one affected animal having a low or absent reserve inclusion cell score (most 0, at most 1). The exception was a soft-shelled lobster with a reserve score of 3.

The association of inflammation with these degenerative changes was reflected in the antennal gland inflammation scores. The total score was 1 for the farmed animals represented a mild cell infiltrate one of the animals showing the nuclear changes, and 21 in the wild animals, mainly in those four groups which showed the highest level of kidney degeneration. One animal showed sufficient inflammatory changes to give a hard nodular appearance grossly. One animal from a group with significant antennal degeneration showed granuloma formation in this organ, without recent degenerative changes in the tubules.

Table 9. Mean group scores for antennal gland changes.

Antennal Gland		Mean Score / submission				
Area	Date	Degeneration & necrosis	Intra-epithelial globules	Inflammation	Coagulated haemolymph	Eosinophilic granular haemocytes
On-shore 1	20/09/2002	0.06	0.38	0.03	0.29	0.00
On-shore 2	1/05/2003	0.00	0.00	0.00	0.00	0.00
Sea-cage 1	15/12/2003	0.00	0.00	0.00	0.00	0.00
Sea-cage 2	9/04/2004	0.00	0.21	0.00	0.18	0.00
Bruny	22/04/2002	0.03	0.03	0.03	0.26	0.13
Bruny	22/01/2003	0.06	0.56	0.06	0.44	0.00
East	19/02/2002	0.00	0.22	0.00	0.33	0.00
East	28/02/2002	0.00	0.21	0.07	0.29	0.00
Flinders	22/03/2002	0.00	0.13	0.00	0.31	0.00
King Is	18/01/2002	0.27	0.00	0.20	0.13	0.00
King Is	16/01/2002	0.50	0.63	0.50	0.25	0.00
South	29/04/2002	0.00	0.21	0.00	0.53	0.16
South	29/05/2002	0.67	0.00	0.00	0.00	0.00
West	11/02/2002	0.00	0.16	0.08	0.08	0.40
West	5/02/2002	0.47	0.73	0.60	0.33	0.33

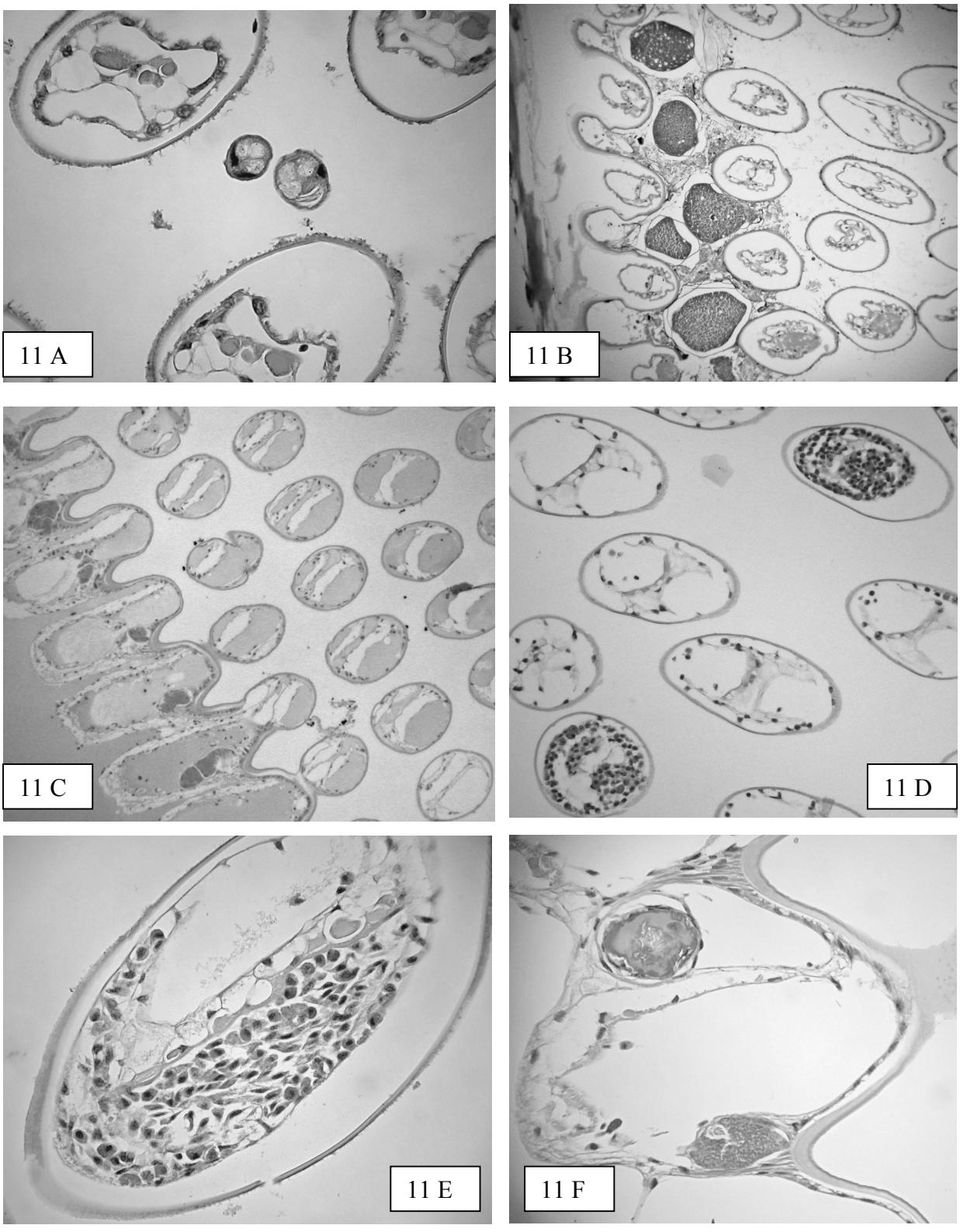


Figure 11. Showing gill pathology. A) Score 1 fouling, with little associated pathology. B) Score 2 fouling round unidentified ova with coagulated haemolymph in some filaments. C) Coagulated or pooled haemolymph. D) Haemocyte reaction in gill filament vessel. E) High power view of C. F) Granuloma in gill.

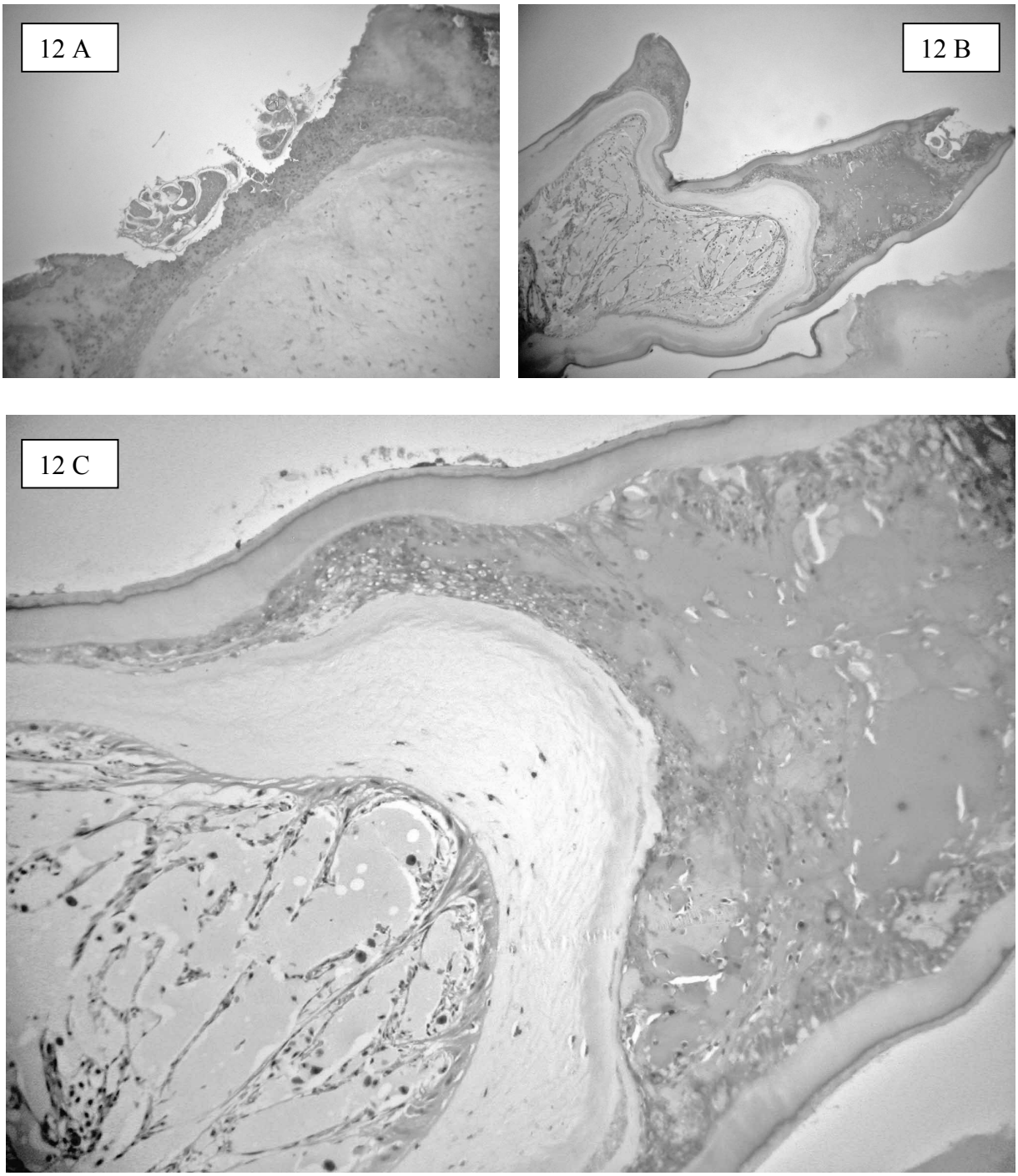


Figure 12. A) & B) show crustacean parasites embedded in cuticle, with melanised reactions. C) A higher power view of B), showing the dilated haemolymph channels and cell reaction, including granular cells.

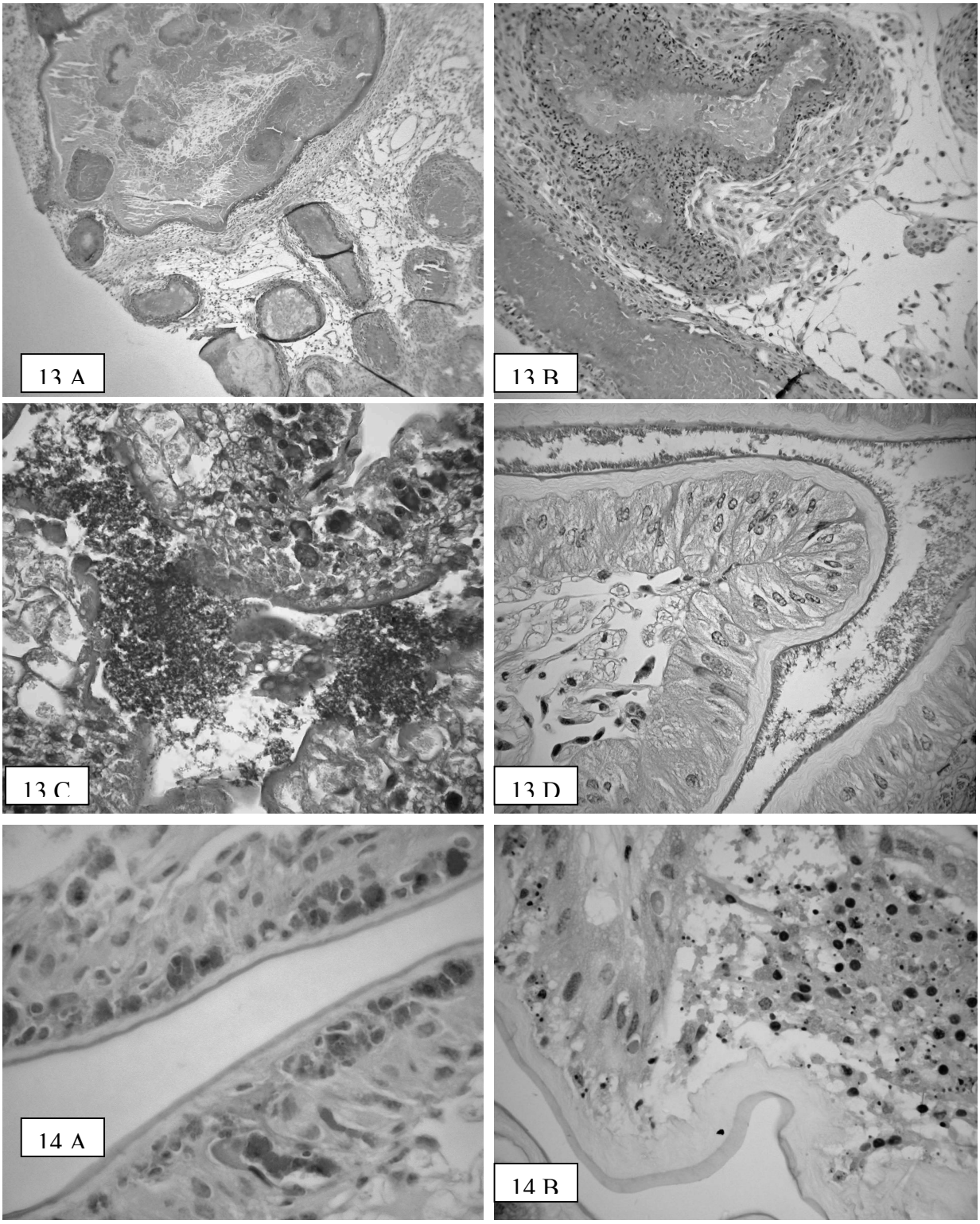


Figure 13 Showing bacterial conditions of the gut. A and B) degeneration, inflammation and melanisation of hepatopancreas, Flinders' Is. C) Bacteria in hepatopancreas, Bruny region. D) Bacteria fouling hind-gut cuticle, on-shore farmed group 1.

