Ballast Water Decision Support System (DSS) Service Level Agreement (SLA) – Part II

Hayes K. R., McEnnulty F. R., Gunasekera R. M., Patil J. G., Green M., Lawrence E., Barry S., Sliwa C., Migus S. and Sutton C.

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Final report for the Australian Government Department of Agriculture Fisheries and Forests

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EXECUTIVE SUMMARY

In January 2004, CSIRO Marine and Atmospheric Research (CMAR) entered into a second Service Level Agreement (SLA) with Australian Government Department of Agriculture Fisheries and Forests (AGDAFF). The aims of the second SLA were to complete tasks that were started but not finished in the first SLA, gather and analyse data to enhance the operational effectiveness of the ballast water Decision Support System (DSS) when applied to domestic vessel/route combinations, and support the development and implementation of the New National System for the Prevention and Management of Marine Pest Incursions. This is the final report of the second SLA project. This report details progress against the project objectives together with additional work tasks undertaken to support the DSS during 2005 and 2006.

The vast majority of the research effort funded under the second SLA has been directed towards the probability that the donor port is infected with a target species (Module A), the probability that a vessel becomes infected during ballast uptake (Module B), and the probability that the target species will complete its life-cycle in a recipient port (Module D). In the context of domestic ballast water management, where journey durations are short, these three modules will provide the best risk reduction returns for research investment.

During the course of this SLA we identified a number of discrepancies between the DSS port infection database (that supports Module A), the National Port Survey Database (NPSD) and other information sources. It is important that these discrepancies are eliminated prior to the implementation of the domestic ballast water DSS, and in this context we recommend that:

- *Carcinus maenas* be listed as present in Sydney and Port Botany, and that the presence or absence of *C. maenas* in Port Kembla and Port Stanvac be confirmed as soon as possible;

- *Crassostrea gigas* be listed as present in Hastings unless the absence of this species here can be verified by a systematic survey of Western port, and that the presence or absence of *C. gigas* in Thevenard, Port Augusta and Port Giles be confirmed as soon as reasonably practicable;

- the presence or absence of *Varicorbula gibba* in Burnie, Hastings and Esperance be confirmed as soon as possible.

Furthermore, if the DSS is used to manage the potential transfer of toxic strains of dinoflagellates between Australian ports we recommend that:

- *Alexandrium catenella* be listed as present in Geelong and Hastings, and that the status of all other ports that the DSS database currently records as infected with *A. catenella*, with the exception of Adelaide and Melbourne, be confirmed as soon as possible, together with the presence or absence of *A. catenella* in Sydney and Port Hacking;

- the origins of the native strains of *Alexandrium minutum* be confirmed and the implications of potentially managing toxic strains of native dinoflagellates within the DSS be given further consideration;

- *Gymnodinium catenatum* be listed as present in Portland, Port Campbell and Port Welshpool, the presence or absence of *G. catenatum* in Hastings be confirmed as soon as possible, and genetic methods to reliably distinguish between the two ecophenotypes of this species are developed and implemented to confirm the absence of tropical ecophenotypes in Australian ports; and,

- site specific information from the national shellfish monitoring program is gathered and used in the DSS port infection database.

The recent advent of genetic probes provides a much more sensitive and reliable mechanism to identify the presence/absence, or quantify the level, of gametes and larvae in the water column. The use of these types of probes, together with more traditional techniques, has provided a much more detailed picture of the planktonic duration of *Asterias amurensis* in the Derwent estuary and Port Phillip Bay. In the Derwent estuary gametes and larvae of *A. amurensis* are now known to be present in the water column from April through to January, whereas in Port Phillip Bay the planktonic duration appears to be two months shorter lasting from May to December. We recommend that the vessel infection database used to support Module B is updated to reflect this information.

The DSS vessel infection database currently records the plankton period of *Crassostrea gigas* to start in October and end in April. Field samples, together with the other information sources collected over the course of this SLA suggest that:

- in the Derwent estuary as a whole, *C. gigas* gametes and larvae may be present in the water column from the end of September through to the end of April, and possibly into May;

- at the site of ballast uptake in the Port of Hobart (Risdon) *C. gigas* gametes and larvae may be present in the water column from November to the end of March; and,

- in New South Wales, *C. gigas* gametes and larvae may be present in the water column from October through to March.

We recommend that the DSS vessel infection database for ports in NSW be amended to reflect this information, but remain unchanged for the Port of Hobart for the moment. We also recommend that all subsequent use of genetic probes to determine the plankton period of target species use real time probes that are capable of quantifying the amount of DNA in positive samples. The limited analysis of journey survival dynamics conducted during this SLA indicates that presence/absence probes are capable of detecting very low levels of gametes and/or larvae that are very unlikely to pose a bio-invasion risk when translocated by ballast water.

Module D of the ballast water risk assessment has been significantly improved during the course of this SLA. The resolution of this Module and the risk benefits that it returns, however, are determined by the proportion of the species life-cycle (the cut-off value) that is considered to pose a bio-invasion risk. The risk assessment approach, and data used to support this approach has been subject to a rigorous independent review. Furthermore, the potential latitudinal distribution of the target species in Australia, particularly for sub-tidal species, compares favourably with the known native and invaded range of the target species. We therefore recommend that management authorities adopt an 80% risk cut-off value for the purposes of the ballast water DSS, and thereby maximise the risk reduction benefits of

the new module. We also recommend that the suggestions made by the independent reviewer regarding the probability of donor port infection be included in an on-going research plan for the new national monitoring strategy.

Most of the issues identified in the independent peer review of Module D have been resolved. There are, however, a number of outstanding issues:

- there is a lack of life-stage specific data for *Varicorbula gibba* and *Sabella spallanzanii* – the conservative approach currently adopted for these species is leading to risk overestimates that could be rectified with additional data;

- the minimum temperature tolerance of *Musculista senhousia* appears to be too high and does not accord with the latitudinal limits of its native and invaded range; and,

- the residuals of the Broome time series model shows periodic autocorrelation at low time lags, suggesting that this model could be improved slightly.

The next SLA will address and improve the Broome time series model prior to the implementation of the ballast water risk tables. We also propose to check for any new literature regarding the life-stage specific temperature tolerances of *Musculista senhousia*, *Varicorbula gibba* and *Sabella spallazanii*. It is important to note, however, that CMAR has conducted extensive literature reviews during the course the last two SLA's and we are very unlikely to discover significant new sources of information for these two species in the literature. We therefore recommend that national and state authorities gather additional tolerance information for these species using experimental approaches.

1. INTRODUCTION

1.1 Background

In 2000, CSIRO Marine and Atmospheric Research (CMAR) developed a quantitative risk assessment framework for ballast water introductions that addresses four steps in the ballast water invasion chain: donor port infection, vessel infection, journey survival and survival in the recipient port (the assessment endpoint). In July 2001 the risk assessment was implemented within a nation-wide ballast water Decision Support System (DSS) applied to all international vessels intending to discharge ballast water in Australia. Initially only two of the four risk assessment modules (donor port infection and survival in the recipient port) were implemented.

In January 2002 CMAR entered into a 12 month collaborative Service Level Agreement (SLA) with the Australian Government Department of Agriculture, Fisheries and Forestry (AGDAFF). The aim of the SLA was to provide technical and scientific advice in relation to the ballast-water DSS to assist its ongoing operation and increase its operational effectiveness. Over the course of this project CMAR staff completed 16 out of the 21 objectives initially set for the project, including the collection and analysis of Lloyds Shipping Data and high quality temperature time series data for twenty ports around Australia (Hayes and Sliwa, 2003).

In January 2004, CMAR entered into a second SLA with AGDAFF that aims to: a) continue the secure level of collaboration achieved between CMAR and AGDAFF in the first SLA; b) complete tasks that were started but not finished in the first SLA; c) strategically gather and analyse data in order to enhance the operational effectiveness of the ballast water DSS when applied to domestic vessel/route combinations; and, d) support the development and implementation of the new national system for the prevention and management of marine pest incursions.

This is the final report of the second SLA project. This report details progress against the relevant objectives together with additional work tasks undertaken (at the request of AGDAFF) to support the DSS during 2005 and 2006. It follows on from the completion of the second interim report in December 2004 (Hayes *et al.*, 2004a) and the third interim report in April 2006 (McEnnulty *et al.*, 2006).

1.2 Objectives and milestones

Table 1 lists the objectives of the second DSS SLA, together with the project milestones, their final status and a summary discussion regarding their status.

Table 1 Progress against objectives of the second DSS SLA and final status of project milestones

Ref	Objective	Milestone	Final status and notes
1	Time series models for 4 out of 20 ports with suitable data	Completion of all outstanding recipient port modelling	Completed: an outstanding item from the first DSS SLA (see Sections 2 and 3 of this report)
2	Analysis and "tidy-up" of temperature and/or salinity data for 6 out of 29 ports for which CMAR currently hold data	Completion of outstanding data analysis from first SLA and review of target species	Completed: an outstanding item from the first DSS SLA
3	Compile world ports list based on AGDAFF and Lloyds Maritime Intelligence Unit data with associated bioregion information	Completion of outstanding data analysis from first SLA and review of target species	Incomplete: a world ports list containing 8232 ports has been compiled but bioregion information and UN codes are missing for some ports. Agreed that this was low priority needing no further action.
4	Identify and rectify errors and omissions in the DSS databases maintained by AGDAFF and CMAR such as the port infection database.	Correct all errors in current port infection status data and complete risk assessment tables	Completed: CMAR has amended the port infection template, and updated port and vessel infection information. Latest data for *Asterias amurensis* and *Crassostrea gigas*, and outstanding issues associated with dinoflagellates, are identified in this report (see Sections 5 and 6 of this report)
5	New recipient port survival models for all target species for all ports which CMAR hold suitable data	Completion of all outstanding recipient port modelling	Completed for all sub-tidal species. On going for inter-tidal species such as *Crassostrea gigas*. Temperature and salinity tolerance data for all species has been checked, reviewed and updated (see Section 3 of this report).
6	New table based approach to the risk assessment within the DSS	Provision of final report	Completed: see Section 3.3 of this report
7	Gather additional data on life-cycle and reproductive behaviour for species/location combinations that lead to high risk assignations on domestic voyages due to current data deficiencies	Update of risk assessment tables for Modules B and C based on existing and newly collected information	Completed for *Crassostrea gigas* in Hobart (see Section 5 of this report). Sampling at Port of Burnie deferred due to logistical constraints and staff availability.
8	Gather additional information on journey survival on vessel/route combinations that lead to high risk assignations on domestic voyages due to current data deficiencies	Update of risk assessment tables for Modules B and C based on existing and newly collected information	Incomplete: Ballast water sampling to test journey survival of *Asterias amurensis* conducted in September 2005, additional trips planned for winter 2006 but not progressed as deemed low priority by AGDAFF. See Section 4 of this report

Table 1 cont…

Ref	Objective	Milestone	Final status and notes
9	In conjunction with BRS personnel, develop new vessel infection and journey survival models to reduce the incidence of Type I errors on domestic voyages	Update of risk assessment tables for Modules B and C based on existing and newly collected information	On-going
10	In conjunction with BRS personnel, investigate residual risk assessment strategies such as CLIMEX, or equivalent approaches based on global sea surface temperature data sets, prior to developing and/or recommending an approach for application within the DSS	Development and implementation of residual risk assessment algorithms	Incomplete
11	Review the status of all introduced and cryptogenic species known to exist in Australia, compare this with the those species currently managed by the DSS and recommend a new target list for coastal translocation risk assessment		Completed: see Section 7.2 of this report together with Hayes *et al.*, (2005a)
12	Assist in the development of emergency management and national control plan criteria for new and existing NIS in Australian coastal waters		Complete: CMAR personnel participated in: a) beneficiaries workshop; b) CCIMPE taxonomy workshop; c) NSW pest workshop; d) risk tables workshop; and, e) two technical workshops to develop guidelines for marine pest monitoring
13	Participate in NIMPCG meetings and contribute as appropriate to NIMPCG/AGDAFF requests relevant to the development and implementation of the national system		Completed: CMAR personnel participated in NIMPCG meetings 9 – 15, 17 and 19 – 21, together with various CCIMPE meetings, and have responded to all information requests.

2. RISK ASSESSMENT MODEL

2.1 Background

The DSS ballast water risk assessment comprises of four modules, A, B, C and D, that each address respectively: the probability that the donor port is infected with a target species; the probability that a vessel is infected whilst ballasting given that the port is infected; the probability that the target species survives the vessel's journey given that the vessel is infected; and, the probability that the species will complete its life-cycle in the recipient port given that it survives the journey and is discharged into the port (Hayes and Hewitt, 2000; 2001). Currently each of these events are treated as independent random variates. The overall ballast water risk is therefore taken as the product of these probabilities

$$\text{Risk} = P(A).P(B).P(C).P(D) \quad [1]$$

Currently three of the four modules are being implemented in the risk assessment – Module C (probability of journey survival) is not used. All species are simply assumed to survive all vessel journeys. This is a conservative approach.

The risk assessment was originally implemented in "real time" but for domestic vessels the preferred approach is a table-based one wherein the risk associated with all routes between Australian ports is assessed and summarised in a series of look-up tables that are periodically updated (Hayes and Sliwa, 2003). In November 2005 the risk assessment approach was subject to independent peer review.

2.2 Response to peer review

The independent review of the ballast water risk assessment raised a number of important issues, most notably:

- the potentially overconservative approach to the probability that the donor port is infected;
- potential dependence between the random variates in Module D and the overall risk calculation, and the sensitivity of the model results to the assumed distributional forms of these variates;
- potential for spatial variation in the temperature profiles of ports; and,
- the assumption that the residual temperature variation in the Module's time series models is approximately normally distributed.

We accept that the current approach to the probability of infection is overconservative for ports which have not been surveyed, but note that for ports which have been surveyed, and found to be free of target species, the assessment may underestimate risk due to the low sensitivity of the baseline port surveys (Hayes *et al.,* 2005b). The effects of this are readily apparent in the results

of the Hastings project (Patil *et al.*, 2004). The reviewer makes a number of very sensible suggestions in this regard which, importantly, could make an important contribution to the development of the new national monitoring strategy. We recommend that these suggestions form part of an on-going work plan for the continued development and improvement of the monitoring strategy.

We accept that Module D currently treats all random variates as independent, and that in reality, some of these variates may in fact be dependant. We propose to explore the effects of potential dependence, and the effect of the assumed distributional form of random variates, over the course of the next SLA. We suspect, however, that the dependence between variates will have only a small influence on the results because the overall proportion of a species life-cycle that is completed in a new location is usually determined by a single (most sensitive) life-stage. Dependence relations between different life-stage variates (e.g. duration or temperature tolerance) is unlikely to have a compounding effect on the overall result. Nonetheless we agree with the reviewer that this should be explored. The effect of different assumed distributional forms for the random variates is likely to have more effect on the overall results of the model. Here, however, we are data constrained for most species. We propose therefore to explore the effects of these assumptions, in the first instance, on a species such as *Asterias amurensis*, for which there is relatively good data.

The potential for micro-environments and spatial variation of temperature in ports was initially raised by Hayes and Hewitt (2001) who proposed dividing ports into homogenous sub-units. With the exception of sub-tidal versus inter-tidal environments (see comments on *Crassostrea gigas* in section 3.2.4), however, we simply do not have enough data to explore this issue. We do not therefore propose to address this further at this stage.

All of the residuals associated with the time series models were tested for normality when the models were originally developed, but only by visual observation. Chatfield (1996) notes a number of statistical tests to detect departures from normality but warns of their low power properties and recommends that the analyst simply examine a time series plot and correlogram of the residuals. More particularly, residual autocorrelation coefficients (r_k) which lie outside the range of $\pm 2/\sqrt{N}$ are significantly different from zero at the 5% level and provide evidence that the time series model may be wrong. The time series model should not be rejected, however, if only a few values of r_k are just significant, at lags which have no obvious meaning (Chatfield, 1996).

The time series plots and correlograms of the residuals from the time series models fitted to the temperature data sets used in Module D are plotted in Appendix A. Some of the residual correlation coefficients lie slightly outside the limits suggested by Chatfield (1996), but, with the exception of Broome, none exhibit any obvious pattern of departure. The Broome correlogram shows periodicity and significant residual departure up to a lag of about 50 days. This suggests that the Broome time series model could be improved. A very small proportion of the model residuals for Burnie, Port Stanvac and Spring Bay show some evidence of seasonal periodicity, suggesting that the time series model may be underestimating the summer temperature extremes. It is important to note, however, that these residuals represent tens of observations over a time series of several thousand observations, and will not therefore have any appreciable effect on the results of the life-cycle completion analysis. Overall, with the possible exception of Broome, all of the time series models are sufficiently accurate for the purposes of the life-cycle completion analysis.

3. MODULE D – LIFE-CYCLE MODELLING

3.1 Background

In August 2002, CMAR began to develop a new model to calculate the probability that a non-native species will survive in a new location (Hayes and McEnnulty, 2002). The modelling approach is still evolving but with the assistance of the Bureau of Rural Sciences (BRS) it has now reached a point where it is capable of providing a much more accurate assessment of the probability of survival in the recipient port (Hayes *et al.*, 2004a). The new model uses information on the duration and temperature tolerance of each life-stage of a species, compares this to the predicted daily temperature range in the new location, and thereby calculates the proportion of the species life-cycle that can be completed in this location. It is important to note that a species must complete all of its life-cycle in order to survive and establish in a new location. The collation, analysis and summary of life-cycle information, for each of the target species, has been an important component of this SLA. In October 2005 the data collected to support the new model was subject to peer review, together with a graphical summary of the new model results for each species. A total of ten species-specific reviews were received covering all the target species except *Alexandrium catenella* and *Alexandrium tamerense.*

3.2 Species specific life-cycle models

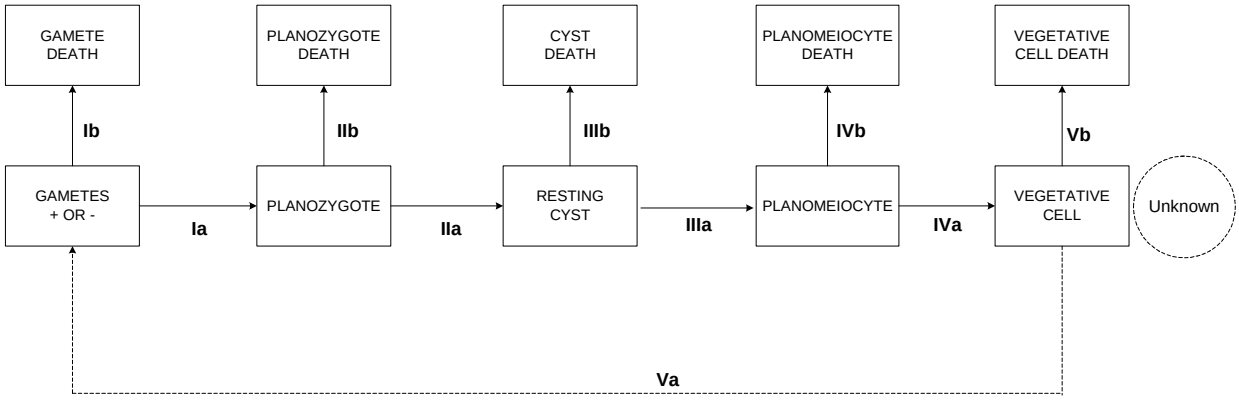
3.2.1 Alexandrium minutum

The independent review of *Alexandrium minutum* provides information on the duration of the three of the species' life-stages transitions and notes that *A. minutum* occurs in Australia as two different genotypes: a (likely) non-native genotype present in the Swan River and Adelaide; and a (likely) native genotype present in Newcastle, Shoalhaven and Port Phillip Bay. The reviewer notes that this information is published in a conference proceeding (de Salas *et al.*, 2001).

We accept the reviewer's suggestions for the three life-stage durations but note that temperature tolerance information is still missing for some components of *Alexandrium minutum's* life-cycle (Figure 1). The possible existence of two genotypes of *A. minutum* in Australia, one native, the other non-native, has important implications for the ballast water risk assessment. The risk assessment does not currently distinguish *A. minutum* genotypes, treating all records of this species as non-native (see section 6.2.2). The National Introduced Marine Pest Information System (NIMPIS) records this species as cryptogenic across its entire native range (Figure 2).

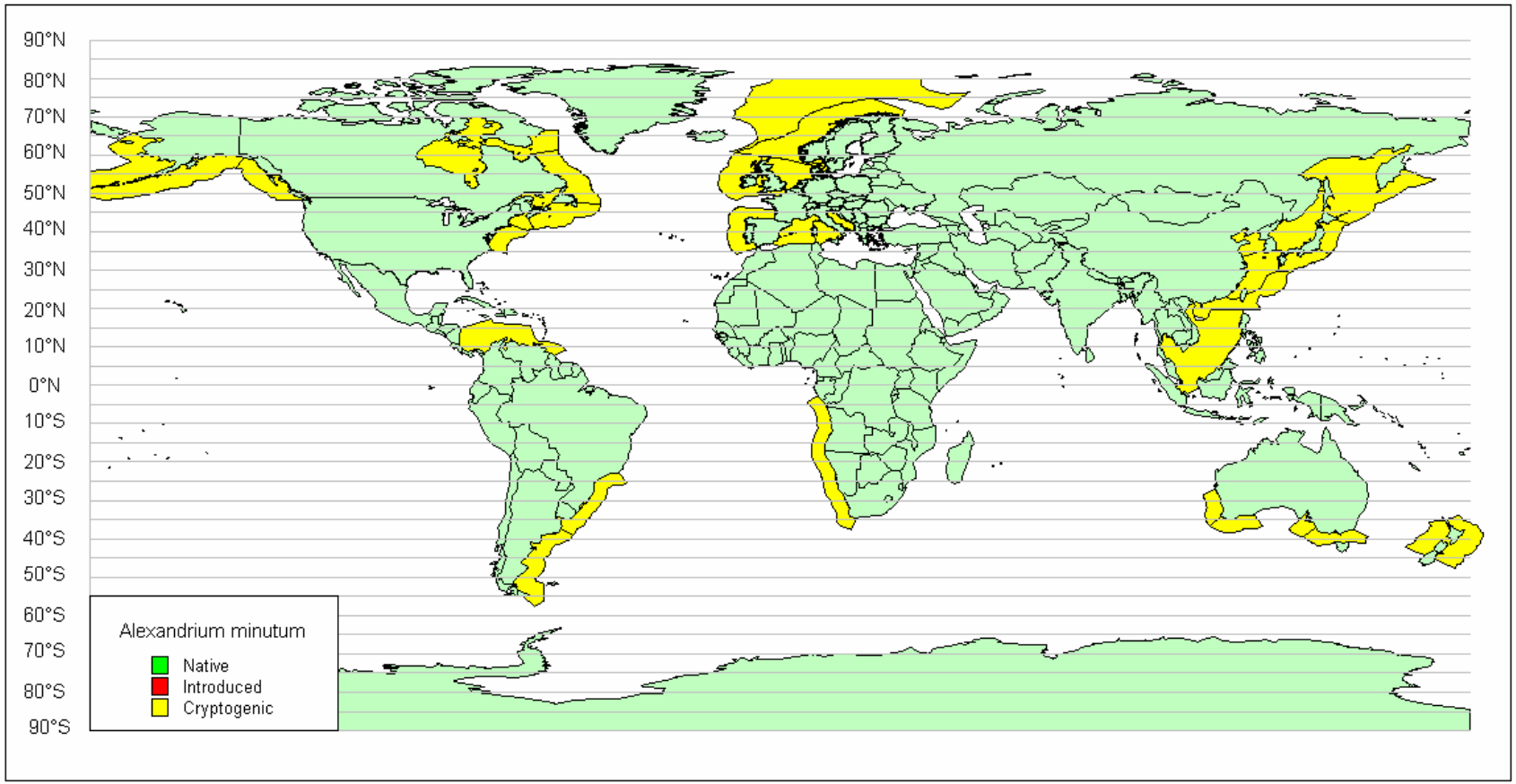
The changes suggested by the reviewer have important implications for the ballast water risk assessment. If the DSS were to change the infection status of Newcastle and all ports in Port Phillip Bay from infected to uninfected, then ballast water from all of these ports would be deemed low risk for this species. It is important that changes such as these are supported by scientifically defensible evidence and agreed to by NIMPCG. We recommend that NIMPCG consider this issue further prior to any changes to NIMPIS and the DSS (see also Section 6.2.2).