Figure 14. Comparing eosinophilic granular cell infiltrates of hind-gut. A) Eosinophilic granular haemocytes infiltrates with no evidence of degeneration. West (Franklin) region. E) Degeneration and inflammation under hind-gut epithelium (D1, I1), King Island.

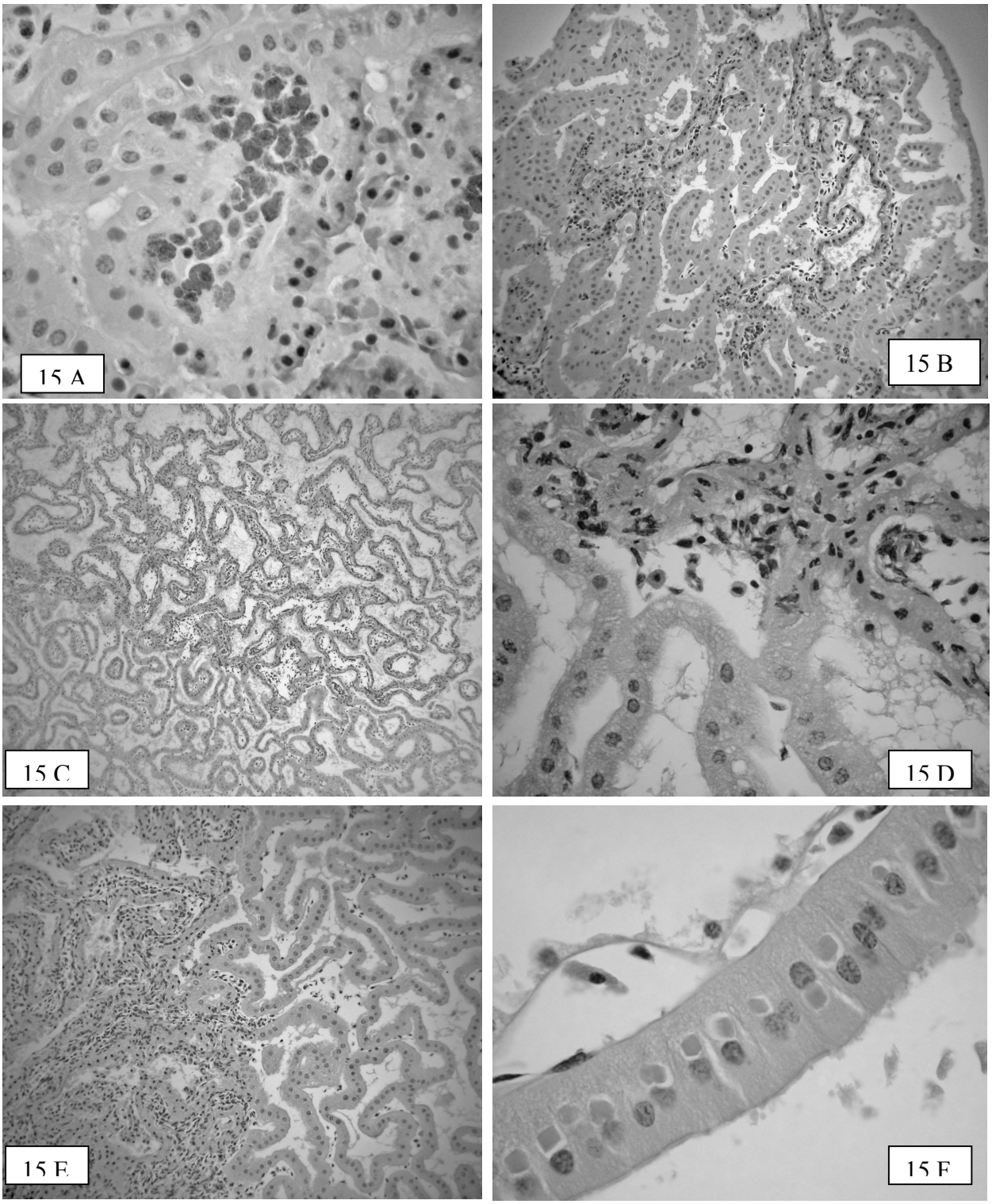


Figure 15. Pathology of the antennal gland. A) Degeneration score 2, inflammation score 1. West Coast (Franklin) region. B) Overview of another West Coast gland. Degeneration 1, inflammation 1. C) Degeneration score 2, inflammation score 2. West Coast lobster from a previous submission. D) Higher power view , cohort of animal C, same scores. E) Low power view of another from this cohort with more marked inflammation. F) Intraepithelial eosinophilic bodies (IEB) score 2.

Haemocyte aggregations which did not appear to be participating in an established inflammatory reaction were also occasionally present in antennal gland, with a score of 4 for the farmed animals (all in one sea farmed location), and a score of 4 for wild animals (scattered over three locations).

Another change that was present in the antennal gland was eosinophilic bodies within the epithelial cells. These appeared to be a hyaline droplet in nature, usually as a large single droplet as per Figure 15F. These were present in both farmed and wild animals with a total score of 20 in the farmed animals from two groups and 43 in the wild animals. There appeared to be no obvious correlation of this change with the other degenerative changes noted.

Bacteria were detected in the antennal gland of one farmed animal from a sea cage system.

Coagulated haemolymph was relatively common, being present in most groups. The sea-farmed system showed this change present in two groups, one on-shore and one in sea cages with mean scores of 0.29 and 0.18 per animal. In the marine system, all but one of the accessions showed this change, though scores ranged from 0.08 to 0.44 per animal (Table 9).

The other cell type irregularly present in the antennal gland were the eosinophilic granular haemocytes. These were not seen in the four submissions of farmed animals, but were present in four of the wild animal submissions with a total score of 23. Reserve inclusion cells were not detected in this organ.

Antennal gland electron microscopy findings

As the nature of the antennal gland degenerative lesions was uncertain, electron microscope examination was carried on archived sea-water formalin fixed material from two affected wild animals, out as a precautionary measure for virus exclusion. In order to locate suitable affected antennal gland material from this small organ (which was difficult to distinguish after fixation and dissection), material was removed from paraffin blocks and reprocessed for electron microscopy. From one animal the original paraffin block was used, the second being from a paraffin block prepared from two-year stored fixed material. No virus or other pathogens were detected by electron microscopy in either sample, though in general the tissue preservation was too poor to determine the ultrastructural basis of the zonal degenerative changes. The globular eosinophilic intracytoplasmic bodies showed an amorphous nature consistent with the protein globule nature suspected by light microscopy.

5.2.5.9 Ventral Nerve:

The ventral nerve chord was examined in approximately two-thirds of the farmed animals from three submissions, but was only rarely recovered from the larger animals from the wild caught and On-shore 1 groups. Apart from autolytic bacterial invasion in farmed animals that were dead on arrival and haemocytes in nerve in one wild animal that were assumed to be an extension from muscle necrosis present in this animal, the only reports of possible pathology noted in this organ were from Sea-cage 2. This showed vacuolation of nerve cells in three animals, multiple small knots of haemocyte aggregation forming an early granuloma in another animal, and numerous animals with reserve inclusion cells in association with the nerve. Reserve inclusion cells were most common in this group, with an average nerve reserve cell score of 1 per animal (score 2 in 8 of 33 ventral nerves examined). Reserve cells in nerves were otherwise seen only at low levels (score 1) in two wild animals from Bruny Island and

two farmed animals from Sea-cage 1. Coagulated haemolymph was also seen associated with the ventral nerve in this submission (total score 5).

The significance of the nerve cell vacuolation (Figure 16) is not known, but is assumed to be an incidental and non-significant finding, with the vacuoles possibly representing swollen organelles such as mitochondria.

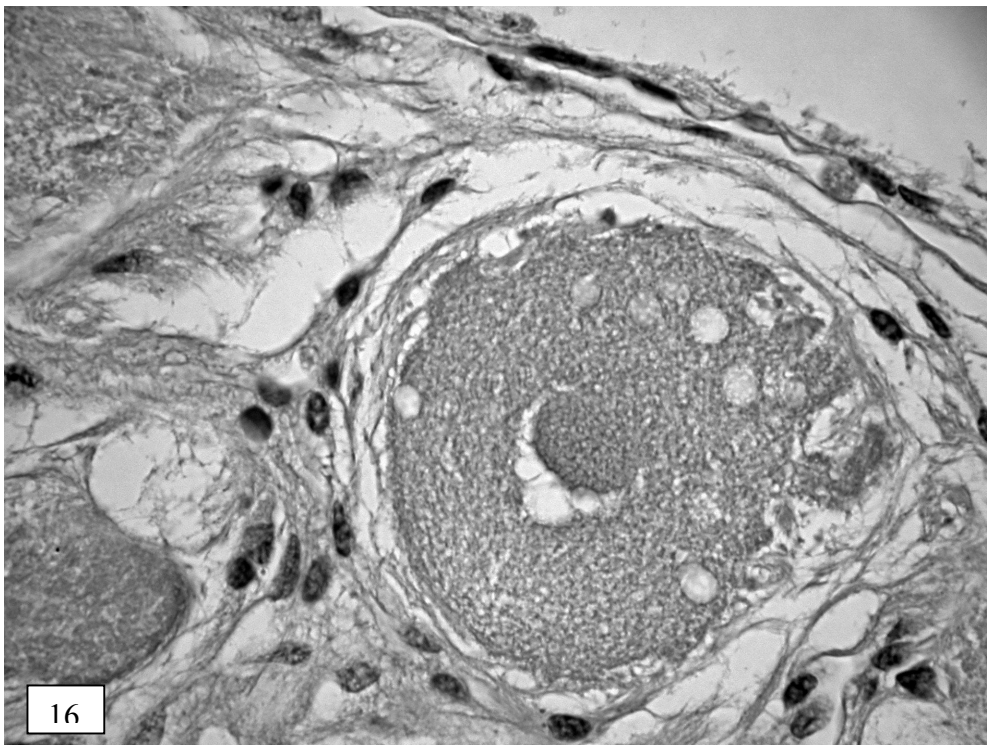


Figure 16. Vacuolated neurone in ventral nerve cord, considered likely to be a non-specific artefact.

5.2.5.10 Turgid lobsters

Seven lobsters were seen with mild haemolymph swelling and bulging, fitting the description of turgid lobster syndrome. All were from one West Coast submission. All showed evidence of muscle damage, some severe. All were phenotypically male (though 2 were classed as female on histopathology). Blisters were present on four animals, erosions on four and leg loss was generally mild. Gills did not show fouling and coagulated haemolymph was absent or at a whole body score of 1. Gill parasites were rare, and there were no eosinophilic granulocyte localisation in gills. Antennal gland inflammation was present in three animals, but degenerative changes were restricted to intraepithelial globules. Reserve inclusion cells were abundant in five of these animals.

5.3 Results by location

5.3.1 Wild samples

5.3.1.1 King Island

Samples were collected from the Christmas and New Year Islands on the 16th and 18th of January 2002 as per Figure 2. There was a total of 41 animals, 26 from the first day and 15 the subsequent day. Animals in the second collection were somewhat larger overall than on the first day with a mean carapace length of 85.6mm compared to 65.6mm on the 16th, with a range on the first day of 33-96mm and on the second day 60-102mm. Thus the major difference was a lack of very small animals on the second day. Due to technical difficulties not all of the weights in access of 400-500grams were recorded as due to equipment failure. These differences should be taken into consideration in assessing any apparent differences between these groups.

Only one animal showed pale blisters and another animal showed erosion. Appendage loss was quite common averaging 1.1 limbs per animal in the first submission and 0.73 limbs per animal in the second submission with an over all submission from this area of 0.98. All but one of the animals under 65mm was immature.

One animal showing red/brown haemolymph was cultured but there was no growth. The haemocyte counts included three from the initial group and seven from the later group, with the animals from the 16th falling within the range of the larger sample. The overall average was 13,575 cells / mm^3 with a range of 5,350 to over 24,000, as shown above (Table 4). This included occasional animals with some of the highest counts from wild lobsters but overall the counts were average for the wild lobster group. There had been a loss of two limbs from the animal with the lowest count, suggesting haemorrhage may have contributed to the low count, but low counts were not seen in other animals with multiple limb loss so significant loss is likely at best to be a sporadic event.

In the tissues, the main changes related to relatively minor fouling of the gill and to changes in the antennal gland. In the gill, fouling was detected in five animals in the second submission, but was not seen in animals from the first group. All of these five animals showed reserve inclusion cells with these generally being visible within the gill, which could indicate the fouling is related to a longer interval since moult. One of these animals showed protozoan and one crustacean, but in general the fouling was bacterial in nature. Three other animals showed an isolated crustacean or crustacean remnant and one animal that did not have fouling showed areas of brown debris within the gills.

General tissue changes included coagulation of haemolymph within the gills of 10 animals from the first submissions and three from the second. This was also occasionally present in other organs such as integument and antennal gland, generally in the same animals, and occasionally hepatopancreas, but less consistently than in the gills.

One animal showed tiny granulomas in the gills. There were negligible changes in the integument with the exception of the animal with the tail erosions detected grossly and one other animal. The animal with gross lesions showed some loss of cuticle, infiltration of eosinophilic granular haemocytes plus pigmentation, plus gill fouling and plus a small haemocyte aggregate in gills. Internally this animal showed no significant lesions, but some reserve inclusion cells ere present. Tail erosion was also noted histologically in another

animal that had multi-focal areas of inflammation and degeneration in the hind-gut plus eosinophilic granular cell infiltration beneath the cuticle. Another animal noted with integument damage showed a massive hole through the encephalo-thorax but showed little evidence internally of ill effects from this trauma.

The two organs showing significant changes were muscle and antennal gland. Muscle lesions were all restricted to the first submission. Most of the 10 animals involved also showed some inflammation. It was noted with several of these that the gills appeared to show bleeding and haemolymph outside the gill lamellae. These did appear to be associated with the muscle necrosis and areas of occasional inflammatory cells. Neither of these changes was noted in the second submission from this area. One animal with muscle degeneration had red flesh.

Antennal gland degeneration and variable levels of inflammation were seen in both submissions generally consisting of well demarcated areas of degeneration with varying levels of inflammation, as shown in Figure 15. Only one animal showed changes in both antennal gland and muscle.

5.3.1.2 Flinders Island

The Flinders Island area was sampled in March 2002. Only 24 lobsters were collected before collection was abandoned due to weather conditions and concern for divers' safety. Because this region is not included in routine stock sampling, the cost of re-sampling this area was prohibitive. Nor was it likely in the short term that puerulus would be collected from this area or juvenile lobsters released to this area.

These animals had a carapace length 60 - 93 mm. Weights were not measured. Sex could be determined on 13 of these animals the remainder being immature. No blisters or erosions were noted. Sixteen of the of the animals showed some loss of limbs, with a total score of 30 limbs lost of which only two were judged to be old lesions. Up to six limbs were lost in one animal.

Haemolymph from five animals were cultured, and there was no significant growth in any of these. Haematology was taken from four animals, and the group mean and three of the animals were within the average range for this project (approximately 16,000 cells / mm^3 or 16 x 10^6 / ml). One animal had a count of 1,050 / mm^3 This animal had significant and apparently long-standing hepatopancreas pathology, as described below.

Fouling was present on the gills of seven animals but was never severe and was not seen on the skin. It was noted that all of the animals showing gill fouling did have reserve inclusion cells in at least some organ (as did a majority of the unfouled animals), but no reserve inclusion cells were seen in eight animals including the animals with low haemocyte counts and hepatopancreas pathology.

Coagulated haemolymph was also a relatively common finding in the gills and present on eight animals, including most of those with gill fouling. However, the correlation of coagulated haemolymph in the gills appeared to be more strongly associated with the presence of reserve inclusion cells. There was a similar trend, but less marked, in other tissues. (This correlation was confirmed in the survey overall, though the association was not absolute.) Interestingly, crustaceans and other parasites were noted in many of the animals that not marked as having bacterial fouling. This was present in nine of the 17 animals marked as not having overt bacterial fouling in the gills. Blackish debris was also marked in the gill chamber of at least three of these animals. Parasites which were present appeared to include

crustaceans and also turbellarians (see photograph). Turbellarians were noted grossly in one animal, and goose barnacles were present in two sections.

There was very little of note in the integument, one animal showed melanisation. This animal had multiple parasites in the gills and did have a degree of coagulated haemolymph and several haemocyte aggregates in the interstitium of the antennal gland, but the integument melanisation appeared incidental.

Muscle degeneration was seen in six animals, one of these with associated inflammatory response. Three of these animals were in the group with significantly fouled gills. All of these animals had parasites but there were no other defining features. The antennal gland showed occasional coagulated haemolymph. The hepatopancreas of one animal showed a haemocyte aggregate not obviously involved in inflammation, and one animal showed eosinophilic spherical bodies suspected to be a protozoan parasite.

The hearts showed variable numbers of reserve inclusion cells and scattered coagulated haemolymph, plus one animal with degenerative changes, one with an accumulation of eosinophilic granular haemocytes and some inflammation, and one animal with granulomas in the heart. The degenerative focus in heart muscle was at the margin of the heart, associated with increased inflammatory cells.

Major hepatopancreas change was seen in the lobster with very low haemocyte count, which showed spectacular changes of inflammation and reaction around virtually all of the sectioned hepatopancreas tubules (Figures 13A and 13B), obliterating the tubules and the surface of the main lumen**.** This animal also showed mild degeneration of the skeletal muscle, gill crustaceans and metazoan and black debris in the gills. Other than this animal, changes in the hepatopancreas were insignificant. Three lobsters showed the presence of low levels of bacteria in or lining the lumens; two also showed external fouling. Two animals showed the eosinophilic spherical suspected parasites. One of those showed an apparent reduction in the height of the epithelium. There were no significant changes seen in the hind-gut.

Very few mid-gut sections were examined. The gonad occasionally showed the presence of reserve inclusion cells. One animal showed an increase in eosinophilic granular haemocytes, plus an overall increase in cellularity in this organ.

5.3.1.3 East Coast samples

Samples from the East Coast region comprised two submissions on the 19/2/2002 and 28/2/2002, both from Butlers Head. The second group had been held for two to five days in tanks following collection before submission. These groups consisted of 21 and 20 animals respectively, both with a mean carapace length of 82 mm, the range for the first group being 59-109, the second 23-104. Weights were only measured for first submission, which showed a mean weight of 213 grams (weights taken on the large balance again rejected). The plot of the weight to length relationship of the 14 smaller animals showed a number of animals which fall well outside the general scatter round the trend line for weight for size. This could be due to loss of weight from limb loss, both directly in the lost limbs and due to dehydration through loss of haemolymph, as some of those outlying animals had lost up to seven limbs. Both groups had a similar level of limb loss overall, with 40 limbs lost from the first group and 41 from the second, from 17 and 16 animals affected respectively, representing a loss of 1.9 and 2.05 limbs per animal.

Tail erosion was present and similar in the two groups, with five and seven animal affected respectively. Tail blisters were present in four animals in the first submission only. Approximately two thirds of these animals were developing gonads and secondary characteristics sufficient to determine the sex, with 14 animals classed as immature, 11 males and 16 females overall.

Haemolymph from five animals were cultured with no growth from any animal. A total of nine haemocyte counts were undertaken with a very similar range across both groups, an overall mean count of 12,572 / mm^3 and a range of 9,200-15,750, falling well within the normal range for this survey.

Gills in this group generally showed little fouling but quite a number of parasites. Only one animal showed a level of gill fouling reaching the magnitude of score 1 (very minor fouling was not scored). In contrast, crustaceans were present in 10 of the animals and other parasites on five, including gill barnacles and turbellarians. This was interpreted as implying that fouling levels remained low, despite a sufficient time since last moult for parasites to re-infect the gills.

Of the general changes, 19 animals across both groups showed reserve inclusion cells in the gills and 22 animals showed coagulated haemolymph with an overall area score for gills of 28 (mean score 0.82, compared to 0.43 from wild animals overall). Eighteen animals showed both changes in the gill. The other four animals showing coagulated haemolymph showed reserve inclusion cells in other organs. The single animal showing reserve cells in the gill but no coagulated haemolymph did show this in the digestive gland. Of the 10 animals from which gills were examined without evidence of coagulated haemolymph, six showed no reserve inclusion cells in any organ. Two showed reserve inclusion cells in other organs without any coagulated haemolymph and two showed reserve inclusion cells and coagulated haemolymph in other organs, so there was again a high but not complete correlation between the presence of coagulated haemolymph and reserve inclusion cells in the body. This was not a reflection of altered reserve cell numbers, as overall the reserve cells were within the normal range: the whole body reserve inclusion score for the total region was 78 or a mean score of 1.9 / per animal (0.3 per organ actually examined), compared to a mean score of 2.1 / animal (0.31 per organ examined) for the survey as a whole.

One gill showed mild degeneration and melanisation. Small areas of haemocyte aggregation were seen in two animals with coagulated haemolymph in the gills. The other change that was noted in the gill was occasional black debris in association with crustacean parasites.

In the integument, three animals showed lesions. Two of these also showed muscle lesions. One of these animals was a tagged animal that showed muscle focal muscle degeneration associated with inflammatory cells. Such lesions have been seen previously and may be associated with bacterial infection. The two other animals showed marked degeneration, inflammation and some melanisation of the integument. In one of these, bacteria were visible in the melanised lesion.

Muscle lesions were evident in approximately half the animals (20) varying in severity to give an overall score for this area of 26 from 37, and a mean muscle damage score of 0.7 per animal. Bacteria were evident in only one of these animals with a suspected possible septicaemia, although inflammation associated with these lesions was present in eight animals (Total score 9). The antennal gland showed scattered coagulated haemolymph. One of these animals showed considerable vacuolated epithelium to an extent that suggested fat storage but

is more likely to represent a mild degenerative change. Whether this is related to the other changes described is uncertain.

The animal with suspected septicaemia, or at least muscle infection, showed some haemocyte aggregation in this organ, as did one other animal with gill barnacles as well as muscle degeneration and inflammation. Intra-eosinophilic bodies were also seen in the antennal gland cells in five animals. Four of these animals showed some muscle degeneration.

Another animal, a female, showed large numbers of haemocytes and eosinophilic granular haemocytes in the interstitial spaces of the antennal gland but no obvious inflammation or degeneration. This animal also showed some filamentous bacteria in the lumen of the gut, some crustaceans and black debris in the gills and intra-cellular eosinophilic bodies also in the non-vitellogenic ova.

The heart showed variable numbers of reserve cells, occasionally in quite large numbers, and coagulated haemolymph. Occasional vacuolated reserve inclusion cells were seen in five animals. Degeneration and inflammation of the heart were seen in one animal that showed multi-focal areas of skeletal muscle degeneration. A general increase in haemocytes without clear cut inflammation was also seen in at least two lobsters. Inflammation with large clots of haemocytes was seen in at least two additional animals. One animal showed diffusely scattered melanin pigment through the myocardium in many areas of the heart. Many reserve inclusion cells and some inflammation were also apparently present. This animal also showed a small focus of inflammation and degeneration in the hepatopancreas. Otherwise the main observations in the hepatopancreas were variable numbers of reserve inclusion cells and occasionally coagulated haemolymph when reserve inclusion cells were present. One animal showed the eosinophilic spherical suspected parasites. These were present only in very small numbers. A few bacteria were also present in the lumen of this animal plus mild increase in eosinophilic granular haemocytes and haemocytes forming occasional light peri-arteriolar reactions in the interstitium in the central area closest to the lumen.

5.3.1.4 West Coast

West Coast samples consisted of two submissions on 11 December 2002 and 5 February 2003 with the first samples being from Davidson's Bay in the Woolnorth region and the second from Couta Rocks area of the West Coast, with 25 and 15 animals respectively. The length and weights of these groups were quite similar with 75mm average length in the first and 81mm in the second, but the level of pathology differed between the groups, especially with regard to muscle lesion which were present in both groups but especially the second submission.

Although a number of these animals were immature, only three animals were so immature that sex could not be determined although a total of 15 animals were classed as immature. Tail blisters were present in eight from the first submission and four from the second submission and tail erosions were present in 15 and six respectively, the average giving mean number of blisters and erosions per animals as 0.31 and 0.6 respectively in the first submission and 0.27 and 0.4 in the second submission. The overall level of limb loss was also sightly higher in the initial submission. The numbers of appendages lost from the first submission was 17 and from the second submission seven, the total number of limbs lost being 28 and 12 respectively, so that the mean number of limbs lost was 1.02 and 0.8. Of the limbs lost, 16 of the first submission were described as old lesions, compared to six old lesions from three animals in the second submission. The number of limbs lost per animal varied from one to five in the

first submissions and one to three in the second submission. Thus the higher level of muscle degeneration seen in the February samples does not appear to be related to either the acute limb loss or more long standing integument lesions.

Haemolymph from seven animals from each submission were cultured with no growth in the majority of animals but low levels of *Vibrio splendidus* I from two animals from the first submission. The two animals with some bacterial carriage included the animal with the highest haemocyte count in submission one, at 18,350, however, the highest animal in the second submission with a haemocyte count of over 20,000 showed no bacterial carriage. Haemocytes were counted from these haemolymph samples, with very similar mean levels of 12,200 and 13,890 (ranges 6,300 to 18,350 and 9,400 to 20,000).

Very little gill fouling was present in the first submission, with low levels from only two animals, whereas six animals showed fouling in the second submission. This could in part reflect the higher temperatures of February compared to December, or this in combination with transport factors.

Apart from fouling, there were a number of parasites present on the gills. These included a score of 13 animals with crustaceans and 17 with other parasites across both submissions. Egg sacs were also detected in four of the animals from the first submission but not from the second submission. Reserve inclusion cells were detected in 13 animals from the first submission and six from the second submission with one animal having very high numbers, (score 2). This animal did have fouling protozoan and crustaceans in the gill and a yellow discolouration of some degeneration in muscle, but was otherwise unremarkable.

The other change noted was coagulated haemolymph from six animals in the first submission, with high levels in the animals which had large numbers of reserve cells, and from three animals in the second submission, all but one of these showing reserve inclusion cells in the gills or other organs as seen previously. Small numbers of haemocyte aggregations were seen in two animals from the first submission and one from the second. One animal from the first group showed mild areas of gill degeneration. This animal had considerable numbers of sessile protozoa and an increase in the cellularity of the gills generally. Another lobster with multiple parasites (unidentified metazoan, a crustacean and some large egg sacs), showed black debris of the non-cellular type in amongst the gills. One animal from the second group with a considerable amount of plant material and other brown debris in the gill also contained a crustacean.

In the integument, reserve inclusion cells were also common and coagulated haemolymph was occasionally detected in the first group. Eosinophilic granular haemocytes were occasionally detected and one haemocyte aggregation. One animal in the first group showed eosinophilic granular haemocytes and haemocytes in an area of the integument associated with debris and compartmentalised algae, although the integument itself appeared normal.