Figure 1 Amended life-cycle model for *Alexandrium minutum*



Transition	Duration	Model	Temperature range	Temperature model
Ia	2 days	2 days	Unknown	Min = ??, Max = ??
Ib	Few hours	<1 day	Unknown	Min < T < Max
IIa	5 days	5 days	Unknown	Min = ??, Max = ??
IIb	Few hours	<1 day	Unknown	Min > T > Max
IIIa	4 weeks	Uniform [21, 35]	4 to 38 deg C	Min ~ Uniform [4, 6]; Max ~ Uniform [36, 38]
IIIb	Few hours	<1 day	4 > T > 38	Min > T > Max
IVa	Unknown	1 day	no data	NA
IVb	Few hours	< 1 day	Unknown	Min > T > Max
Va	Unknown	1 day	10 > T > 30 deg C	Min ~ Uniform [10, 12]; Max ~ Uniform [28, 30]
Vb	Few hours	< 1 day	10 > T > 30	Min > T > Max

Figure 2 The IUCN bioregion distribution of *Alexandrium minutum*



3.2.2 *Asterias amurensis*

Two independent reviews of the *Asterias amurensis* life-cycle model were completed. One reviewer also commented on the potential distribution of *A. amurensis* in Australia as predicted by Module D, noting that the predicted distribution of *A. amurensis* in Australia was consistent with his understanding of this species' temperature tolerances. Both reviewers identified a number of important issues with the life-cycle model, most notably:

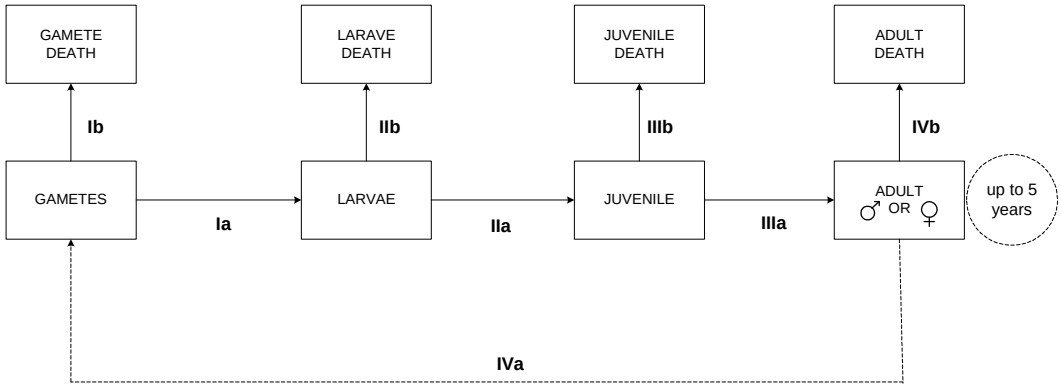
- the onset and cessation of the planktonic period;
- the duration of the juvenile period; and,
- maximum temperature tolerance of larvae.

In the original model the onset and cessation of the *Asterias amurensis* larval period is recorded as May to November inclusive. Both reviewers questioned these limits, suggesting that May could be too early and that the larval period could extend into December (respectively). Since the development of the original model CMAR staff have collected additional field data on the presence/absence of *A. amurensis* larvae in the Derwent estuary, for example by undertaking the empirical validation projects (Hayes *et al.*, 2004b; 2007). This field work suggests that, at least in the Derwent estuary, *A. amurensis* larvae may be present in the plankton from April to January, leaving only a two month window when larvae are not in the water column. This data is further supported by unpublished data collated by the Victorian Government Department of Sustainability and Environment (Hough and Dommisse, 2004). We recommend that Module B is amended to reflect these results for the Port of Hobart, whilst retaining the original data (May to December) for Port Phillip Bay (but see also Section 5.2).

The juvenile duration in the original model was represented as a uniform distribution on the range 110 to 240 days. One of the reviewers, however, provided extensive (published and unpublished) data and recommended a much longer juvenile duration. We accept these recommendations and subsequently changed the juvenile duration to a uniformly distributed variable on the range 270 to 730 days (Figure 3 and Figure 4). We suggest, however, that in areas with abundant food supply, juvenile growth can be slightly faster (*pers comm.* C. Sutton, CMAR, 23.05.06*)*, hence the model may be a little conservative in this regard.

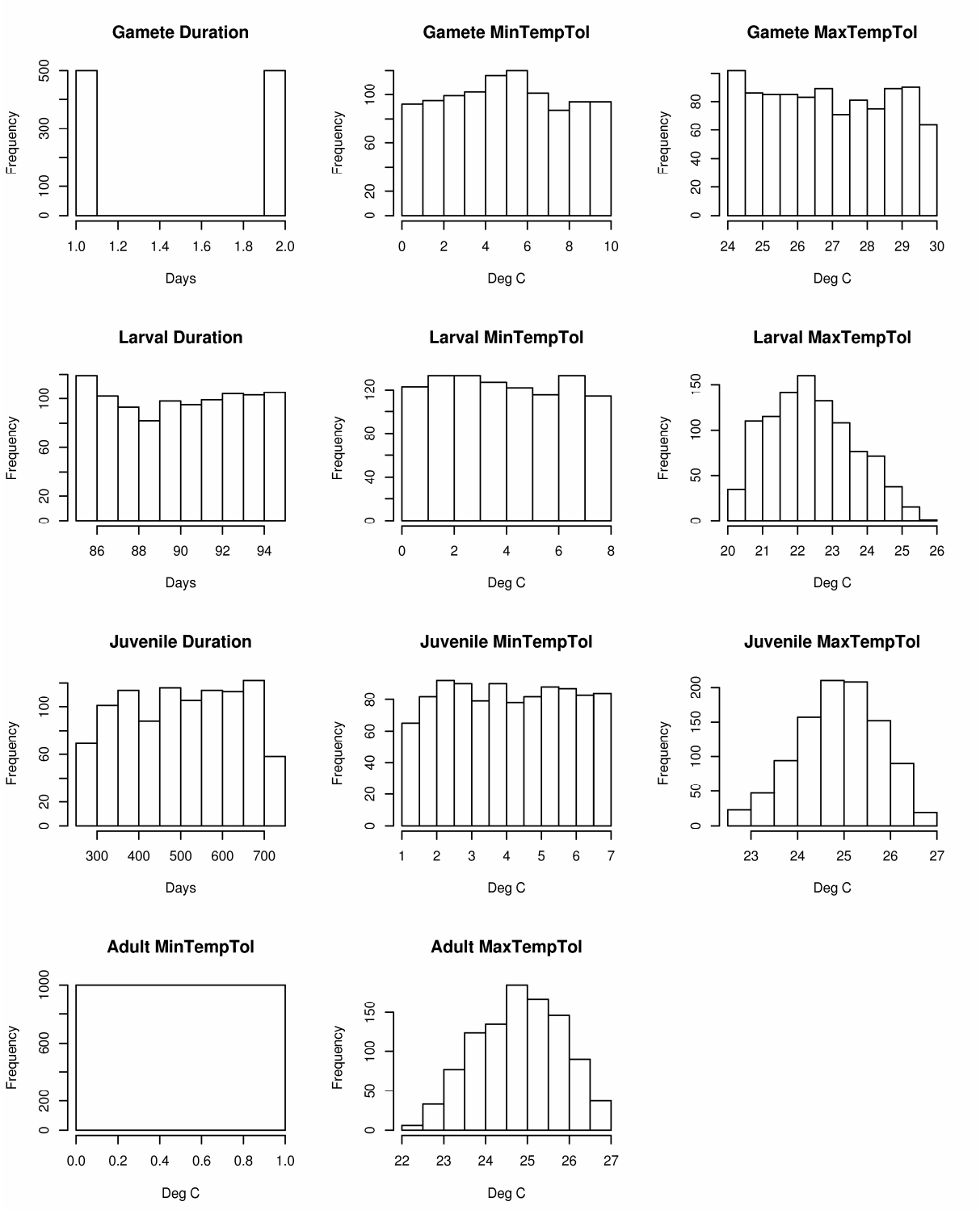
In the original model, the maximum temperature tolerance of *Asterias amurensis* larvae was represented as a Pert distribution on the range 22 – 27°C with a mode of 25°C (and a weight parameter of 3). One of the reviewers questioned the accuracy of the references from which these limits were derived, suggesting that the experimentally derived limits of Sutton and Bruce (1996) are more reliable. We accept that experimentally derived tolerance information can be more reliable than field-based observations but note that experimentally derived tolerance limits can also be confounded by a number of factors such as acclimatisation and nutritional status. We have checked and (where necessary) altered our interpretation of values in the literature and subsequently reduced the maximum larval temperature tolerance to a Pert distribution on the range 20 – 26°C with a mode of 22°C (Figure 3 and Figure 4).

Figure 3 Amended life-cycle model for *Asterias amurensis*



Transition	Duration	Model	Temperature range	Temperature model
Ia	1 to 3 days	Uniform [1, 3]	0 to 30 deg C	Min ~ Uniform [0, 10]; Max ~ Uniform [24, 30]
Ib	Few hours	<1 day	0 > T > 30 deg C	Min > T > Max
IIa	D = f(T)	D = exp(-0.11T + 5.68)	0 to 26 deg C	Min ~ Uniform [0, 2]; Max ~ Pert [20, 22, 26]
IIb	Few hours	<1 day	0 > T > 26 deg C	Min > T > Max
IIIa	270 to 730 days	Uniform[270, 730]	1 to 27 deg C	Min ~ Uniform[1, 7]; Max ~ Pert[22, 25, 27]
IIIb	Few hours	<1 day	1 > T > 27 deg C	Min > T > Max
IVa	Few hours	< 1 day	5 to 23 deg C	Min = 5; Max = 23
IVb	Few hours	< 1 day	1 > T > 27	As for IIIb

Figure 4 Amended life-cycle variates of *Asterias amurensis* based on 1000 simulations of the life-cycle model



The effect of the changes made to the duration of the juvenile life-stage and temperature tolerance of the larval life-stage are summarised in Table 2. The overall effect is a modest reduction in the proportion of the life-cycle that is completed in sub-tropical and warm temperate regions of Australia, that tends to reach a maximum in the winter months. A very slight rise is recorded in Port Kembla (February and March) and Thevenard (December) but this is well within the random difference that could occur between the two models based simply on a difference between two stochastic simulations.