The skeletal muscle damage score in the second submission was the highest of any group of lobsters examined and from the first submission was the third highest (0.146 per animal for the second group and 0.96 lesions per animal for the first). One animal in this group had a maximum muscle degeneration score of 3. Inflammation was associated with muscle damage in both groups with a score of six for the first submission and seven for the second submission (or 0.23 and 0.47 per animal). Haemocyte aggregations were quite rare with only one in the second submission. Eosinophilic granular haemocytes were also only rarely seen with a score of two in the first submission and none in the second.

Both submissions showed considerable levels of muscle degeneration. The total score for this change in the first submission was 25 for the 26 animals, presenting 18 animals with lesions, the maximum level of degeneration being three (in one animal). These muscle lesions typically consisted of white muscle streaks with focal muscle degeneration being present histologically, with or without inflammatory reactions. Several of these animals also showed antennal gland inflammation or degeneration. In one animal muscle lesions were associated with a large wound on the ventral area of the distal tail muscle. All but one of the animals from the second submission showed histological and / or gross evidence of muscle degeneration, remaining animal had darkening of the gut and other changes suggesting possible starvation.

Seven animals in the second submission also showed bulging of the inter-segmental membranes, with a suggestion of mild turgid muscle syndrome. All showed evidence of muscle degeneration, often seen grossly as white streaking in the muscle (in one animal the lesions were only seen grossly, not in the areas sectioned), but as virtually all these animals showed muscle degeneration, the relationship may be tenuous. Bulging muscle segments or turgid lobster syndrome was not detected in the December submission. However two of the animals showed a yellow discolouration of muscles which otherwise showed degenerative lesions and one of these showed orange haemolymph. The animal with the orange haemolymph was one of those from with low levels of carriage of *V. splendidus*.

In the antennal gland, the first group showed very little pathology other than the occasionally eosinophilic granular cell noted and one granuloma in one animal (with the highest levels of eosinophilic granular haemocytes). In the second submission, six of the 15 animals showed antennal gland degeneration with one animal having a score of two. Associated with this was inflammation (total score of 9). All but one animal showing degeneration had detectable inflammatory changes. Inflammatory changes were also detected in one gland that had no noticeable degeneration.

Coagulated haemolymph and intra-epithelial bodies of the antennal gland (IEB) were seen in the second submission, the latter in five animals, three of these also with coagulated haemolymph. These were not seen in the animals undergoing acute antennal gland zonal degeneration, although interestingly the animals showing inflammation without degeneration did have these globules. Of the animals with IEBs, all showed the bulging membranes of turgid lobster syndrome except for one animal which was reported to have a hard white nodular antennal gland, and showed predominantly inflammation of apparently longer standing. Degenerative changes of the antennal gland of these submissions were quite high, almost the highest counted, and were the highest encountered for eosinophilic granular haemocytes. In the second submission the inflammation was the highest level encountered.

The heart showed reserve inclusion cells and eosinophilic granular haemocytes at levels similar to other submissions. Vacuolated reserve inclusion cells were present in two animals from the first submission. Reserve inclusion cells were also fragmented in an animal with orange haemolymph and yellow muscle streaks, this animal was not cultured. In another animal with large areas of muscle necrosis, fragmented reserve inclusion cells were seen and IEB in the antennal gland. The February submission had perhaps the second highest level of reserve inclusion cells seen in any submission, but only moderate levels were present in the first submission.

The hepatopancreas showed only moderate levels of coagulated haemolymph, one with eosinophilic granular haemocytes from the second submission. One animal in the second

submission, the one with the large wound, showed a large metazoan parasite, suspected tapeworm with minimal host reaction within the hepatopancreas (Figure 9). This appeared to be an incidental finding.

The hind-gut showed one animal with inflammation in the first submission and a total score of nine for the fifteen animals for the second submission. The eosinophilic granular score was also increased at 0.71 per animal compared to 0.24. These two submissions had the two highest levels of eosinophilic granular haemocytes, with the next closest mean score being 0.07 per animal. The second group inflammation score was also almost ten times higher than any other group. One animal in this group was noted to have multi-focal areas of increased cellularity with many eosinophilic granular haemocytes beneath the cuticle and the underlying tissue in the hind-gut with large plant material dominating the lumen. The overall appearance of this animal, the dark brown hepatopancreas, suggested some degree of starvation, although multi-focal areas of inflammation and degeneration were also present in the antennal gland. Dark discoloured hind-gut inflammation plus dark discoloured antennal glands was also noted in another animal which had small multi-focal lesions with inflammation in the muscles and bulging membranes, or turgid lobster syndrome. Some eosinophilic granular haemocytes and the large amount of plant debris were also noted in a third animal, which had muscle streaking and a pale hepatopancreas and bulging inter-segmental membranes, although there was no antennal gland changes noted in this animal.

Mid-guts were not examined. Gonad was examined from 13 animals without significant findings, except for some IEB and eosinophilic granular haemocytes. Tiny IEB were seen in the gonad of three animals with bulging of the inter-segmental membranes, giving a granular appearance to these organs. Two of these animals had been classified as male grossly, but were proved to be female on histological examination. All other animals were either not examined or correctly escribed on gross examination. 19 nerves were examined without any significant findings.

5.3.1.5 South Coast – Davey Region

The south coast or Davey regions samples consisted of two submissions; 22 animals from Mouldies Hole on the 29/4/2002, and five additional animals from Trumpeter Bay on 29/5/2002 which had been held in tanks at Taroona for several days. The mean length range of the first submission was approximately 81mm (range 50-98mm) with the additional submission falling within that range at 47-75mm. The majority of animals were sufficiently mature for sex to be determined, with only two animals classed as immature.

There were no blisters or erosions externally on the initial April submission. Erosions were present on four of the five animals that had been held at Taroona, with blisters in one of these. The total number of limbs lost was 31 and eight respectively (overall level of loss per animal examined was very similar at 1.4 and 1.6 respectively. It was noted that losses marked as old were present in both submissions. In the first submission, 17 of the 31 lost limbs were old, 11 recent and three as very recent. In the second submission, all of the losses were marked as old.

Haemocyte counts and bacteriology were carried out on five animals from the first submission. Haemocyte counts only were carried out on all five animals from the additional submission. The initial April submission showed haemocyte counts ranging from 6,775-16,3223 cells / mm^3, and no growth from any of the haemolymph cultured. The animals held for several days showed a relatively narrow range of haemocyte counts, though these were at a somewhat higher level, with a mean count of 19,269 / mm^3 and a range of 14,275-23,025.

The gills of the April submission showed low levels of fouling on six animals with a mean fouling score of 0.32 per animal. Note that in this group, crustacean parasites were only noted in two animals, only one with obvious fouling. Other parasites, particularly apparently encysted turbellarians were commonly noted, being present in nine of these animals. In several of these animals, eosinophilic granular haemocytes were elevated in the gills. Of the five animals with elevations of these cells, only one did not show these parasites in the section examined. Other inflammatory changes were quite rare, only present in one heavily infected animal. Most of the animals with the embedded parasites did show reserve cells. The second land held submission showed fouling in three of the five animals with a high level in one animal, which also showed a high level of crustaceans, and some other parasites. One of these showed eosinophilic granular haemocytes, but in general no reserve inclusion cells or coagulated haemolymph was seen in the gills of these five animals.

In the integument, degenerative changes one animal was noted in the second group, though no significant changes were seen in the first submission. The affected animal showed black/brown discolouration of one side of the gill area. The right side gills showed a slight greening of the gills, and integument erosive lesions. It was noted that reserve inclusion cells were only seen in the healthy areas of integument. There was also an area of degeneration and increased cellularity in the antennal gland of this animal.

Degenerative changes in the skeletal muscle were relatively common averaging 0.79 in the major submission, but 1.2 per animal from the small group held on-shore, though this represented lesions in only three of the five animals. One of these animals having a very high score of degeneration associated inflammation and eosinophilic granular haemocytes. Inflammation and occasionally eosinophilic granular haemocytes were present only in those muscles with degenerative changes but not present in all such animals. Regenerative changes were present in affected areas of muscle in two of the animals from the first submission, suggesting a more chronic cause than the collection period.

In the first submission, there were no degenerative changes in the antennal gland, one animal showing increased apparent interstitial storage in this gland and a second animal showing slight increase in contents in the lumen of this gland suggesting some sloughing of cells. There was no other pathology evident in this animal. In the second land held submission, two animals showed degenerative changes in the antennal gland. One of these showed some skeletal muscle degeneration and gill fouling, the other only low levels of gill fouling and some parasites, plus a high haemocyte count, discolouration of the gill areas and some integument erosion. The animal with muscle degeneration showed a very soft shell and yellow discolouration of the muscle round the walking legs, with multi-focal degeneration.

Coagulated haemolymph and eosinophilic granulocytes were occasionally present in the gland but appeared to have no relationship to pathology except for the presence of IEBs which were present in some animals showing coagulated haemolymph or muscle degeneration. These were also associated with areas of increased cellularity locally, but without obvious degeneration of the gland cells. Coagulated haemolymph was relatively common in animals with significant numbers of reserve cells. The number of reserve inclusion cells varied, especially in hearts from the main submission. It was noted that neither reserve inclusion cells nor coagulated haemolymph were seen in the smaller land held submission. Reserve inclusion cells were occasionally vacuolated. There was no other heart pathology noted.
Reserve cells, coagulated haemolymph and occasionally vacuolated reserve inclusion cells were also variably noted in the hepatopancreas, unassociated with any pathology. The

suspected protozoan cells were noted in three of the animals from the first submission only. One hepatopancreas showed a mild increase in eosinophilic granular haemocytes in the interstitial tissues near the lumen. Another showed a mild decrease in epithelial height, although no other pathology was noted.

In the hind-gut, occasional reserve cells, and coagulated haemolymph were noted. One animal in the first submission showed bacterial foci lining the lumen. This animal did not have noticeable bacterial gill fouling but did have multiple parasites present in the gills, suggesting no recent moult.

Reserve inclusion cells were the only finding noted in the mid-gut region. In the gonad, some degeneration of ova was noted in one female with mild integument damage and a large number of recently lost limbs and one animal from the first submission with mild degeneration and gill fouling. Overall, very little pathology of significance was seen.

5.3.1.6 Bruny Island Region

42 animals were submitted in April 2002, from Adventure Bay in Bruny Island and near by Blackman's Bay, plus an additional submission of 18 animals from the Bay of Islands area (near the southern margin part of this and the Davey zone) in on 22/1/2003. Animals from the first submission were of an average length of 70.8mm, with a range of 35-108mm and 49-105mm in the second. Only nine were classed as too immature to distinguish the sex. Externally erosions but no blisters were present in five from the first submission. Erosions were present in seven animals January 2003 submission, occasionally severe, with blisters present on two of these. One of these showed a high level of both blisters and erosions. Blisters were only present on those animals that also showed erosions.

In the first submission, a total of 23 animals showed some appendage loss, representing a total of 40 limbs lost or a mean loss of 0.95 per animal. Severity ranges from 1-4 limbs lost and the majority, 36 of these 40, were classed as old lesions. In the 2003 submission, 29 limbs were lost, representing 1.6 per animal, with only five marked as old and most were marked as recent losses.

Haemolymph was collected from five animals for haemocyte counts and bacteriology and ten animals from 2003 submission. The external tail erosion from one animal from the initial submission was also cultured, with mixed Flavobacterium-like organisms were present in large numbers from this lesion. One 2002 animal showed low levels of *Vibrio navarrensis*, the other animals showing no growth. Four of these haemolymph samples were suitable for haemocytes counts, with a mean count of 9,281 / mm^3 and a range of 2,925-14,250. The animal with a maximum count had no limb lost, whereas two animals showing loss of limbs showed counts of approximately 9-10,000 cells / mm^3. No limb loss and no bacteria growth, and no pathology other than very slight muscle degeneration, were present in the animal with a very low haemocyte count. The lower count in this animal may reflect overnight rehydration in the holding system as not all animals could be processed on the day of submission.

The January 2003 submission showed no growth in seven animals and *Vibrio splendidus I* in low levels in three animals. Of these, one showed a very low haemocyte count of 1100 / mm^3. The counts in remaining animals varied from 9,750 in another animal showing bacterial carriage to 28,150 in an animal showing no growth with the third positive animal showing 19,800 haemocytes. The mean haemocyte count in this submission was 15,345. All of the animals with *Vibrio splendidus* carriage showed recent muscle degeneration present grossly as

muscle streaking and histologically as degeneration with occasional inflammatory cells in two animals. However these changes were also seen in other animals in the group which had been cultured and were negative on culture.

There were few changes noted in the gills. In the initial 2002 submission, reserve inclusion cells were relatively common, present in 20 of the 41 gills examined. Coagulated haemolymph was present in 11 of these, usually at low levels. It was noted that reserve inclusion cells were present in at least some organs in all of the animals with coagulated haemolymph. These were present in the gills in all but four animals. Bacterial fouling was noted (mostly at scores of level 1) in 15 animals, all with reserve inclusion cells present and usually within the gills. Crustacean parasites were detected in six animals and egg sacs in three, with 14 animals showing other parasites present in the gills. The most frequent other parasites seen were large sessile protozoa, however, turbellarians were also occasionally detected, sometimes apparently encysted or embedded.

In the January 2003 submission, fouling was present in only one animal, though crustacean, metazoan and protozoan parasites were evident in a number of animals (eight with crustaceans, six with other parasites noted).

The integument showed a melanised inflamed lesion associated with surface metazoans in one animal from the initial submission, and degeneration was also noted in one animal from the later group. Otherwise, two animals showed moulting changes and reserve inclusion cells were relatively common in the skin. Of the two animals with integument lesions in 2003 submission, one had only very minor lesions with increased numbers of eosinophilic granular haemocytes. The other animal showed more areas of degeneration and inflammation with some melanisation particularly in the uropods. This animal also showed the loss of five limbs, although all losses appeared to be recent. Reserve inclusion cells were fairly common in both animals.

In the 2002 sample, 11 animals showed degeneration of the skeletal muscle, with degeneration usually being severe when present to give a total score of 20, or a mean score of 0.5. Some but not all of these animals had also lost limbs with old lesions present. Four of the seven animals with severe lesions showed inflammatory changes. There appeared to be no commonality between the animals that showed muscle degeneration and no inflammation, regardless of the level of degeneration seen, which is consistent with this reflecting handling damage rather than underlying health status. All of the four animals, which showed severe deep muscle degeneration with inflammatory changes, did show reserve inclusion cells and bacterial fouling in the gill. There were no significant changes in the antennal glands in these animals. Coagulated haemolymph or reserve inclusion cells were the only changes noted in other organs. One animal, which did not show the recent degenerative changes, showed a granuloma in the muscle section. This animal had several superficial metazoan parasites but was otherwise unremarkable.

Degenerative changes in the skeletal muscle were also relatively common in the 2003 submission averaging 0.61 per animal.

Changes such as coagulated haemolymph and eosinophilic granular haemocytes were occasionally present. Similar changes were also present scattered through the antennal gland where the only significant pathology was degeneration and inflammation in one animal which had a puncture wound in the gills judged to be of some duration. Other changes present in antennal glands from 2003 include the occasional IEB and some vacuolation in two animals

with clumped bacteria in the lumen of the hepatopancreas and a decreased height, but otherwise little change. One animal with bacterial fouling on the gill and parasites showed swollen antennal gland cells, plus multi-focal haemocyte aggregation in the heart and coagulated haemolymph. Another unremarkable animal showed the presence of sloughed spherical fragments in the lumen of the antennal gland suggesting some cell sloughing.

Antennal gland degeneration was present in three animals in the 2003 submission, none of these having gill fouling integument damage, but two animals having marked muscle degeneration. Both the animals with muscle degeneration showed inter-epithelial globules in the antennal gland as well as demarcated areas of gland with increased cellularity. Intra-epithelial bodies were also present in the third animal that had a distinct demarcated area of degeneration and increased cellularity in the antennal gland.

The heart showed variable numbers of reserve inclusion cells and coagulated haemolymph. The heart also showed small inflammatory infiltrates in two animals with mild muscle lesions. One of these also showed a degenerative focus, and integument degeneration and melanisation. Six animals also showed changes suggestive of storage organ cells. Another animal showing little except several lost limbs and small areas of muscle degeneration showed very sparse heart muscle fibres and haemocytes in the nerve tissue. The hepatopancreas showed occasional presence of reserve cells, coagulated haemolymph and occasionally a decreased epithelial height (four animals). Eosinophilic spherical bodies suspected to be possible protozoa were present in five animals, always in low numbers. Bacteria were present in lining the hepatopancreas lumen also associated with it in four animals.

In the hind-gut, coagulated haemolymph and bacteria were seen in one animal from the 2002 submission, multi-focal layer of eosinophilic granular haemocytes underlying the cuticle in another. Bacteria without other changes were also detected in one other animal. The 2003 January submission showed one animal with vacuolation of the epithelium of the hind-gut, plus marked muscle degeneration and inflammation and a demarcated area of increased cellularity of the antennal gland. Bacteria were also present lining the lumen in two animals in this submission. Neither showed gill fouling, although both showed some evidence of parasites in the gill.

Mid-gut sections were not recovered. There were no significant lesions in the gonad and the only change present in the seven nerve chords examined was the association with haemocytes in one animal as already discussed.

5.3.2 Farmed samples

5.3.2.1 On-shore flow through culture system sample 1 (2002)

The first farm sample received on 20/9/02 consisted of 30 animals collected during the preceding season and held for 12-15 months, and ten lobsters that had been collected the previous season and held for 22-27 months. Both were from batches with a planned release at Adventure Bay, Bruny Island. Lobsters from the two year classes were held separately. The small size-class were collected predominantly from salmon cages at Nubeena, although about 10% originated from Marine Research Laboratories' collection sites at Recherche Bay, Bicheno and the Derwent River. The older animals were all collected from Marine Research Laboratory's sites.

Smaller animals having a carapace length of averaging 30mm (range 24-35mm) and the larger animals having an average carapace length of 67mm (range of 64-70mm). The older animals did show some gonad development, but gonad was really seldom detectable or immature in the younger animals as might be expected.

Both groups showed frequent erosion of the external surfaces or appendages (Table 2). Actual blister formation was relatively common in the older animals, but not in the younger ones. Appendage loss was present in both groups but particularly the younger animals. Haemocyte count averages were similar across the two groups.

This submission had one of the highest level of biofouling, and the highest level of shell erosion. The biofouling levels were similar in both of the age groups. Crustacean levels were present in both groups but were slightly higher in the older animals, averaging a score of 1 per animal. Overall the level of other parasites in the two groups were similar.

In the integument, inflammation, degeneration and melanisation were all more common in the older animals reflecting the levels of gross pathology. The two groups showed some similar levels degree of degeneration of skeletal muscle. Moderate integument biofouling was evident in one of the younger animals.

Two of the younger animals showed slight changes in the antennal gland. Both showed epithelial erosions of the tail. Both showed small foci of degeneration plus inflammation or increased interstitial cellularity, and one also showed foci of degeneration of skeletal and heart muscle. Muscle degeneration was seen in both age groups. Coagulated haemolymph was seen eight of the 10 older animals, often at high levels, but in only five of the younger animals, mostly at low levels. These older animals also showed a higher level of intraepithelial globules in the antennal gland (seven animals, two at high levels) although these were also present in five of the 30 younger animals. All of the animals with antennal gland globules showed significant biofouling (on the gills of 10, in the hind-gut only in one), and all but one showed evidence of tissue damage and / or coagulated haemolymph. In general, the antennal intra-epithelial globules tended to correlate with relatively high levels of coagulated haemolymph.

Coagulated haemolymph was also seen in the heart of some of the larger animals and in the hepatopancreas. The latter was a rare finding in the younger group. The only other difference in the hepatopancreas was a slightly higher incidence of score for reserve inclusion cells in this organ in the older animals. Reserve inclusion cells in the older animals averaged 1, and in the younger animals only a fifth of that.

In the hind-gut, bacteria were occasionally present lining the lumen, with slightly higher levels in the older age group. This appeared to reflect the general level of biofouling in this group.

5.3.2.2 On-shore flow-through culture sample 2 (2003)

The second on-shore farm submission from May 2003, were lobsters for release at Stapleton Point, Orford and Safety Cove, Port Arthur. All were held at the Marine Research Laboratory for 12-15 months and they were sourced from the Marine Research Laboratory's collector sites outlined above. The mean carapace length was 27.9mm, which is slightly smaller than the animals of the preceding year. These animals showed only immature gonads. External findings included tail erosions in 13% of the 42 animals but no tail blisters were present.

Appendage loss was very slight with one old and one recent loss. Haemocyte counts within the ten animals counted were very variable with the highest recorded count of over 25,000 cells / mm^3. Haemolymph was cultured from two of these animals with counts in excess of 23,000 and from one of the animals with a very low haemocyte count of 2,350 / mm^3 as well as animals in the intermediate range. No bacteria were cultured from any of the haemolymph.

Apart from variable evidence of recent or imminent moult, there was very little to distinguish this group except for bacterial fouling on the gills. Fouling was occasionally present in the hind-gut, particularly in those animals with high levels of bacterial fouling of the gills. Fouling on the skin was only detected at low levels in two animals. Another animal showed integument granulomas. Reserve cells, usually at low numbers, were present in gills, integument, and occasionally heart in ten animals, all of which showed appreciable levels of gill fouling. This may indicate these are animals that have not recently moulted. Inflammation was present in the gills of two animals with high levels of gill fouling. This plus the granulomas in the integument of one animal mentioned above were the only evidence of inflammatory changes in this group, which suggests that inflammation is not a significant influence on the varying haemocyte numbers.

Coagulated haemolymph was also a rare event being present in one integument and three hearts. The only other findings of note in this group were slightly brown haemolymph in one animal approaching moult; flukes and egg cases inside the gill chamber in another animal; and the dark tail muscle in another two animals undergoing moult (one with a thin shell and one with a distinctly soft body). Black dirt was noted in gills of one of these animals.

Overall the results suggest better culture conditions and handling than for the previous year.

5.3.2.3 Sea-cage culture system 1 (2003 sample)

The sample size of the first sea-caged group was influenced by the difficulty encountered by prospective farmers in puerulus capture and the subsequent changes to the overall program. While commercial production and release of 10% of the animals was abandoned, the remaining farms continued to on-grow stock to obtain growth data for the future, when such problems could be overcome and commercial ongrowing resumed. Only 19 animals were received from this 2003 population as the number of animals captured was very small compared to expectation and the farm was reluctant to submit samples as numbers were regarded as barely sufficient for their own experimental purposes. Nevertheless, to ensure a sound basis for assessment in the future, the 10% of animal that would have been released under the original licence conditions were directed into this survey.

These animals were quite small, with a mean carapace length of 28mm and a mean weight of 11.4grams. This figure under-represents the true weight to some extent as seven animals were dead on arrival and of these, the majority showed large numbers of lost legs, up to all legs being lost. Tail blisters were absent and tail erosion present in only two animals. The animals were generally all immature although gonad development could be seen commencing in several animals.

Dead animals were not included in those for haematology and culture. Of the ten animals cultured bacteria were only isolated from one, a *Vibrio splendidus* I at low levels. This animal did have a high haemocyte count of 28,500, but showed no significant lesions other than mild gill and integument fouling. Bacteria were not isolated from other animals with high haemocyte counts and a similar level of fouling. Haemocyte counts were relatively high

relative to the wild animals, with four of the nine animals counted having levels of greater than 20,000 / mm^3. The two highest levels were 28,500 and 29,500 cells / mm^3. One of these animals showed mild muscle degeneration, the other showed low levels of the *V. splendidus* I, otherwise they were unremarkable compared to the group.

The group as a whole consistently showed gill fouling, varying from low to medium in severity, and frequently also showed integument fouling. Bacteria lining chitinous surfaces were also seen in the hind-gut in four animals, only two of which were dead, the pattern appearing to be independent of survival on arrival. Bacteria were also seen in several hepatopancreas but only in those animals that had died. Low levels of degeneration were seen in skeletal muscles of most of the animals that had died, but not in other animals. Only one small crustacean parasite was seen in the gills. The only other finding of note was aggregation of eosinophilic granular haemocytes and some haemocytes in multi-focal areas of the hind-gut of one animal. This animal also had vacuolated antennal gland epithelium, as did one other animal. The trend line of the weight to carapace length showed considerable scatter along the trend line, which is likely to reflect differences in moult stage.

5.3.2.4 Sea-cage culture sample 2 (2004)

Similar constraints relating to the low rate of puerulus collection and contraction of the expected rate of aquaculture development applied to the second of the submissions of sea-aged lobsters. Samples were unavailable the first year, due to very low numbers collected. Animals from both groups were made available after the second year of collection proved no more successful. Given the low numbers held, and that the re-release of animals from these populations was no longer required, only one combined sample was submitted.

This sample consisted of three sub-groups: 14 three-year-old on-grown product fed mussels and mackerel, 17 two-to-three-year-old on-grown product fed mussels, and 7 one-to-two-year-old lobsters fed mackerel only. (Additional animals died due to misadventure between collection and receipt). The length and weight of each group clustered along the trend line with similar slopes to other groups, but with considerable scatter within each group (indicating variable moult stage). Mature and immature groups were discernible within each of the sub-groups.

Gross external observations of each group included spirorbid fouling on the dorsal surface of all groups. Each group showed one animal with goose barnacles on the gills, two high levels. One animal in the oldest group also showed a large mussel attached to the ventrum.

The higher haemocyte counts in 2-3 year animals fed mussels has been discussed above. There were slight differences in scores for reserve inclusion cells between the groups, no obvious differences in bacterial fouling. All groups contained similar levels of gill parasites and other gill findings. There were no marked differences between the sub-groups in the integument except for coagulated haemolymph which was only present in the older animals but was still at low levels and sporadic. Three animals in group 2 (mussels only), showed a high level of muscle degeneration. In the heart, reserve inclusion cells were less commonly seen in the smaller animals. In the mid-gut bacteria were more common in group 2, the mussel fed group, and there were no other significant changes in the other organs.

5.3.3 Summary of general pathology

A summary of the tissue changes from all animals is given in Table 10.