The IUCN bioregion distribution of *Asterias amurensis* is summarised in Figure 5. The native range of *A. amurensis* is thought to extend south to approximately 25^0N. It is important to note, however, the limitations associated with the resolution of the IUCN bioregions in this context. There are, however, several reliable observation of *A. amurensis* populations in locations south of 35^0N – for example Nagasaki and the Ariake Sea (Nojima *et al.*, 1986). This southward extent of the *A. amurensis* native ranges compares favourably with the predicted potential distribution of *A. amurensis* in Australia based on the amended life-cycle model (Figure 6). The amended life-cycle model suggests that 30 – 35^0S is the furthest extent North in Australia that *A. amurensis* will be able to complete a significant proportion of its life-cycle ($\geq$ 80%). The model results therefore suggest that the potential distribution of *A. amurensis* in the Southern hemisphere is likely to mirror its distribution in the Northern hemisphere.

3.2.3 Carcinus maenas

The independent review of the life-cycle data and potential distribution of *Carcinus maenas* concluded that the life-cycle data is correct and an accurate reflection of the current literature, and that the distribution of *C. maenas* in Australia, as predicted by the life-cycle model, is supported by the literature and field surveys. Consequently no changes have been made to the life-cycle model. Figure 7 shows the (unamended) model, whilst Figure 8 illustrates the results of the simulations of the life-stage variables used in the port survival module.

The IUCN bioregion distribution of *Carcinus maenas*, and its predicted potential distribution in Australia (based on temperature tolerance alone) are summarised in Figure 9 and Figure 10 respectively. Again it is important to note the scale of the IUCN bioregions when comparing these figures, and unfortunately the problems associated with this scale are particularly prominent in this example. The south-east African distribution of *C. maenas*, for example, is based on a single record from Madagascar (Christiansen, 1969 cited in Le Roux *et al.*, 1990). The relevant IUCN bioregion (EA-III), however, spans almost 30^0 of latitude, and extends well into the tropics. Similarly, the south-west African record is from the Cape Peninsula, South Africa (Le Roux *et al.*, 1990) but the relevant bioregion (WA-IV) extends from 35^0S to almost 5^0S. The north-west African record is from Morocco and the northern border of Mauritania (Carlton and Cohen, 2003) but the relevant bioregion (WA-I) starts at 35^0N and extends 30^0 of latitude south. The recorded occurrence of *C. maenas* in the tropical East and West coasts of central America and Brazil (IUCN bioregions CAR-III, SEP-H and SA-III) date back to 1968 (Crothers, 1968) and 1930 (Ruthbun, 1930 cited in Le Roux *et al.*, 1990). In these cases, however, the population status of these records is unknown – i.e. it is unclear whether these records represent established populations. These records are also inconsistent with the recorded range of *C. maenas* elsewhere in the world and are therefore likely to be incorrect (*pers comm.* R. Thresher, CMAR, 22.05.06).

Table 2 Difference between the original and the amended proportion of *Asterias amurensis* life-cycle predicted to be completed in 20 Australian ports by month of introduction

Port name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Abbot Point	0.00	0.00	0.00	0.00	0.01	0.06	0.06	0.03	0.01	0.00	0.00	0.00
Brisbane	0.00	0.00	0.05	0.29	0.37	0.31	0.21	0.12	0.06	0.02	0.00	0.00
Broome	0.00	0.00	0.00	0.00	0.00	0.04	0.06	0.04	0.01	0.01	0.00	0.00
Burnie	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cape Flattery	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Dampier	0.00	0.00	0.00	0.00	0.01	0.06	0.07	0.04	0.01	0.00	0.00	0.00
Darwin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Esperance	0.00	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.07
GrooteEylandt	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Haypoint	0.00	0.00	0.00	0.00	0.13	0.17	0.11	0.06	0.02	0.00	0.00	0.00
Hobart	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lucinda	0.00	0.00	0.00	0.00	0.00	0.04	0.05	0.03	0.00	0.00	0.00	0.00
Mourilyan	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Port Kembla	0.00	-0.04	-0.01	0.03	0.15	0.17	0.15	0.15	0.14	0.11	0.07	0.03
Portland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Port Stanvac	0.03	0.01	0.09	0.07	0.11	0.12	0.13	0.12	0.12	0.12	0.11	0.11
Spring Bay	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Thevenard	0.06	0.06	0.05	0.06	0.07	0.06	0.06	0.06	0.05	0.05	0.04	-0.01
Thursday Is.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Townsville	0.00	0.00	0.00	0.00	0.00	0.09	0.14	0.09	0.05	0.01	0.00	0.00

Figure 5 The IUCN bioregion distribution of *Asterias amurensis*

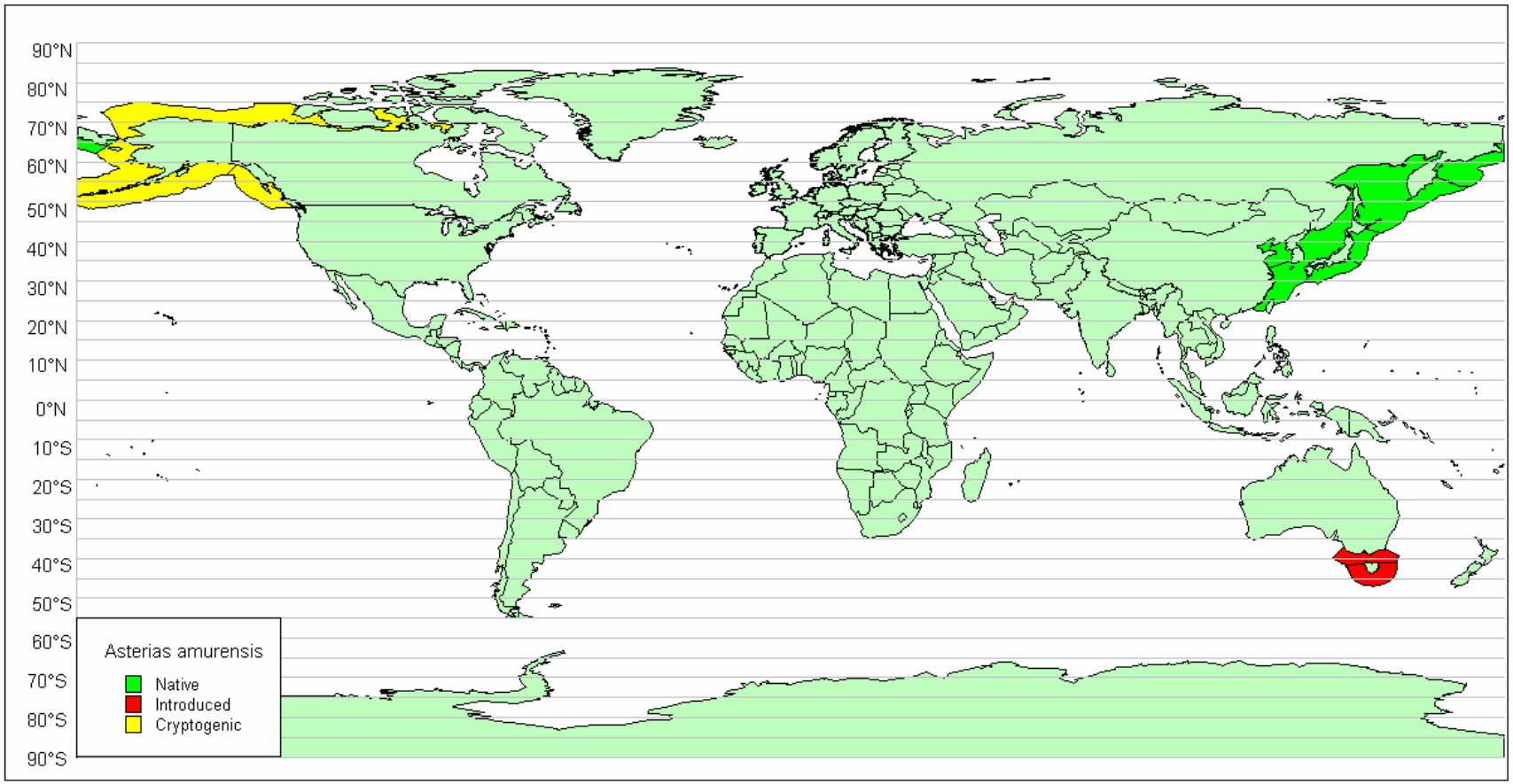


Figure 6 Predicted proportion of *Asterias amurensis* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)

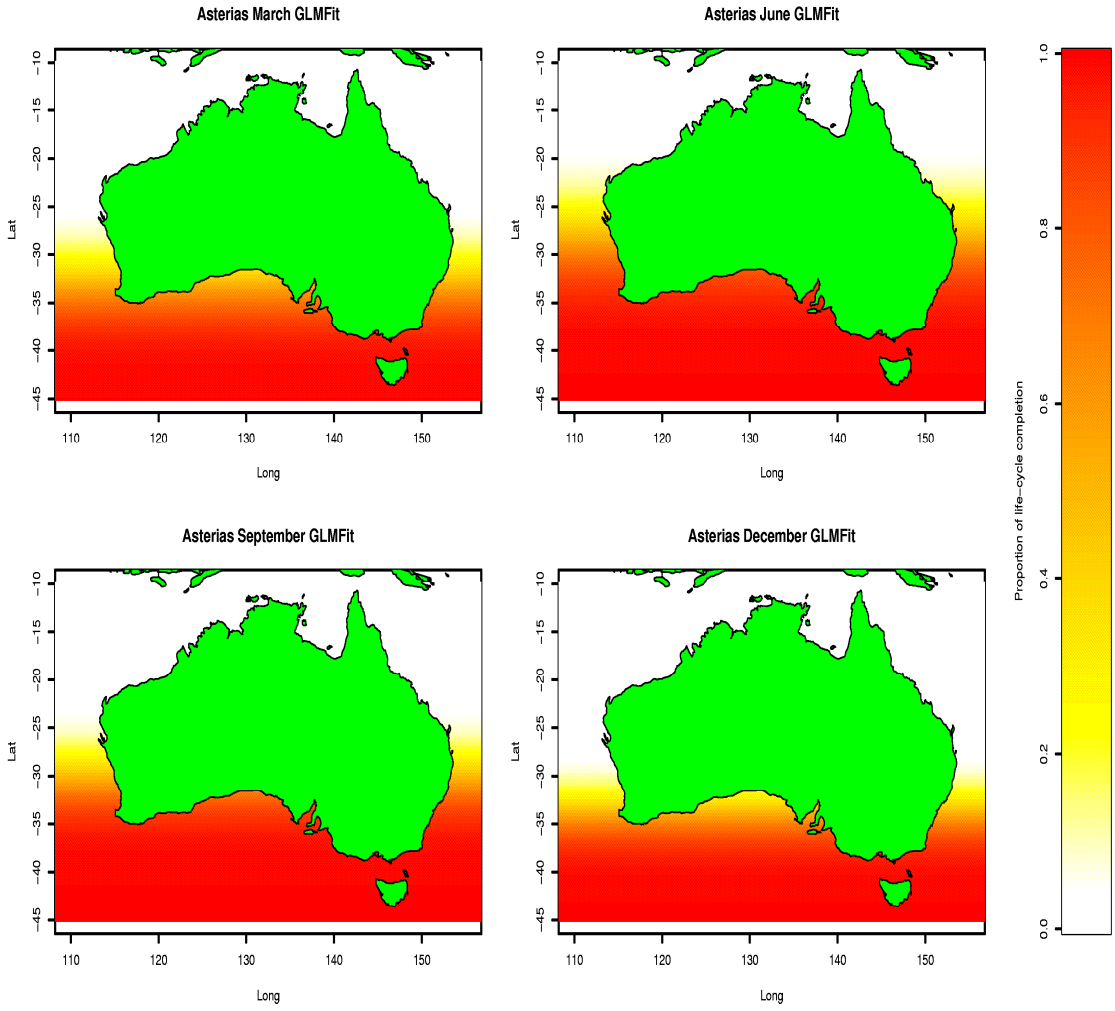
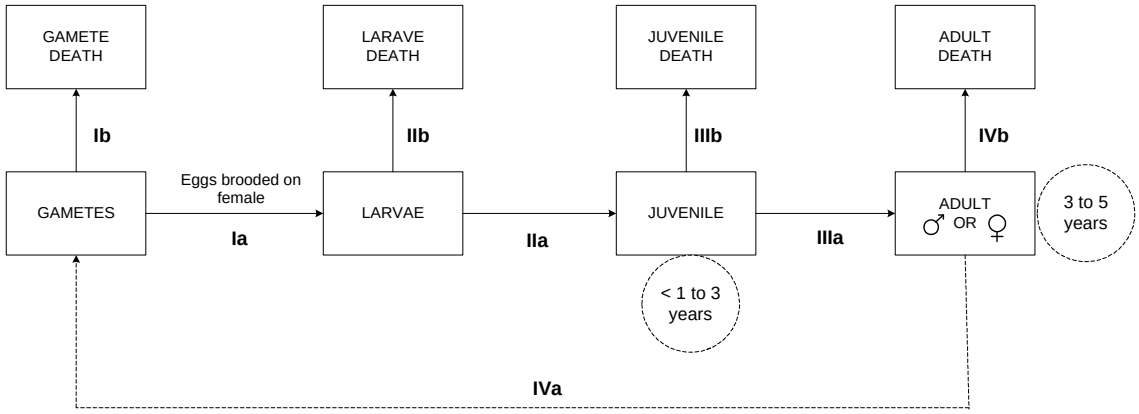