Table 10. General pathological changes and indicators of overall health identified in the Tasmanian rock lobsters *Jasus edwardsii*

Disease Disease agent	West	King Is	Flinders Is	East	SE / Bruny	South Coast	Farmed on-shore 1	Farmed on-shore 2	Farmed Sea-cage 1	Farmed Sea-cage2
General external lesions										
Gill bacterial fouling	+	+	+	+	+	+	++	+	++	+
Shell Blisters	+ (12)	+ (1)	+ (4)	+ (3)	+ (3)	+ (1, held)	+ (6)	0	0	+ (13)
Shell erosions	+	+ (1)	0	+	+ (12)	+ (4, held)	++ (39)	+ (13)	+ (11)	+ (21)
Appendage loss	+	+	+	+	+ (39)	+ (15)	+ (24)	+ (2)	++ (38)	+ (18)
General internal lesions										
Coagulated haemolymph	+	+	+	++	++	++	++	+	0	++
Muscle degeneration / inflammation	++ (47)	+ (11)	0	+ (26)	+ (31)	+ (21)	0	+/- (1 sl)	+ (3)	+ (10)
Antennal Gland degeneration	+	+	0	0	+	+	+ (2 sl)	0	0	0
Antennal Gland inflammation	+	+	+	+	+	0	+	0	0	0
Antennal gland globules	+	+	+	+	+	+	+	0	0	+
Hard nodular antennal gland	+ (1)	0	0	0	0	0	0	0	0	0
Vacuolation of reserve cells	+	+	0	+	+	+	+	+	+	+
Granulomas	+ (1)	+ (2)	+	0	+ (1)	0	+ (6)	+ (6)	0	+ (6)
Turgid lobster syndrome	++ (1 accn only)	0	0	0	0	0	0	0	0	0
Red-brown haemolymph	+	+	0	0	0	0	0	0	+	+
Hepatopancreas mummification										
Other tissue changes (including non-pathogenic)										
Accumulation of reserve cells										
Accumulation of eosinophilic granular haemocytes	0	0	+ (1)	0	0	0	0	0	0	0
Haemocyte aggregations	+	+	+	+	+	+	+	+	+	+
Melanin in myocardial cells	0	0	0	+ (1)	0	0	0			

5.4 Discussion

Overall the number of wild samples approximated the planned sampling with the exception of reduced numbers of lobsters from two of the five regions due to adverse weather collections truncating the planned sampling and the high cost of re-sampling from these areas. The number of samples from the farmed groups was considerably reduced due to a contraction of the planned aquaculture ventures, due to difficulties in collection of puerulus in sufficient numbers and other constraints. Several of the licence holders pooled efforts and the small number of puerulus collected were raised on one on-shore facility (the marine research laboratories at Taroona), with the overall results of this forming part of another study. The only other groups of lobsters that were available to this survey were two groups from sea-farm systems which allowed a comparison of these two farming systems, as well as the primary purpose of this survey which was to compare the health of wild and cultured lobsters due for release.

The primary finding from this survey was the lack of serious disease in either group.

Given the low level of disease encountered, and the considerable data accumulated to reach this conclusion, considerable effort was concentrated on the comparison of general health indicators and an understanding of the histological variations seen, and their pathological interpretation. Because this was not primarily a comparison for such purposes, conditions such as sampling and transport conditions were not standardised, and therefore an absolute comparison between these groups in statistical terms, is unwarranted, and results have been presented in a descriptive manner. Nevertheless, all data is available for reassessment in the future if and when required. Another constraint in the interpretation of these findings is a difference in size range between some of the farmed groups and the wild lobsters. Very small animals were rarely detected and captured in the wild and more common in those animals which had been held for the minimum time in culture.

This study has allowed a collation of findings from nearly 400 lobsters, although not undertaken under as controlled conditions as the previous small studies of adult wild-caught lobsters (Evans et al, 2004) of this species, but covering a broader health spectrum than the previous morbidity based study of cultured juveniles (Handlinger et al, 1999). This considerably expands knowledge of the pathology of *Jasus edwardsii*. It was not the intention to undertake such a study specifically, however, the high staff turnover and large number of people involved in assessing these animals dictated the form of results and has resulted in a comprehensive database from which current and future comparisons can be made.

The limb loss and culture pattern indicate that collection and transport stress is likely to have been significant in most, if not all, samples. This needs to be taken in to account in assessment of results such as the bacteriology and haematology.

Bacteriology results indicate that the prevalence of bacteraemia was considerably less than the post-transport levels detected in the Evans et al (2004) study, in which bacteraemia in adult lobsters of this species increased from 4% to 40% after transport. Nevertheless, the high level of muscle necrosis and associated lesions in this study overall and the relatively recent nature of most of those lesions and the association of bacterial carriage with muscle lesions suggests a significant level of transport stress overall. This is consistent with the results and views expressed at the International Symposium of lobster health management, that

conditions of transport had a major effect on the health of spiny lobsters received into holding systems. The bacterial levels seen and the lack of detectable directly associated lesions in these animals suggest that most could be classified as a bacteraemia rather than a septicaemia. There was no evidence that internal bacterial infection had been contributing significantly to ill health in lobster from either location, prior to sample collection.

Bacteriology results suggest the overall bacterial carriage load of juveniles of this species to be considerably less than reported from the South Australian transport studies of adult *Jasus edwardsii* by Evans et al. *Vibrio splendidus* I has been previously isolated from disease in a range of aquatic animal species, including *Jasus edwardsii* in New Zealand (Diggles, 1999) and has also been regarded frequently as an opportunist, as appears likely in these occasions. *Vibrio navarrensis* is a much less frequent isolate, and could only be regarded as an opportunist in a stressed animal, given the overall findings in this animal.

With the possible exception of bacteria in the hepatopancreas, the most obvious difference in bacterial profiles between farmed and wild lobsters were different levels of bacterial fouling and associated changes, which were considerably higher levels in farmed groups than wild groups. This is likely to indicate a real compromise in the robustness of farmed groups and this may have been reflected in the number of animals from one of these groups that died during submission. Differences in minor skin erosions appeared to reflect the gill fouling and probably reflect the same deficiencies in culture conditions.

The haematology results overall are consistent with the South Australian study by Evans et al, 2004, of a smaller number of adult rock lobsters of this species. They showed a considerably higher total haemocyte count in *Jasus edwardsii* than in *Panulirus cygnus*, the lobster species from which haematology has been best studied in this country. The results from this survey are of a similar order to their findings of a total haemocyte count of $13.17 \pm 1.01 \times 10^6$ cells / mm^3. The overall counts for the farmed animals were slightly higher than those from wild animals and there was more scatter of results. The high overall level of scatter is likely to be due to differences in moult stage, as the numbers of circulating haemocytes increase just after moulting and later decline. It was considered whether the higher levels overall in farmed animals might be due to the differing size ranges in these groups, but plots indicated any such relationship was slight. However, comparison of groups from one location with a variety of age and diet groups suggested the differences could be diet related. This is consistent with the findings from Evans et al (2004), who found considerably greater variation in haemocyte counts in mussel fed *J. edwardsii* than in animals on other more natural diets. Norton and Linton, 2004, also found total haemocyte counts in the tropical lobster (*Panulirus ornatus*) varied between diets.

These results are a useful guide to interpretation of haemocyte counts in animals from either groups which have undergone transport, which has been shown to increase the total count in intermoult animals (Evans et al, 2004). These variations reduce the immediate diagnostic value of haemocyte counts if moult stage is unknown, though these could be valuable in assessing the severity of disease retrospectively if assessed in conjunction with moult-stage. The lack of overt ill-health in these animals overall also made it more difficult to determine variations of haemocyte counts which were clearly associated with disease processes.

Of the parasites detected, none were present at levels which would be regarded as a health risk in either farmed or wild animals, and only gill protozoa, generally sessile peritrich ciliates, were more numerous in farmed animals than the wild. The latter is likely to reflect the overall level of gill fouling and, like bacterial fouling, is likely to reflect the intensity of

the holding system, and is likely to be readily corrected following release of animals to the wild. No specific pathology could be ascribed to the ciliates, which are not known to have even locally invasive potential. Such organisms are regarded as commensals and no pathogenic affect was present.

Mild gill reactions were sometimes associated with crustaceans on the gills, a variety of free-crawling crustaceans, smaller examples of which could not be identified. The largest crustaceans were goose barnacles that were identified in only low numbers in three of the wild groups and one of the farmed. In this survey, goose barnacles were only detected on the gills, and from relatively low numbers. Field reports, (Gardner, unpublished), suggest these are common, and usually attach to any hard surface of lobsters but especially on the ventral surfaces. The level of infection seen in the farmed animal in figure 7C would be rare. As this animal had been held for some time in a sea-cage site, this raises the possibility that under such intensive conditions, this parasite could build up in the immediate environment, and increase the level of infection. Nevertheless, no major pathology was associated with these parasites, and there was only one lobster seen with these levels of infestation.

Turbellarian parasites of lobsters do not appear to be previously reported from lobsters, although this group of parasites is common in molluscs. Turbellarians were some of the most common parasites detected in the gills in this survey and were often closely associated with gill surfaces. There is a possibility that they are geographically restricted as all of the wild examples and wild examples were from the eastern and southern regions, with none detected in the Western (Franklin) or King Island areas. Farmed animals indicated that like other parasites these were generally associated with the older animals examined. This is to be expected as, the older animals are likely to moult at less frequent intervals, allowing a greater build up of commensal parasites. This was most evident in one farmed group that showed a number of animals with spirorbid fouling on the external surfaces, also likely to indicate a relatively long time since last moult and hence slow growth in culture. This was a September sample, which suggests that the slow growth rate may be a winter feature. The presence of a quite large mussel (approximately 2 cm) on the ventral surface of one of these animals is consistent with such a view.

The egg cases seen attached to gill filaments on 18 occasions were not identified. They were most frequent co-existent with small unidentified and possibly variable crustaceans on the gills, suggesting these could represent egg sacs of crustaceans, but this is uncertain. The egg sacs are unlikely to be related to the turbellarians, as although these have a direct like-cycle and are reputed to glue their eggs to their host, the morphology is not consistent with a flat-worm egg (pers comm, Marty Deveney).

Of tissue changes seen in the gill, bacterial fouling and the pathology associated with high levels of this is a well recognised feature in crustaceans generally and in previous studies of this species (Handlinger et al, 1999; Diggles and Handlinger 2004). The finding of significant bacterial fouling in the hind-gut in groups which showed gill fouling indicates that this can be regarded as another site for general fouling with a build-up under conditions of intensification, restricted flow, and/or a delay in moult. However, the time since moult alone does not appear to be reflected in the level of fouling as many wild animals and some farmed animals showing no indications of recent moult, and with other parasites present, did not show these changes. Low levels of melanosis and granuloma formation in the hind-gut in the farmed groups, but not the wild groups, may indicate that such fouling may be associated with erosion and reaction if severe, as has been reported for other sites such as the gill. A similar pattern was seen in the integument. Small cuticular erosions (generally on pleopods or other

small appendages) were also high in the heavily fouled groups and are likely to reflect a similar pathogenesis.

Overall the integument findings are consistent with those of the gills, the conclusion being that there is a mild background increase in superficial damage associated with increased fouling in the farmed groups, especially those larger animals which have been held loner in culture system. Cultured animals appear to suffer only a mild decrease in environment quality due to the culture systems. There was considerable variation between individual groups, rather than any obvious difference between on-shore and cultured systems.

The necrosis, melanisation and virtual mummification in the one animal seen from Flinders Island is consistent with the report of Diggles (1999) of a similar hepatopancreas disease in *Jasus edwardsii* in New Zealand lobsters held in a holding system. They found that condition to be diet related. The end result was similar to the lesions we saw in this animal, however they were able to detect small protozoa, possibly flagellates as well as gram-negative bacteria within the degenerating tubules, whereas we found only occasional tubules with bacteria. The exact role of diet and these organisms is uncertain. Failure to digest an adequate diet was also implicated in a similar condition of bacterial necrosis and mummification of hepatopancreas tubules of *Cherax tenuimanus* (Langdon et al, 1992). Obviously, we have no knowledge of the diet of this wild animal. This change has not previously been seen in *Jasus edwardsii* from Tasmania, although a bacterial infection of the gut has been seen in a recently collected puerulus (Handlinger et al, 1999). Whether the bacteria present in the lumen of two wild animals is a related condition is also uncertain. This was the only significant pathology seen in the digestive system other than the changes already mentioned in conjunction with fouling and the association of granulocytes with hind-gut epithelium which may be moult related.

A level of muscle degeneration is consistent with previous reports, but does appear overall to be high, particularly given the low levels of reactions seen in other organs overall in this study. The majority of lesions appear to be recent and presumably related to collection and transport although at this stage we have no detailed figures of time between collection and processing for most groups. The more severe lesions were seen from the Franklin area, which appear likely to have the longest transport time.

This Western group of lobsters also displayed relatively mild symptoms of haemolymph swelling referred to as turgid lobster syndrome. This had previously been reported in experimental holding tanks in which bacteria, including *Vibrio harveyii* and *Vibrio splendidus* I had been isolated (Diggles, 1999). Diggles reported an increase in the number of circulating granulocytes and pre-granulocytes in these animals as well as the bacterial isolation, but does not report on whether histological examinations were done on these animals. Whether muscle lesions occurred in these animals is unknown. The cause of the turgid lobster syndrome was reported as unknown at that time, not associated with salinity fluctuations, handling or other known stresses. They did suspect that animals in the early stages of pre-moult are more likely to be affected. This condition has been reported in other species, including the tropical rock lobster, *Panulirus ornatus* (Stephens et al, 2004), again without a definitive cause being identified. However Chen and Chen 1996, reported that a similar condition could be induced in the prawn *Penaeus japonicus* by ammonia, and Mugnier and Justou, 2004, showed both that ammonia influenced osmoregulatory control in the blue shrimp *Litopenaeus stylirosris*, and that the magnitude of this effect was influenced by moult stage. However the range of factors that could influence osmoregulatory control is not known. The high levels of reserve cells found in these animals, probably indicating an approaching moult (see later discussion), may indicate a significant increase in vulnerability to development of this condition. Another

possible influence is the relatively greater transport time and distance for these animals, if this resulted in a decline in water quality (probably with increased ammonia), or overall increase in stress levels. Our examples showed muscle degeneration, antennal gland globules but not zonal degeneration, and little haemolymph coagulation though reserve cells were high in five of the seven animals. It is possible that muscle degeneration due to handling has released tissue factors that interact with reserve cells to promote a premature release of volume expanding proteins, or alternatively that the muscle degeneration is secondary to such a process. Comparison with other affected groups would be of interest.

Perhaps the most significant pathology seen, mainly in wild animals, were the antennal gland degenerative changes that appear to be of two types. The light microscopy and electron microscopy appearance of the intra-epithelial bodies is of a protein globule. The exact nature of this is uncertain, although animals with high levels of these bodies did tend to have overall high scores for degenerative changes and/or coagulated haemolymph. Relatively high levels of coagulated protein were occasionally seen in animals without these bodies in the kidney, but the uncertainty of timing and the crude nature of our scoring system does not preclude drawing the obvious conclusion that these are protein droplets similar to those seen in vertebrate animals. In fish these may result from environmental changes compromising the overall health, such as prolonged carbon dioxide build up in the environment. Such globules in other species often but not always represent resorption from tubules of protein when the level of protein in solute is increased. As such, they have been interpreted as a change reflecting degenerative or inflammatory processes elsewhere in the body. It is expected that these cells would return to normal once the high protein solute resolves.

The zonal changes in the antennal gland however, are less readily explained, particularly the zonal nature of the degeneration with large areas of the gland being affected, and an abrupt transition to normal tissue. Clearly the degeneration occurs prior to the inflammatory infiltration.

A similar pathology has once been reported in lobsters in Australia in a single tropical lobster (*Panulirus ornatus*) by Norton and Linton (2004), and in the western rock lobster, *Panulirus cygnus* (Stephens et al, 2004). These were noted but the cause was not identified. Aiken and Waddy, 1986, also report that a low level of mortality can occur from a disease of unknown origin that causes necrosis and melanisation of the antennal glands, destroying osmoregulatory function and causing death. They found this condition in both culture and impounded lobsters. A condition having some resemblance with regard to the zonal distribution has been seen as a serious disease in the summer of 2002 in American lobsters (*Homarus americanus*) as published by Dove et al, 2004, which they have called excretory calcinosis. The condition they described includes calcification of the kidney that was not seen in this case, and the animals they studied were all inter-moult or pre-moult hard shell lobster stages, lethargic or moribund, with distinctly orange abdomens and orange haemolymph. It was noted that while the same groups of lobsters contained some animals with orange haemolymph, these were not represented in the samples with severe degenerative changes in the kidney. The animals they described also contained high levels of granulomatous gill disease, whereas these animals affected here showed very little gill pathology. Overall the cause of this condition remains unresolved, with the only obvious correlation recognised being a very low level of reserve cells. Electron microscopy being carried out on this group was done purely as a precautionary measure as the primary aim of this study was to assess any risks involved with translocation of lobsters within Tasmania. While viral disease was excluded, the samples were inadequate to resolve the nature of these degenerative changes.

Discolouration of haemolymph was seen in this survey however, with the colour varying from red-brown to orange, but these instances may not all reflect the same changes. Red haemolymph is seen not uncommonly and has been interpreted as due to egg-resorption by mature females (MacDiarmid and Butler, 1999). However, of the seven animals showing discoloured haemolymph or flesh, only three were recognisably female. Discoloured haemolymph has been described with a number of infections (reviewed by Stephens et al, 2004), such as pink discolouration with the bacterial disease gafffkaemia and orange with the dinoflagellate *Haematodinium* infections. In this survey, one case of discoloured tissue was apparently due to autolysis with bacteria present, and not regarded as typical of the other animals. One of the animals classified grossly as in immature male showed low levels of *Vibrio splendidus* carriage and widespread muscle degeneration, but muscle degeneration was not present in all animals. Pink flesh during moulting may be due to astaxanthin (Paterson et al, 2004), with the levels sufficiently reliable to provide an indication of the stage of the moult (Musgrove, 2001). Thus haemolymph discolouration may represent more than one change. It is unclear whether the specific colours released are sufficiently consistent and differentiated to provide an indication from gross examination of the likely cause of the discolouration in individual animals.

Apart from these specific conditions, much of the effort in this analysis has been directed towards understanding the pathological processes involved in the observed general processes. The pathological processes need to be differentiating from changes due to the moult cycle. The most frequent variations which could not be directly ascribed to degenerative or inflammatory processes were visible coagulation of the haemolymph, and large variations in reserve inclusion cell numbers (not be confused with the hepatopancreas epithelial reserve cells) and variations in eosinophilic granular cell number and distribution.

It appeared likely that the coagulated haemolymph seen commonly in the gills, especially in the two groups with heavy biofouling was part of the early stages of a general response in animals which may have been undergoing high levels of oxygen deprivation stress during transport, though this warrants further assessment. This was rarely seen unless at least moderate numbers of reserve cells were present.

Eosinophilic granular haemocytes seen in association with the hind-gut epithelium and integument, on the other hand were more common in the wild groups, and do not appear to be related to this process. Clear criteria for differentiation of moult-related movements into epithelium from inflammatory processes is necessary for diagnostic pathology assessment, as apparently well developed inflammatory reactions were also seen in this location, possibly as an extension from excessive fouling in a similar manner to gill and surface erosions. In the muscle, however, these appeared only where degeneration was present.

The roles of these cells, particularly the reserve inclusion cells appear poorly defined, while an appreciation of the known effects of moulting status is also required for other cells. There is recognised to be increased abundance of circulating haemocytes just after moulting, and a subsequent movement into the epithelium of the exoskeleton. Large numbers of haemocytes accumulate near the epidermis during ecdysis and early post-ecdysis and appear to release cytoplasmic constituents that harden the exoskeleton. Granulocytes reported predominate prior to ecdysis and contribute protein necessary for the formation of the new exoskeleton. Hyaline haemocytes are most abundant during post-ecdysis, when they are reported to release tanning substances. Following sclerotinisation, the number of haemocytes declines both in the haemolymph and in the epidermis.

Review papers by Martin and Hose (1992, 1995) and by Aiken and Waddey (1995), describe the inclusion cells as putative haemocyanin producing cells. These cells are rarely seen in the blood but may be present in haematopoietic tissue and in organs such as the heart. Earlier papers, such as Ghiretti-Magaldi et al, 1977, postulated that reserve inclusion cells derived from haemoblasts, migrate from the haematopoietic tissue while still undifferentiated, and complete their differentiation in the adjacent connective tissue. However, they were unable to find maturing reserve inclusion cells within the haematopoietic tissue of any decapod they studied. Other papers such as Johnson, 1980, suggest that reserve inclusion cells possess some phagocytic capabilities, although their main function was described as both producing and storing haemocyanin.

The role of these cells, and in particular a possible involvement in the moult process was raised when earlier studies, Diggles and Handlinger, 2004, indicated these cells varied from very low to very large numbers in young juveniles of this species. The current study concurs in that the number of these cells in highly variable, from undetectable to very abundant. Their possible secondary role in the moult process relates to the increase in haemolymph volume which rises from approximately 30% of the animals weight to 55% immediately after moulting, and then decreases as the animal grows. It is likely that this results is a considerable release of haemocyanin to return the levels of this to normal quite rapidly. However, Senkbeil and Wriston 1981, suggest that haemocyanin does not appear to function in osmoregulation since haemocyanin production is not enhanced when lobsters are placed into water of abnormally low salinity.

Review of the moulting process, such as that by Anger 2001, do not entirely resolve this process, as they tend to concentrate on changes in the integument. Changes in haemolymph are discussed, but the origin of such changes has not been emphasised.

Blood serum protein is regarded as typically increasing throughout the pre-moult stages, but this is described as a consequence of partial resorption of carticular protein and the level of haemocyanin is not discussed. Osmolarity of haemolymph is known to be minimal in early post-moult, and during pre-moult water losses may be reduced by lowering the rates of urination and ammonia excretion. This may result in increased metabolic vulnerability of the antennal gland at these times.

It is usual in controlled studies of haemocyte responses to exclude animals close to moulting because of these variations in haemocyte numbers, however this is of little value to the diagnostic studies where animals of any stage may be presented. (Even in the American lobster, *Hamarus americanus*, the role and identity of reserve cells is still not entirely resolved because the relationship to other cell types is still uncertain, thus species variations may be expected to influence the histological presentation of lobster responses). These studies would not be inconsistent with the reserve cells fluctuating with the moult cycle, although there's insufficient data on moult stage to verify this, however, their presence in moderate number appears to be a prerequisite for extensive coagulation of haemolymph and the significance of this is still uncertain. It is known that coagulation of haemolymph occurs in contact with seawater, and it is anticipated that it may be initiated by other tissue damage events. A similar pattern of coagulation of haemolymph has been seen with multiple types of insult in other invertebrates such as abalone (Handlinger, unpublished, and Mouton, pers com).

Clearly, a full understanding of these processes, and the boundaries of normal variation, will require further assessment. It is hoped that the data from this survey can add to that process.

6 RISK ASSESSMENT FOR RELEASE OF ONGROWN JUVENILES

Risk assessment is defined by the OIE Aquatic Animal Health Manual as the evaluation of the likelihood and the biological and economic consequences of entry, establishment, or spread of a *hazard* within the territory (*of concern*). The AQIS risk assessment handbook defines risk assessment is the process of estimating the risk presented by a hazard, in qualitative or quantitative terms. For the purpose of this IRA (which relates to deliberate release of animals), the risk of entry of a hazard known to be associated with these animals is 100%. The risk of establishment and spread depends inherently on whether the agent is already present in that area (i.e. established), as well as any risks of increased spread associated with a differential load in endemic and released populations.

The initial step of a risk assessment consists of hazard identification, which is the process of identifying the hazardous biological agents that could potentially be introduced with the commodity of concern. A hazard is defined as a biological agent which may have an adverse effect and risk as the integration of the likelihood of the occurrence and the magnitude of the consequences of an adverse event to animal or human health in the . (*recipient area*). Hazard identification is defined as a categorisation step, identifying biological agents dichotomously as potential hazards or not.

The first step in hazard identification, according to the AQIS Handbook, is to list the pests and diseases that have been identified as being potentially associated with the .. *goods*: in this case those pests and diseases which have been identified by this project's disease survey. The second task is to categorise these pests and diseases (in some cases in a preliminary manner) according to whether the evidence presents a *prima fascia* case that these represent a risk and as need to be considered in the subsequent risk assessment. Pests and diseases are to be categorised conservatively. Agents thus classified are termed 'potential hazards'.

6.1.1 Hazard identification for release of on-grown puerulus from these batches

Pests and disease agents identified, and their apparent distribution and severity are listed above in Tables 3 and 6. General pathological changes and indicators of overall health, which could reflect pathogens present but not identified in the survey, are summarised in Table 10.

The OIE Code states that to be identified as a potential hazard, a pathogenic agent should be appropriate to the animal species to be imported, or from which the commodity is derived, and could produce adverse consequences in the importing country (*i.e recipient area*). The pathogenic agent may be present in the exporting country (i.*e source population*), but if present in the importing country, should be associated with a notifiable disease, or should be subject to control or eradication measures. Clearly, the latter is not the case for any potential risks identified by this survey. While the rules for international trade should be a guide only to best practice management of local resources, an agent identified by this survey could only be regarded as a potential hazard to wild stocks if:

> 1) it was absent from an area, ***or*** present at a significantly higher level in farmed stocks;

and 2) had been demonstrated or suspected to produce adverse consequences in recipient populations.

None of the agents identified in Tables 3 and 6 meet the criteria of being present only in farmed stock, or at a significantly higher level in farmed animals.

Two parasites (goose barnacles and turbellarians) may be absent from some bioregions. (That is, they could fulfil criteria 1 for these regions). An assessment of pathology associated with these parasites does not indicate a significant effect of either parasite on the health of affected lobsters. (That is, criterion 2 is not fulfilled). Furthermore, the anticipated release from these batches was restricted to 2 on-shore batches, to areas within the same zone as the culture facility and the collection sites of the majority of the animals, where the status of these parasites was the same as the cultures groups.

Examination of Tables 3, 6 and 10 indicate one group of parasites (sessile peritrich protozoa on gills), and bacterial fouling generally, and in one culture group shell erosions, were at higher levels in some cultured stocks but widely distributed in wild stocks. Both types of fouling are a regarded as a complex of ubiquitous organisms widely distributed on a variety of surfaces in all environments and likely to reflect density of animals and water flow in the immediate environment. They are likely to dissipate to normal levels at the first moult after release. The level of shell erosion is likely to reflect the overall level of external fouling. As these bacterial and protozoan agents may attach to a variety of surfaces, the contribution of the fouling on released animals to the recipient environment would be minimal, that is, the agents are already established and criteria 1 is not met. Furthermore the pathology associated with these agents was minimal, and would not extend beyond the next moult in animals currently affected. Criterion 2 is not met.

Conclusion: The hazard identification process identified no potential hazards.

The OIE and AQIS guidelines indicate that the risk assessment may be concluded if hazard identification fails to identify potential hazards associated with the importation.

The risk assessment for these batches is therefore concluded.

6.1.2 Comparison of on-shore and sea-cage culture systems

Comparison of Tables 3, 6 and 10 and the more detailed results above indicate a much wider variation between batches within each culture system than between culture systems with regard to general indicators of health such as gill fouling and shell erosion. The indications are that animals may be kept in a satisfactory manner in either system, but that either system has the potential to provide a less than satisfactory environment, especially with prolonged culture. Diet also has the potential to impact on general health status, through alterations in immune competency. The potential for this to occur with puerulus culture was shown by dietary effects on circulating haemocyte levels. Thus further work to optimise the culture conditions for juvenile rock lobsters should include both system and dietary aspects, to minimise the likelihood of disease occurrence in culture.

6.2 General recommendations on pre-movement testing for southern rock lobsters

The specific concern leading to the development of this project related to the desire for developmental aquaculture based on wild puerulus collection be stock neutral. As wild

harvest is already limited for sustainability, and the potential to collect puerulus without affecting the wild population size at maturity or harvest, it was aimed to ensure this by return a proportion of on-grown farm stock to the wild after approximately one year of on-growing. The proportion intended to be returned from these culture batches was set at 10%, to ensure that the number to be reseeded would exceed the number estimated to have survived naturally. To achieve neutrality these animals needed to healthy, both to survive post-release and to ensure they did not compromise the health of other lobsters in the area of release. Cultured animals also need to be healthy to ensure adequate productivity and long-term commercial returns.