Figure 7 Unamended life-cycle model for *Carcinus maenas*



Transition	Duration	Model	Temperature range	Temperature model
Ia	21 to 133 days	Uniform [21,133]	1 to 26 deg C	Min ~ Uniform [1, 3]; Max ~ Uniform [23, 26]
Ib	Few hours	<1 day	1 > T > 26 deg C	Min > T > Max
IIa	36 to 68 days	Uniform [36, 68]	6 to 26 deg C	Min ~ Uniform [6, 10]; Max ~ Uniform [23, 26]
IIb	Few hours	<1 day	6 > T > 26 deg C	Min > T > Max
IIIa	< 1 to 3 years	Uniform [300, 1095]	-1 to 36 deg C	Min ~ Uniform [-1, 6]; Max ~ Uniform [30, 36]*
IIIb	Few hours	<1 day	-1 > T > 36 deg C	Min > T > Max
IVa	Few hours	< 1 day	3 to 26 deg C	3 < T < 26
IVb	Few hours	< 1 day	-1 > T > 36 deg C	As for IIIb

*Inferred from adult data

Figure 8 Unamended life-cycle variates of *Carcinus maenas* based on 1000 simulations of the life-cycle model

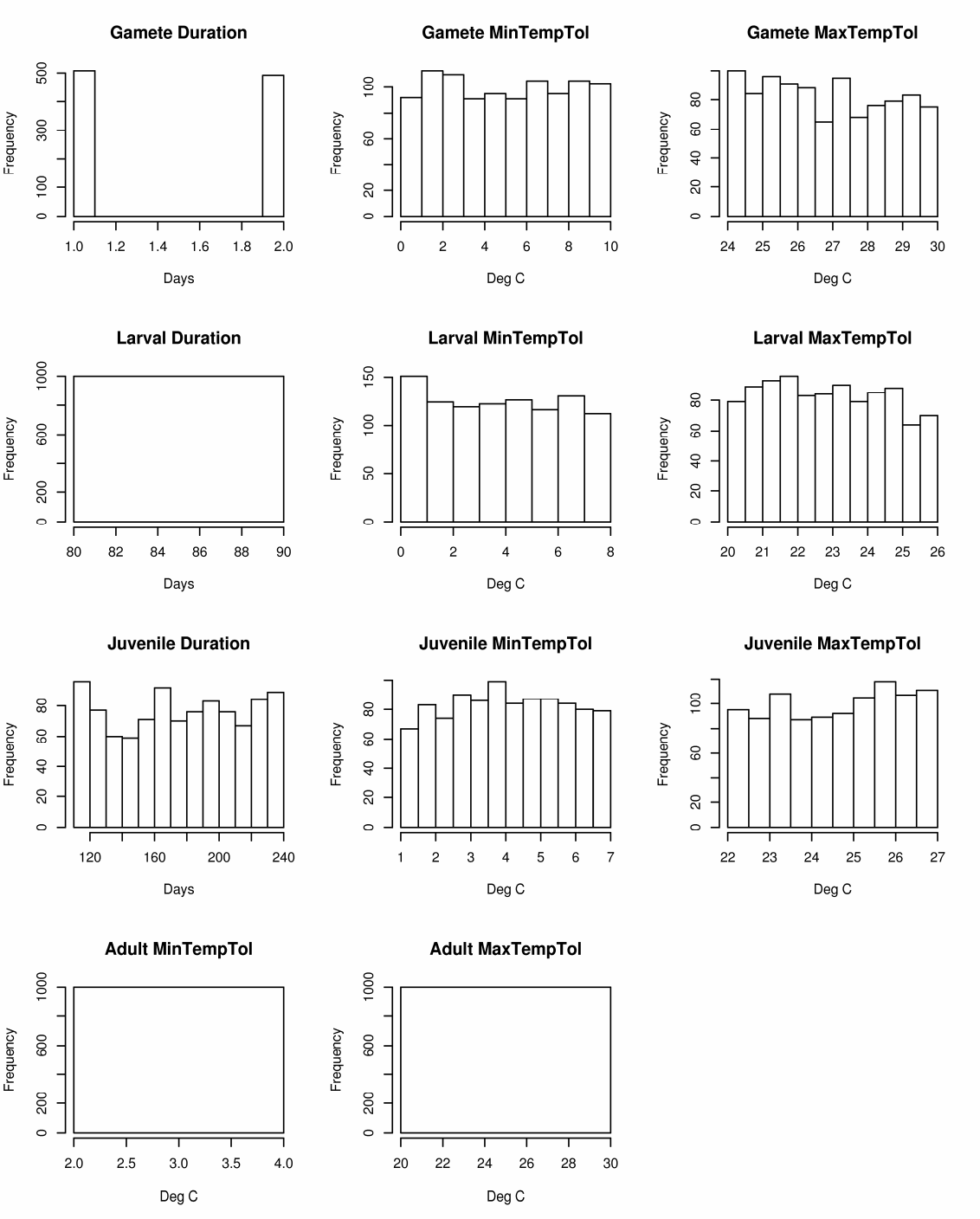


Figure 9 Global IUCN bioregion distribution of *Carcinus maenas*

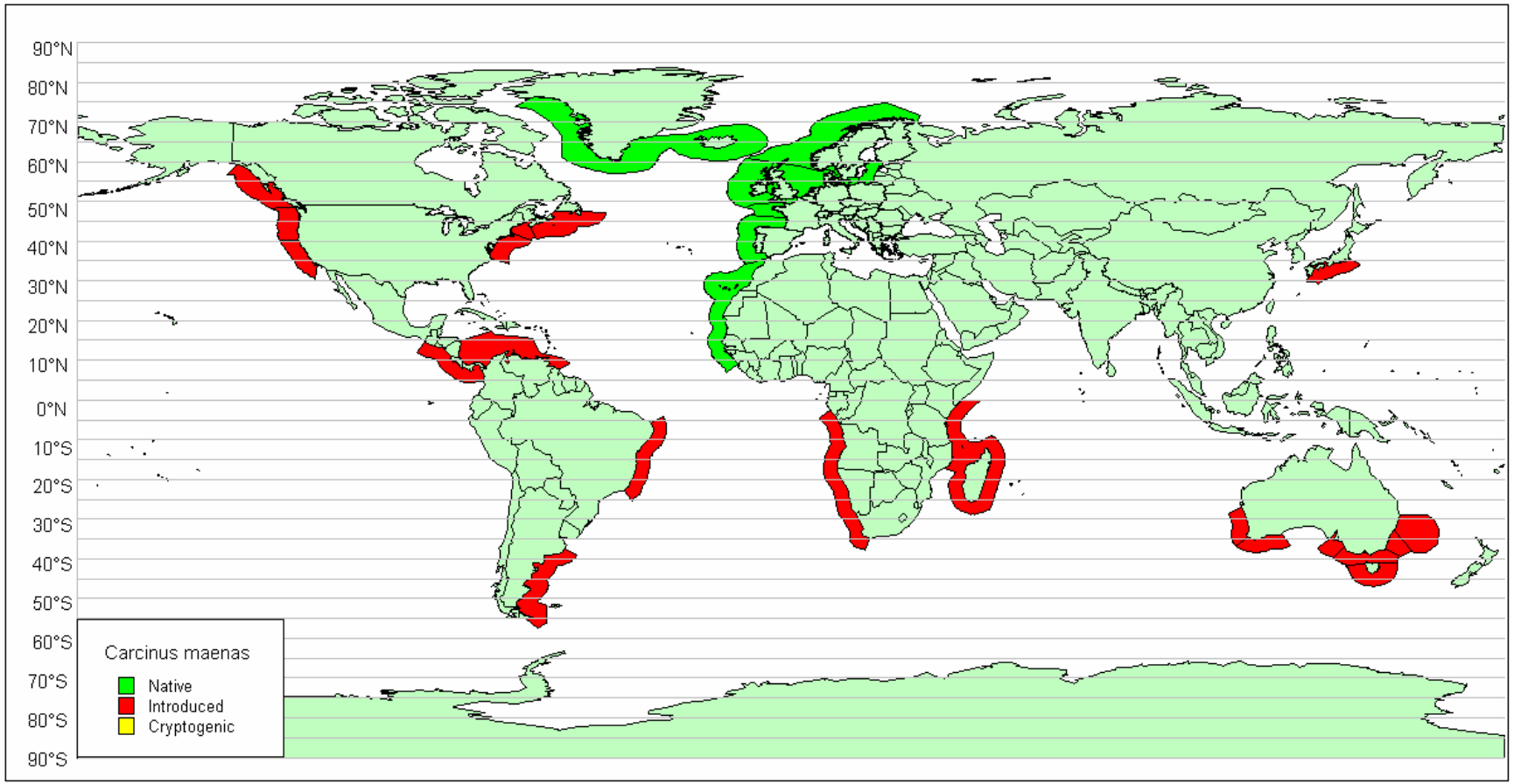
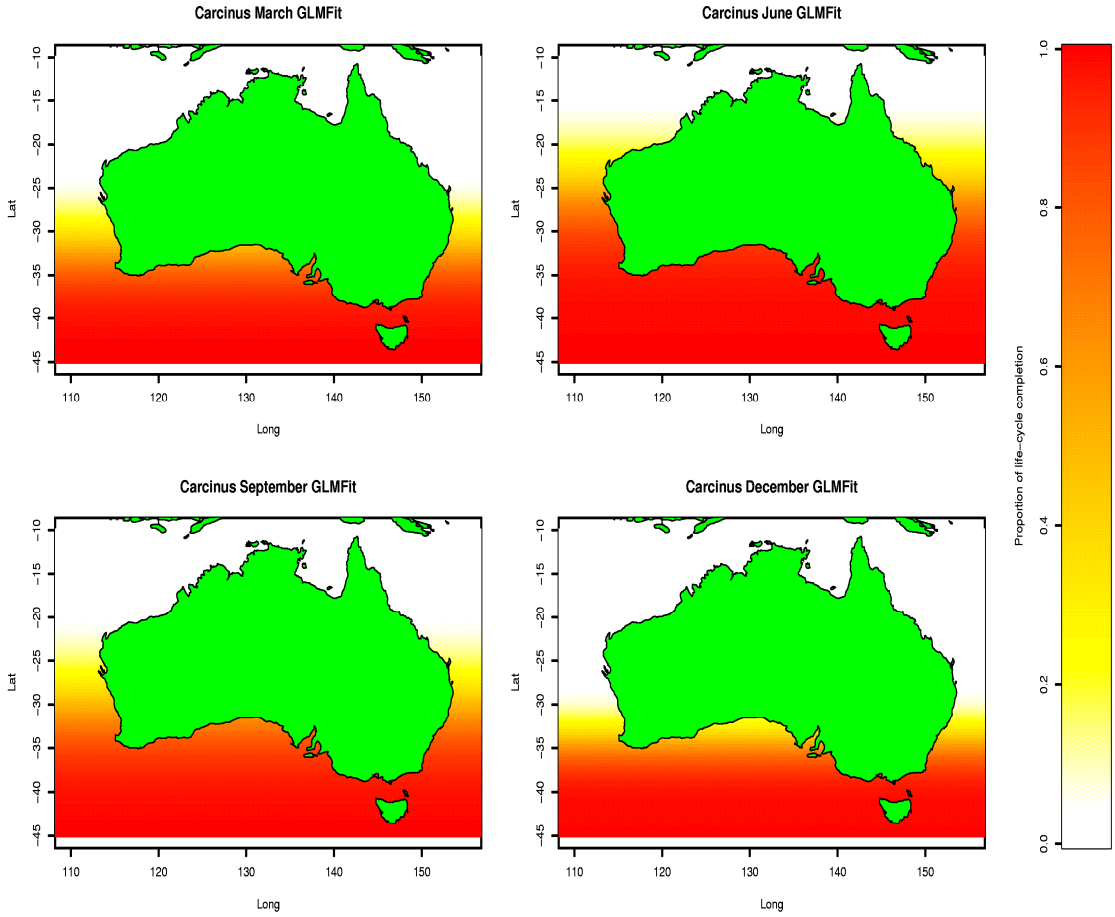


Figure 10 Predicted proportion of *Carcinus maenas* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)



The unmodified life-cycle model predicts that *Carcinus maenas* will be able to complete a significant proportion of its life-cycle (≥80%) in all of the temperate regions of Australia, extending North to approximately 28^0S. This is consistent with its native distribution in Europe and its invaded distribution in North America and Japan. This is also consistent with point source information from Africa and Argentina but is inconsistent with the (possibly erroneous) records from central America and Brazil (see above).

3.2.4 Crassostrea gigas

The independent reviewer of *Crassostrea gigas* noted that the life-stage specific temperature tolerances of the original model (Figure 11 and Figure 12) are broadly correct but the predicted distribution in Australia is incorrect. The reviewer (correctly) suggests that this error has resulted because the temperature information used in the model was taken from sub-tidal stations and does not therefore accurately represent the temperature extremes experienced by inter-tidal habitats that are periodically subject to much higher air temperatures. Southwood (1958) demonstrated that the temperature of the inter-tidal zone varies dramatically depending on the state of the tide, humidity, wind and sunlight. Moreover, the body temperature of sessile inter-tidal organisms, such as barnacles and limpets, exposed to the air generally have body temperatures that are $6 - 7^{\circ}$C greater than would be predicted by the air temperature, largely because of the warming effect of the sun. Potter and Hill (1982) measured a similar effect in the Sydney rock oyster, *Saccostrea (Crassostrea) commercialis*, in Ningi creek, Queensland, recording maximum tissue temperatures approximately 10°C above maximum air temperature.

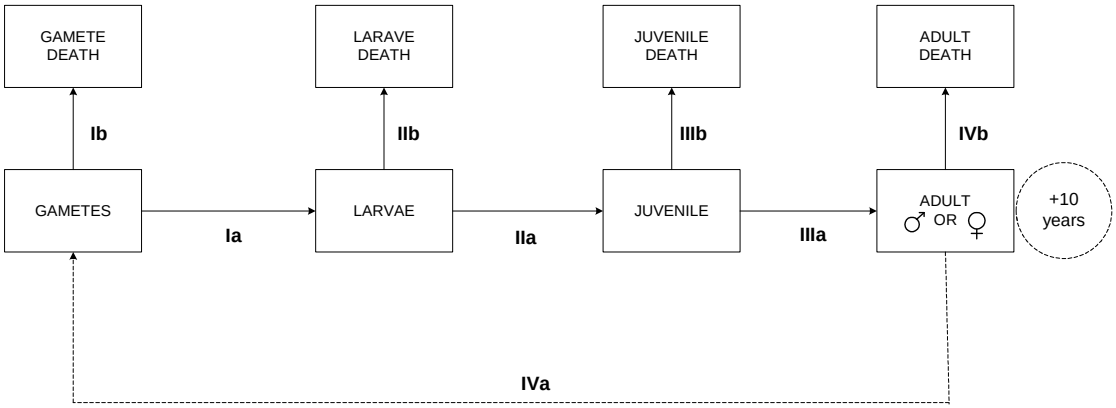
This empirical information confirms that the maximum and minimum temperatures experienced by sessile inter-tidal organisms cannot be accurately predicted from either air or sea temperature, and is generally significantly higher because of the warming effect of the sun. The amount of solar insolation[1] at any time and place on the earth's surface can easily be determined from the solar constant[2], latitude and time, but the relationship is made significantly more complicated by atmospheric variables such as cloud cover. We propose to investigate this relationship further in the next SLA as part of the on-going development of Module D for inter-tidal organisms. In the meantime, however, the simulation model has been amended by adding 5°C to the moving average (or rolling mean) maximum air temperature, in an attempt to more correctly represent the temperature extremes experienced by inter-tidal organisms such as *Crassostrea gigas*. It is important to note that this approach is intended as a temporary measure.

Figure 13 shows the global IUCN bioregion distribution of *Crassostrea gigas*. In this context it is important to note that the temperature tolerances of inter-tidal organisms is often more closely related to vertical distribution on the shore than to latitudinal distribution (Southwood, 1958). Hence, the global distribution of an inter-tidal organism may not provide an accurate indication of its potential temperature tolerance, because its distribution is confounded by vertical height and the extent to which the organism is exposed to sunlight and air temperatures at low tide. The broad distribution of *C. gigas* reflects the issues associated with the scale of the IUCN bioregions (as noted above) together with the effect of its vertical distribution.

[1] Defined as direct solar radiation at the earth's surface

[2] Defined as the amount of energy received at the top of the earth's atmosphere on a surface orientated perpendicular to the sun's rays

Figure 11 *Crassostrea gigas* life-cycle model



Transition	Duration	Model	Temperature range	Temperature model
Ia	Few hours	< 1 day	10 to 35 deg C	Min ~ Uniform [10, 15]; Max ~ Uniform [30, 35]
Ib	Few hours	<1 day	10> T > 35 deg C	Min > T > Max
IIa	D = f (T)	D = -0.77T + 36	6 to 32 Deg C	Min ~ Uniform [6, 15]; Max ~ Uniform [30, 32]
IIb	Few hours	<1 day	6 > T > 32 deg C	Min > T > Max
IIIa	152 to 365 days	Uniform [152, 365]	3 to 30 deg C	Min ~ Uniform [3, 12]; Max ~ Uniform [25, 30]*
IIIb	Within duration	MA(AirTemp, Duration)	3 > T > 30 deg C	Min > T > Max
IVa	Few hours	< 1 day	15 to 30 deg C	Min ~ Uniform [15, 18]; Max ~ Uniform [26, 30]
IVb	Within duration	MA(AirTemp, Duration)	2 > T > 32 deg C	Min ~ Uniform [2, 4]; Max ~ Uniform [30, 32]*

*Air temperature extremes

Figure 12 Life-cycle variates of *Crassostrea gigas* based on 1000 simulations of the life-cycle model