Release sites were generally chosen to be within the area of collection and / or culture, to minimise the possibility of translocation of disease agents. Nevertheless, there was recognition by all parties that only animals that were generally healthy and of at least the same health status with regard to infectious diseases should be returned to the wild. Although there was regarded as only a small risk of spread of disease by this process, the consequences of such spread are unknown. This survey was a standard, responsible action to avoid regional shift of diseases.

This study did not find risks were posed by release of ongrown animals from the specific batches examined. In any case changes to the program during the survey removed the requirement for release of animals from these batches in any case. Despite the low level of disease found in this survey, and the lack of demonstrable risk, the general question of disease risks from movement of *J. edwardsii* within its range still need to be addressed, given the limitations of the data from this survey. The limited number of sites sampled may not provide an adequate indication of health status in all areas. It is probable that agents affecting rock lobsters within Tasmania are common to all areas. Nevertheless, the recognition of specific bioregions, the possible restriction of some pathogens by micro-environments and the population based adaptation of invertebrates (as distinct from development of individual immunity), all support a precautionary approach to live lobster movements. It should also be recognised that movement of lobsters to holding facilities may also pose a risk of movement of diseases between areas, if animals, water or other contaminants are released from these facilities. It is therefore recommended that general health surveillance continue for all lobster populations subject to movements, and that any deliberate relocations to wild populations (including any future proposed re-release of culture juveniles) be subject to further testing commensurate with the magnitude of the movement.

This recommendation is made in recognition that the data from this survey remains limited and neither precludes regional disease shifts with future movement, nor fulfils international criteria to demonstrate freedom from diseases (OIE, 2005). However it does provides data that contributes to the ability to fulfil these criteria in the future.

Recent changes to the OIE recommendations for demonstration of freedom from specific diseases provides a realistic framework, even for wild populations, for achieving this status by accumulating data over time (up to 20 years), utilising a variety of data sources including field observations. However this data must meet certain criteria, including but not restricted to, the overall amount of data, and an effective and demonstrable early warning system for disease incursions and the recognition of new diseases. A necessary condition for an early warning system in the absence of on-going extensive disease surveys, is demonstrable overall health surveillance to a level which provides an assurance that if significant disease is present it will be brought to the attention of the responsible authorities. As well as providing for safe movement of specific batches tested, the recommended testing would contribute towards the

overall data required for recognition of freedom from specific diseases, and provide one essential component of an early warning system for a broad range of new diseases and major disease incursions. The other essential component is to ensure that an early warning system exists for lobster disease occurrence outside the context of lobster movements. This question if further considered below.

It is highly likely that risks recognised in the future will be diseases not currently recognised. Although comprehensive pathological examination, plus associated tests such as bacteriology and haemolymph examination where indicated, is currently the only practical means of assessing rock lobster health, this type of test program provides considerable long-term advantages over testing for specific disease agents (if and when these should become available). Comprehensive pathological examination provides a basis for retrospectively demonstrating whether newly discovered diseases were likely to have been present within the samples previously examined. This may be important where the range of diseases considered as having a potential local impact (either within Tasmania / Australia as a growing region, or by its markets), is considerably broader than those diseases recognised by OIE listing as representing an international risk. Such a situation has not been uncommon when Australia has considered the risks associated with other types of animal imports.

7 GENERAL DISCUSSION

Although collection based on large-scale puerulus collection did not proceed within the anticipated timeframe, this species is still regarded as a potential aquaculture species of the future. Whether or not aquaculture production will in the future be based on return of on-grown stocks is not known, but there is also a potential for release and / or relocation for stock enhancement, so these concerns regarding regional shifts of disease still apply.

It is typical of a wild harvest industry that only major disease outbreaks or chronic diseases are detected, especially if examination is restricted to the general health of animals meeting harvest criteria. Culture of aquatic animals is likely to unmask disease because of crowding and possibly stress, but also because closer observation. Knowledge of the more common diseases, as well as potentially major hazards, is essential to manage the health of stock in culture.

The rock lobster health issues addressed by this project therefore included those common to a mature wild harvest industry with little history of disease investigation as well as those common to new aquaculture species. There is, worldwide, an increasing emphasis on demonstrable knowledge of health status where this may impact on trade access. While the specific question of risk associated with management of re-release of juveniles from these ongrown animals have been addressed above, this section considers the long-term aspects of health surveillance for rock lobsters under this scenario, and in particular how best to maximise the benefits from this project.

That few diseases of lobsters are known confirms the aquaculture potential of this species, (with regard to robustness). This is in part a reflection that few lobster disease studies have been undertaken, as shown by the emergence in recent years of significant new diseases, such as herpes virus infection and the apparently non-infectious excretory calcinosis. It is likely that more diseases will emerge in the future, though in this survey the only previously unrecorded findings were minor parasites such as turbellarians and goose barnacles. Early

recognition of emerging diseases is essential to minimise their impacts, when they do occur. This is potentially far more important than the value of an early warning system to fulfil international criteria for demonstration of disease freedom. Early recognition requires both a submission pattern likely to ensure early cases are examined, and laboratory resources familiar with the current disease patterns to ensure changes are promptly recognised. It is essential, therefore, that any future aquaculture ventures, and preferably the wild fishery, participate in sufficient health monitoring to provide both the producers and the health regulators with sufficient information on health status to recognise change.

There was a long-term expectation that a down-stream outcome of the project would be an on-going part-industry funded surveillance program for aquaculture, similar to other industries. Base-line data, of both wild and cultured stock, is needed for such programs. While the rate of aquaculture development has slowed, it is essential that on-going health monitoring be maintained in all relevant sectors. As risks are shared between both sectors of the industry, ensuring that effective health monitoring includes the wild fishery in the interim would have long-term advantages to both industry sectors, such as supporting trade access and quarantine policy, even if no new diseases emerge here.

Long-term requirements to maximise benefit from disease surveillance programs

As well as the immediate benefits to producers and disease control agencies in limiting the immediate impact of diseases, disease monitoring and surveillance programs can provide data for recognition of freedom from specific diseases. Recent changes to OIE protocols for recognition of disease freedom (Anon, 2003), have introduced a great deal more flexibility in the type of surveillance activity that can be used to verify disease freedom. The general principles underlying this change are that the absence of infection over a long period of time in susceptible *populations* can be substantiated by effective disease investigation and reporting by the Competent Authority of the Member Country. A variety of data, from structured surveys such as this, routine laboratory investigations of possibly minor disease outbreaks, and health observation reporting by industries may contribute to this. Historical freedom or eradication can be accepted on this basis, provided that the *prescribed biosecurity conditions* have been in place continuously [in the country, zone or aquaculture establishment] for at least the previous 10 years. Underpinning all of these data sources is a general requirement to assess the level of **confidence in the surveillance system**, that is, the **likelihood that the system would detect the disease if it was present,** and that this can be substantiated by the Competent Authority of the Member Country.

Regardless of whether data is collected by a rigorous and expensive test program over at least 2 years, or at the other end of the scale by passive surveillance over a long period (e.g 25 years of apparent absence, backed up by 10 years of passive surveillance), there is a need to have effective biosecurity measures in place, which include an effective early detection system for diseases.

The prescribed biosecurity measures required to meet OIE conditions for disease freedom are defined by OIE as [*comments in italics mine*]:

> "A set of conditions applying to a particular disease or infection, and a particular zone or country, required to ensure adequate biosecurity, namely:
>
> a) the disease is legally notifiable to the Competent Authority;
> b) an early detection system is in place within the zone or country;
> c) no vaccination against the disease is carried out; [*Not relevant to rock lobsters*]

d) infection is not known to be established in wild populations;
e) import requirements to prevent the introduction of disease or infection into the country or zone, as outlined in the *Aquatic Code,* are in place. [*In general for Australia, yes*]"

Note for a) that specific status as "Notifiable" can only be put in place once specific disease risks are identified. To identify such new diseases, Tasmanian legislation requires that all new diseases are reportable, but there is a need to ensure practical means to translate this into practice for this industry. The exact requirements may vary between Australian States, though the underlying principals apply across Australia.

An early warning system implies that there are competent and effective personnel of the Competent Authority able to investigate, diagnose and report disease or infection, if present. The OIE definition for this is:

"an efficient system for ensuring the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease* situation, or unexplained mortality, in *aquatic animals* in an *aquaculture establishment* or in the wild, and the rapid communication of the event to the *Competent Authority,* with the aim to activate diagnostic investigation with minimal delay.

Such a system will include the following characteristics:
a) broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in *processing*, of the characteristic signs of the *listed diseases;*
b) veterinarians or aquatic animal health specialists trained in recognising and reporting suspicious disease occurrence;
c) ability of the *Competent Authority* to undertake rapid and effective disease investigation;
d) access by the *Competent Authority* to laboratories with the facilities for diagnosing and differentiating *listed* and *emerging diseases*."

The implication is that program activity needs to be sufficient to ensure ongoing adequate availability of trained specialists and adequate laboratory facilities, and an adequate, demonstrable level of awareness in personnel of aquaculture and wild harvest establishments. This survey has provided considerable information on lobster responses and general pathology, but continued laboratory submission is required to maintain and expand this knowledge in the face of the sometimes rapid turnover of the small pool of expertise available in any area (as the staff turnover during this project demonstrated), and to increase our understanding of lobster pathology. Regardless of the need to maintain specialist health expertise, maintaining an awareness of health status for rock lobsters within the industry must be regarded as a shared responsibility, as it is only the industry sectors that can provide the eyes and ears to raise disease issues and utilise this expertise.

Farm licence conditions could provide a framework for ensuring adequate awareness in this sector, by specifying the level of interaction required of this sector. Another essential component of achieving a framework capable of meeting this objective overall is increased interaction of the wild harvest and animal health services, preferably within a formal framework, to provide an effective and on-going means for the industry to become the "eyes" for lobster health surveillance. For this to have international validity (in terms of demonstration of the health of this industry for market purposes), both must be aware of health issues, be alert for apparent changes in lobster health, and have both a commitment to

investigation of any diseases seen, and an active and ongoing awareness of the mechanism to initiate this process. Even though the level of disease in this industry is low (and perhaps more so under these circumstances), there is long-term value in establishing an education process and a formal agreed and documented process to increase the industry role in health surveillance.

The recent changes in international criteria for health assessment recognises the value of documenting observations of the lack of disease as a valuable source of information for comparing animal health between regions, but also recognises that "the lack of data does not equate to lack of disease". It is essential that the framework exists to maintain industry awareness and ensure that any changes in the current low level of disease are speedily recognised and investigated.

8 BENEFITS

The major benefit of this work was to ensure that lobsters can be released from culture systems during these studies, and into the future without compromising the health of recipient wild populations, and with a health status that does not compromise their survival in the wild.

This work also increased our knowledge of the background pathology and parasites of the southern rock lobster *Jasus edwardsii,* as an aid to diagnostic and general health assessments of either cultured or wild stocks in the future. This information on background pathology can also be expected to benefit health monitoring for translocation operations.

This survey also increases the knowledge of the health status of *J. edwardsii* generally, and specifically with regard to Tasmanian stocks, data that can contribute towards a demonstration of freedom of these stocks from specific diseases, providing there is ongoing disease awareness and investigation and biosecurity measures are maintained.

9 FURTHER DEVELOPMENT

Although the rate of development of lobster aquaculture has declined, there is much interest in the translocation of wild lobsters to increase yield. The background information on pathogens obtained through this research, and the monitoring protocols, will be of value for these translocation operations.

10 PLANNED OUTCOMES

The risk of re-release of on-grown lobsters has been assessed, and this information has been provided to the Chief Veterinary Officer of Tasmania, to enable a decision to release animals as planned from the culture groups examined.

11 CONCLUSIONS

In *Jasus edwardsii* that had been collected from the wild as pueruli and on-grown in culture facilities for a minimum of six months, no diseases of concern with regard to translocation were identified which were not already present in wild stock.

The overall level of disease in both wild and cultured stocks was low. Gill parasites levels in culture were initially low, but increased with age and time in holding to levels approximating those in wild stocks. Gill parasites detected included turbellarians, which appear not previously to have been recorded from lobsters.

More gill fouling and background integument damage was present in current culture systems, but the overall effect on lobster health appeared slight, though results suggest this may predispose to transport stress. Individual batch history including the length of time in holding, rather than culture system, was the main determinant of this effect.

Significant unexplained pathology was rare but included zonal antennal gland degeneration, mainly in wild animals.

This study increased our understanding of disease processes and provided background data for future investigations. Apparent associations which may warrant follow up assessment include muscle damage as a component of post-transport pathology; an apparent association of muscle damage, intraepithelial globules of the antennal gland with the development of mild turgid muscle syndrome in one group; and the relationship of reserve inclusion cells numbers with coagulation of haemolymph at examination and with the moult cycle.

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13 APPENDIX 1 (Intellectual property)

No intellectual property issues associated with this project have been identified by either party.

14 APPENDIX 2 (Staff)

Handlinger, Judith - Principal Investigator
Carson, Jeremy - Co-investigator, microbiology
Caleb Gardner - Co-investigator, wild collections
Stephen Pyecroft - Co-investigator, pathology
Jane Sammons - Pathology
Taylor, David - Pathology (limited period)
Les Gabor - Pathology (limited period)
Bradley Chadwick - Pathology (limited period)
Richmond Loh - Pathology (limited role)
The late Barry Munday - project development
Dane Hayes - Technical support & electron microscopy
Mark Lleonart - Technical support
Belinda Williams - Technical support
Shane Fava - Specimen collection
Pip Cohen - Specimen collection
Sam Ibbott - Specimen collection

15 APPENDIX 3 (Raw Data)

Data base of all primary data and additional photographs defining scoring system to follow in electronic form.