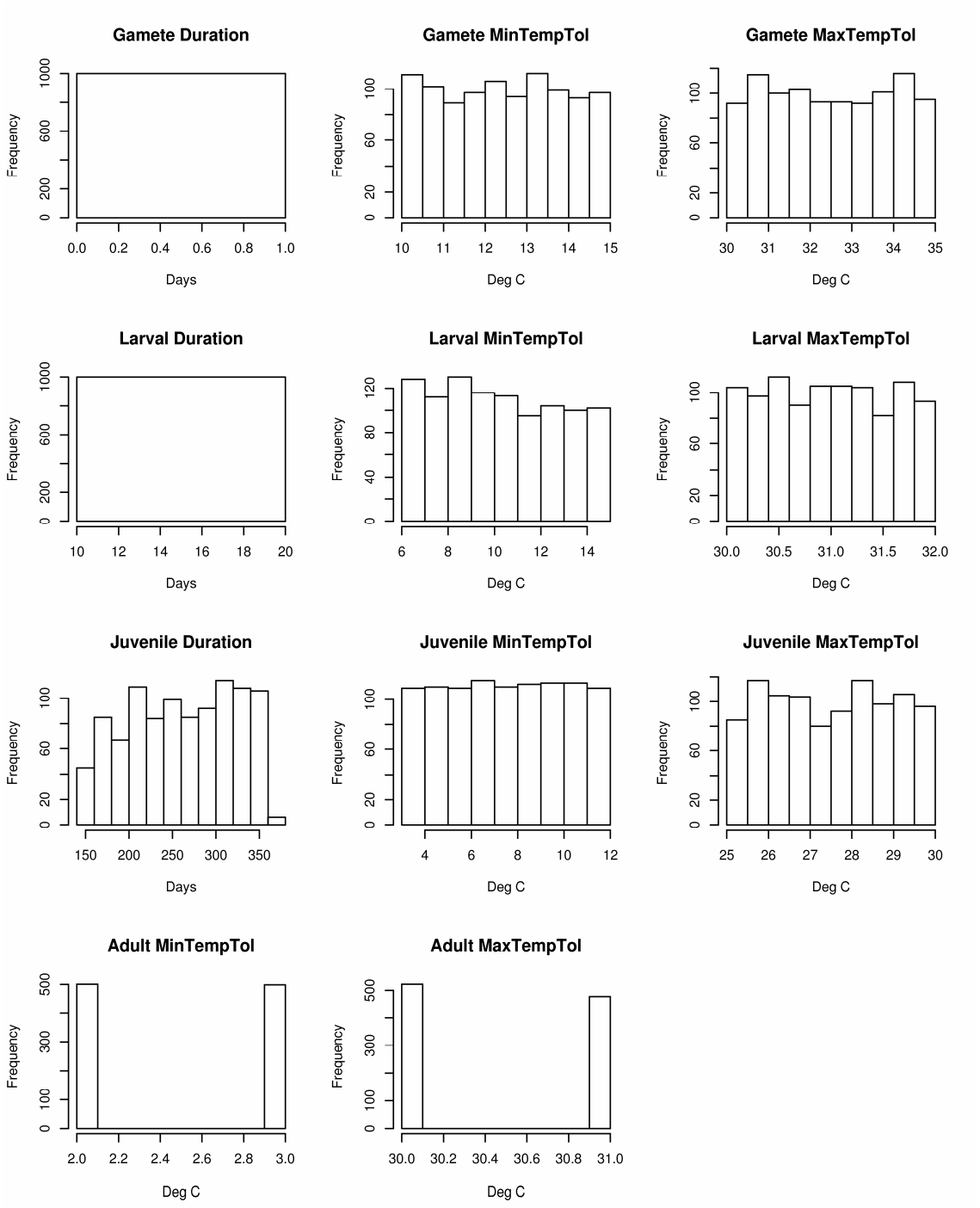


Figure 13 Global IUCN bioregion distribution of *Crassostrea gigas*

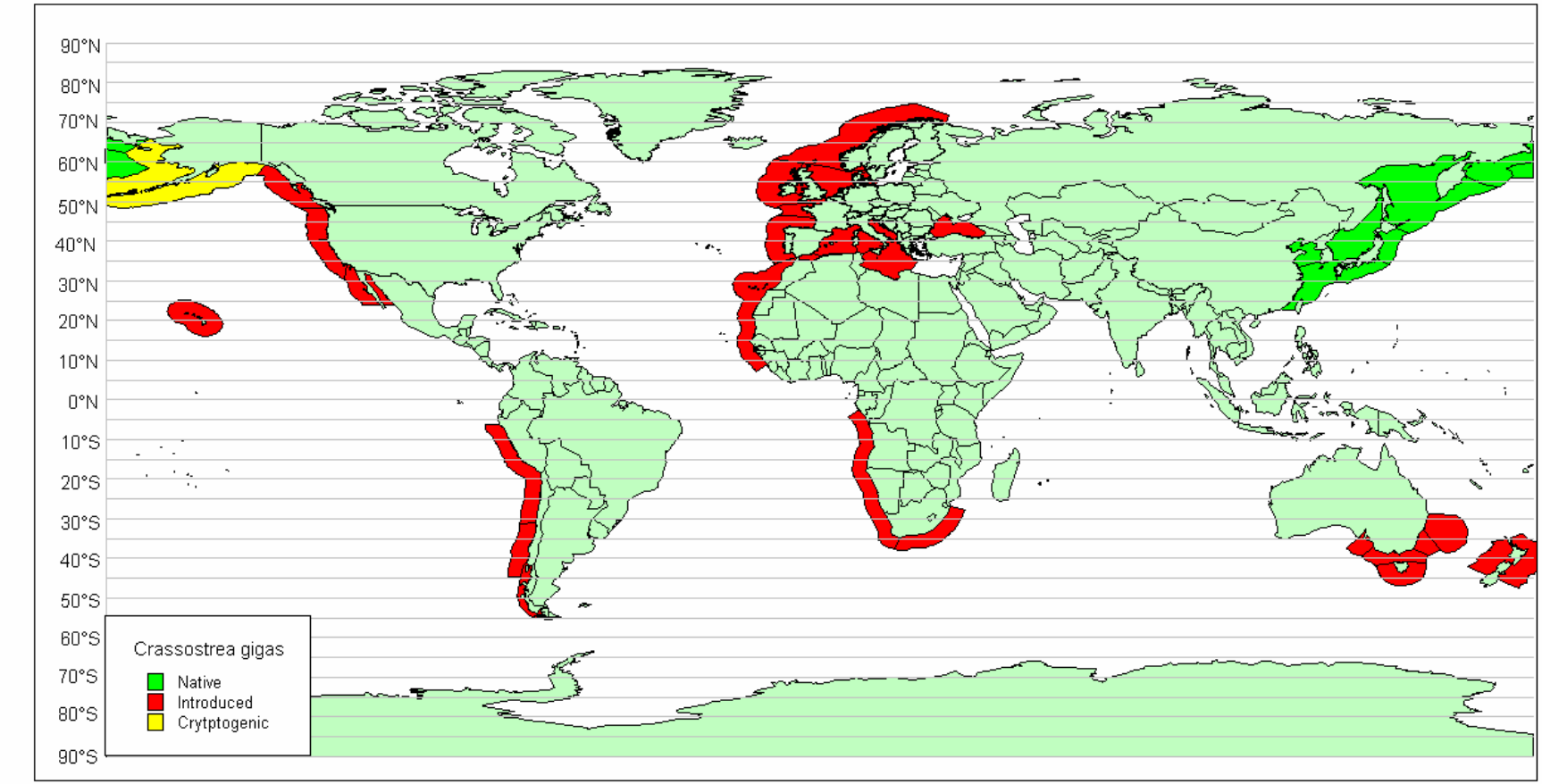
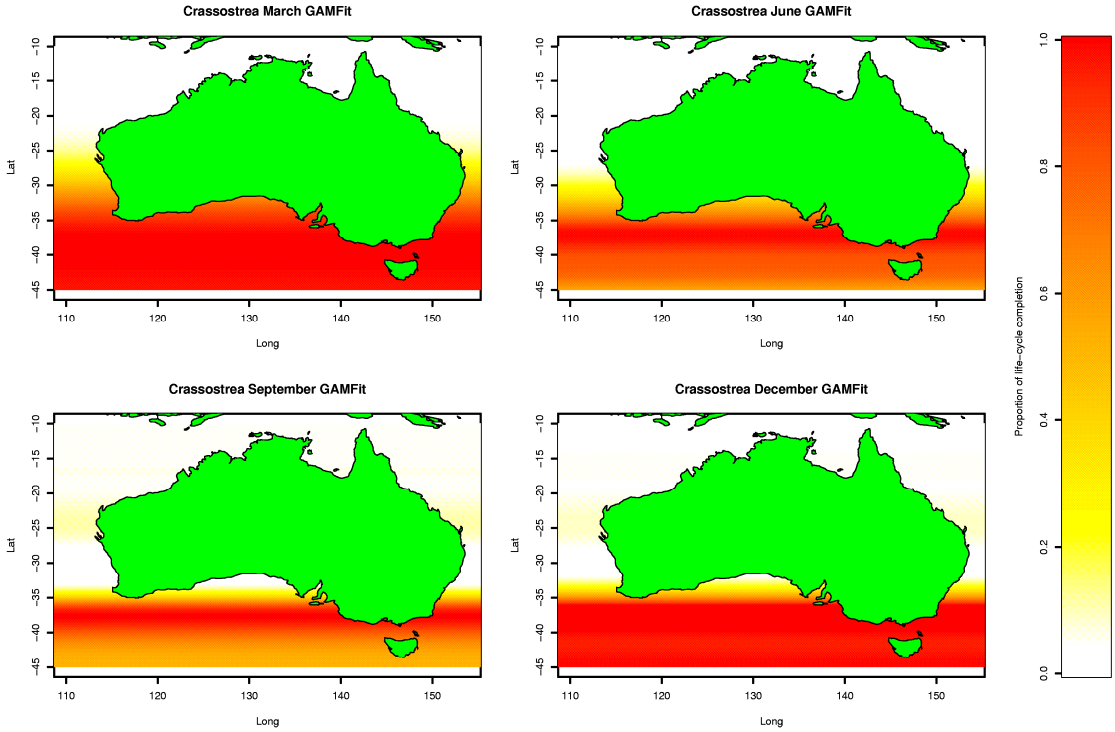


Figure 14 Predicted proportion of *Crassostrea gigas* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)



The results of the life-cycle simulation model for *Crassostrea gigas*, based on the modified temperature simulation model and the unmodified life-cycle model, suggests that *C. gigas* will only be able to complete a significant proportion of its life-cycle (≥80%) in the temperate regions of Australia, extending no further North than approximately 32^0 S (Figure 14)[3]. This prediction, however, assumes that the adult life-stages are periodically exposed to sunlight at low tide. The distribution of *C. gigas* could potentially spread further North than this if it were to occupy a very low (or even a sub-tidal) position in the inter-tidal shore. The prediction as it currently stands, however, accords with the known distribution of *C. gigas* in Australia which has not been recorded in any major port north of Newcastle, New South Wales (32^0 56'S) (Patil *et al.*, 2004). In the oyster leases of Port Stephans ($32^0$43'S), *C. gigas* is known to occupy the lower intertidal zone and the estuarine conditions of the inner harbour. Sydney rock oysters, *Saccostrea (Crassostrea) commercialis*, are more evenly distributed on the foreshores of both the inner and outer harbour of Port Stephens, and also survive in greater numbers in the higher intertidal zone (*pers. comm.* John Nell, NSW Fisheries, 20.05.04), suggesting that the temperature tolerance of *C. gigas* may be lower than that of *S. commercialis*.

3.2.5 Gymnodinium catenatum

The independent review of the *Gymnodinium catenatum* life-cycle model raises a number of important issues, most notably:

- the emphasis in the current model on the sexual, as opposed to vegetative, life-cycle of *G. catenatum*;
- the duration of the transition times from planozygote to resting cyst and from resting cyst to planomeiocyte;
- the distinction between the distribution and temperature tolerance of warm-temperate versus tropical ecophenotypes;
- possible bias in culture-recorded temperature tolerance.

The *Gymnodinium catenatum* life-cycle model has been adjusted to place greater emphasis on the vegetative life-cycle, allowing for continuous indefinite cell division every three days. We have also accepted the reviewer's suggestion to shorten the transition time from planozygote to resting cyst, and increase the viability of cysts to at least a year following a 14 day mandatory dormancy period (Figure 15).

The distinction between warm-temperate and tropical ecophenotypes of *Gymnodinium catenatum* is critical to the future development and implementation of the ballast water risk assessment for this species. The reviewer questions the broad potential distribution predicted by the model suggesting that the optimum temperature range of the warm-temperate ecophenotype (12 – 18°C) restricts its potential distribution in Australia. It is important to note, however, that the reviewer subsequently confirmed that all references, and the tolerance information therein, that supported the original model refer to the warm-temperate phenotype (*pers comm.* G. Hallegraeff, UTAS, 19.05.06).

[3] The discontinuous predictions in September and December are an artefact of the unconstrained General Additive Model used to fit the predictions of the time series/life-cycle model to latitude.

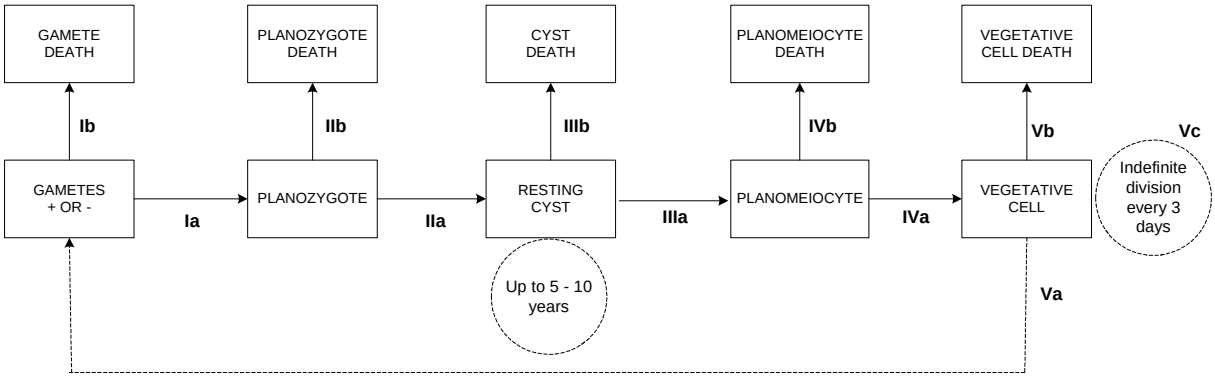
The reviewer's comments also suggest that: a) tropical ecophenotypes of *Gymnodinium catenatum* are not present in Australian waters; and, b) a domestic ballast water risk assessment should be restricted in scope to the warm-temperate ecophenotype (presumably until such time as the tropical ecophenotype is introduced into Australia). We stress that the implementation of the risk assessment in this manner would only be tenable if the DSS, and associated monitoring strategies, are able to confirm the absence of tropical ecophenotypes in Australian ports and reliably distinguish between the two ecophenotypes into the future (see also Section 6.2.7). In this context it is important to note that there is currently no morphological or genetic way to distinguish between the two ecophenotypes (*pers comm.* G. Hallegraeff, UTAS, 19.05.06). The reviewer also points to the existence of a well known "culture phenomena" wherein algal species raised in culture exhibit warmer temperature optima than in the wild. This phenomena can cause an upward bias in laboratory measurements of temperature tolerance (Karentz and Smayda, 1984).

The existence of ecophenotypes of *Gymnodinium catenatum*, and to a lesser extent the culture phenomena, are relatively new discoveries and they are not currently well understood. Recent data from Australian strains of *G. catenatum* confirm their potential to germinate and grow at 25°C, possibly higher. This data is relevant even in light of the culture phenomena (*pers comm.* S. Blackburn, CMAR, 22.05.06). We have made a small reduction to the maximum temperature tolerance of the dormant cyst and minimum temperature tolerance of the gametes, in light of the reviewer's comments, but do not propose to substantially amend the data in the original model without additional guidance and/or directed research effort on the temperature tolerances of the each of the ecophenotypes, and the potential bias of laboratory measurements of *G. catenatum* temperature tolerances.

The amended life-cycle of *Gymnodinium catenatum* is summarised in Figure 15, whilst Figure 16 shows the life-cycle variates generated by the simulation model. The amended model predicts that *G. catenatum* is capable of completing its entire life-cycle up to approximately 25^0 South (Figure 17). This distribution is broader than the predictions of the independent reviewer who suggests that temperate Tasmanian populations of *G. catenatum* could not spread north of the New South Wales/Queensland border on Australia's east coast and Geraldton on the west coast (*pers. comm.* G. Hallegraeff, UTAS, 22.11.05). This predicted range, however, is latitudinally narrower than the recorded global distribution of *G. catenatum* (Figure 18) but as noted above this information may reflect the presence of two ecophenotypes.

The life-stage duration and temperature tolerance changes made to the model have the effect of increasing the proportion of life-cycle completed in the lower latitudes, whilst eliminating any seasonal effect (Table 3). These effects appear to be due to the substantial increase in the viability of cysts which has been increased in the model from 90 days to several years. This also appears to have eliminated any seasonal effects in the model's results. In effect the model allows the cysts of *Gymnodinium catenatum* to smooth out seasonal effects in the water temperatures by simply waiting until the temperature falls within the germination range of the dormant cysts.

Figure 15 Amended life-cycle model for *Gymnodinium catenatum*



Transition	Duration	Model	Temperature range	Temperature model
Ia	3 to 28 days	Uniform [3, 28]	12 to 27 deg C	Min ~ Uniform [12,14]; Max = 27
Ib	Few hours	<1 day	12 > T > 27 deg C	Min < T < Max
IIa	Hours to days	Uniform [1, 3]	no data	Min ~ Unifom [12,14]; Max = 27*
IIb	Few hours	<1 day	no data	Min > T > Max
IIIa	14 days to years	Min duration = 14 d	no data	Min = 12, Max = 27**
IIIb	Few hours	<1 day	4 > T > 38 deg C	Min ~ Uniform [4, 6]; Max ~ Uniform [32, 35]
IVa	up to 36 hours	Uniform [1, 2]	no data	Min = 12, Max = 27**
IVb	Few hours	< 1 day	no data	Min > T > Max
Va	Few hours	< 1 day	4 > T > 30 deg C	Min ~ Uniform [4,10]; Max ~ Uniform [25, 30]
Vb	Few hours	< 1 day	4 > T > 30 deg C	Min > T > Max
Vc	Indefinately	Min duration = 3 d	4 > T > 30 deg C	Min ~ Uniform [4,10]; Max ~ Uniform [25, 30]

*Inferred from gamete data

**Inferred from vegetative cell data

Figure 16 Amended life-cycle variates of *Gymnodinium catenatum* based on 1000 simulations of the life-cycle model

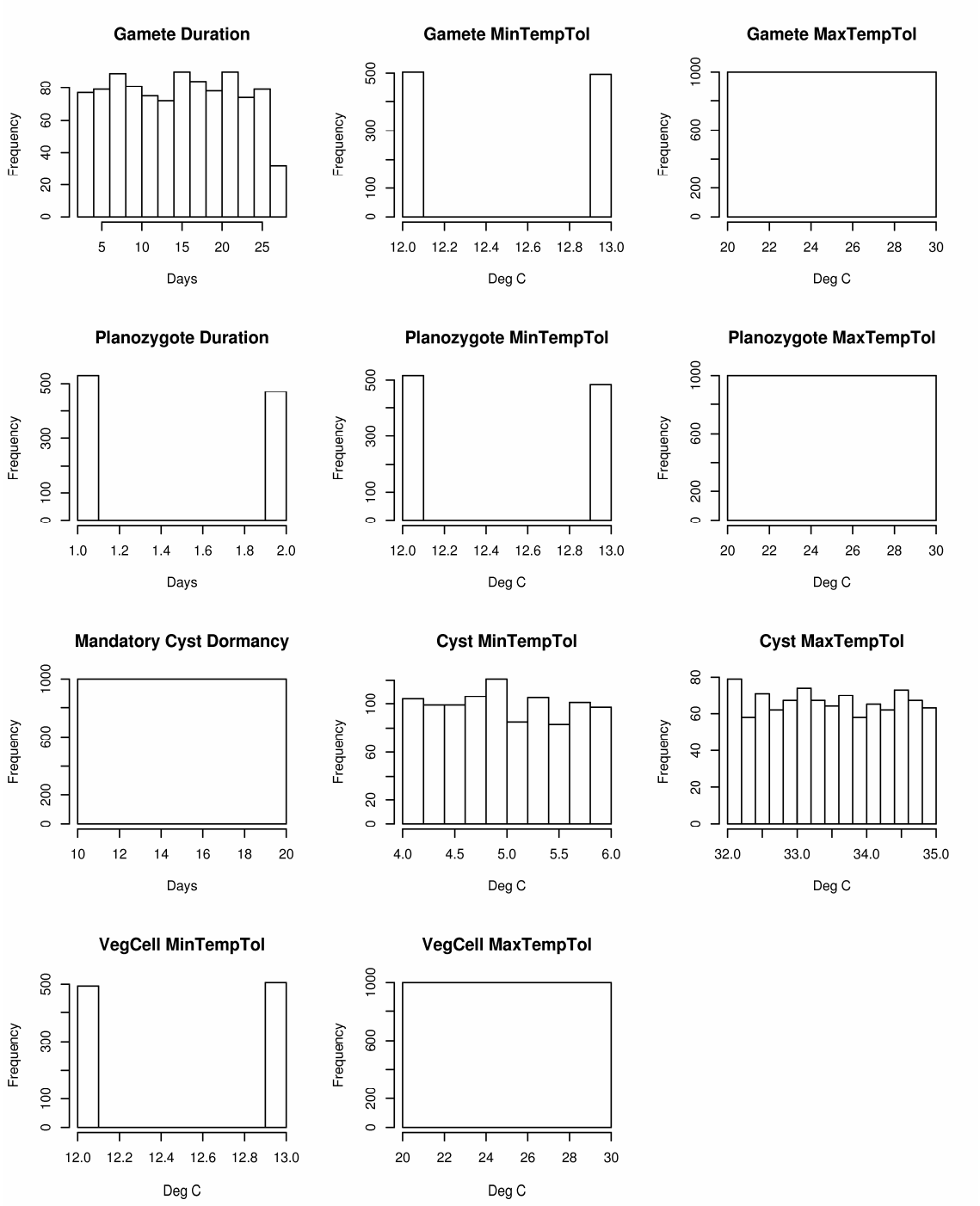


Figure 17 Predicted proportion of *Gymnodinium catenatum* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)

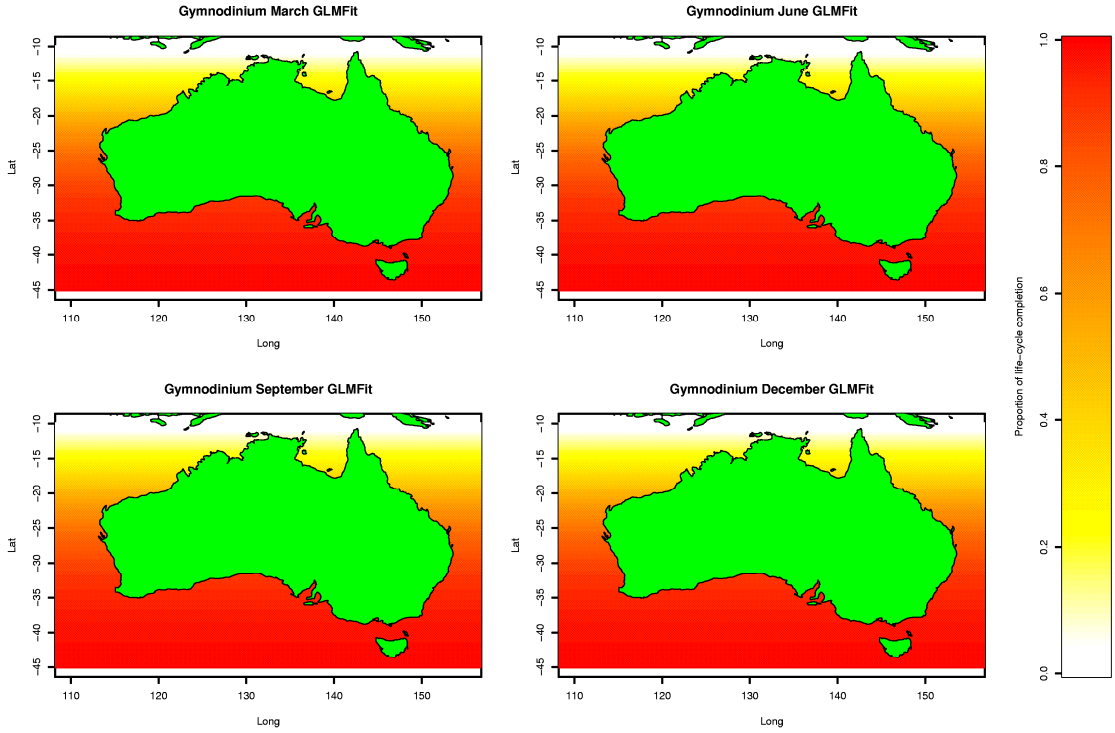


Figure 18 Global IUCN bioregion distribution of *Gymnodinium catenatum*

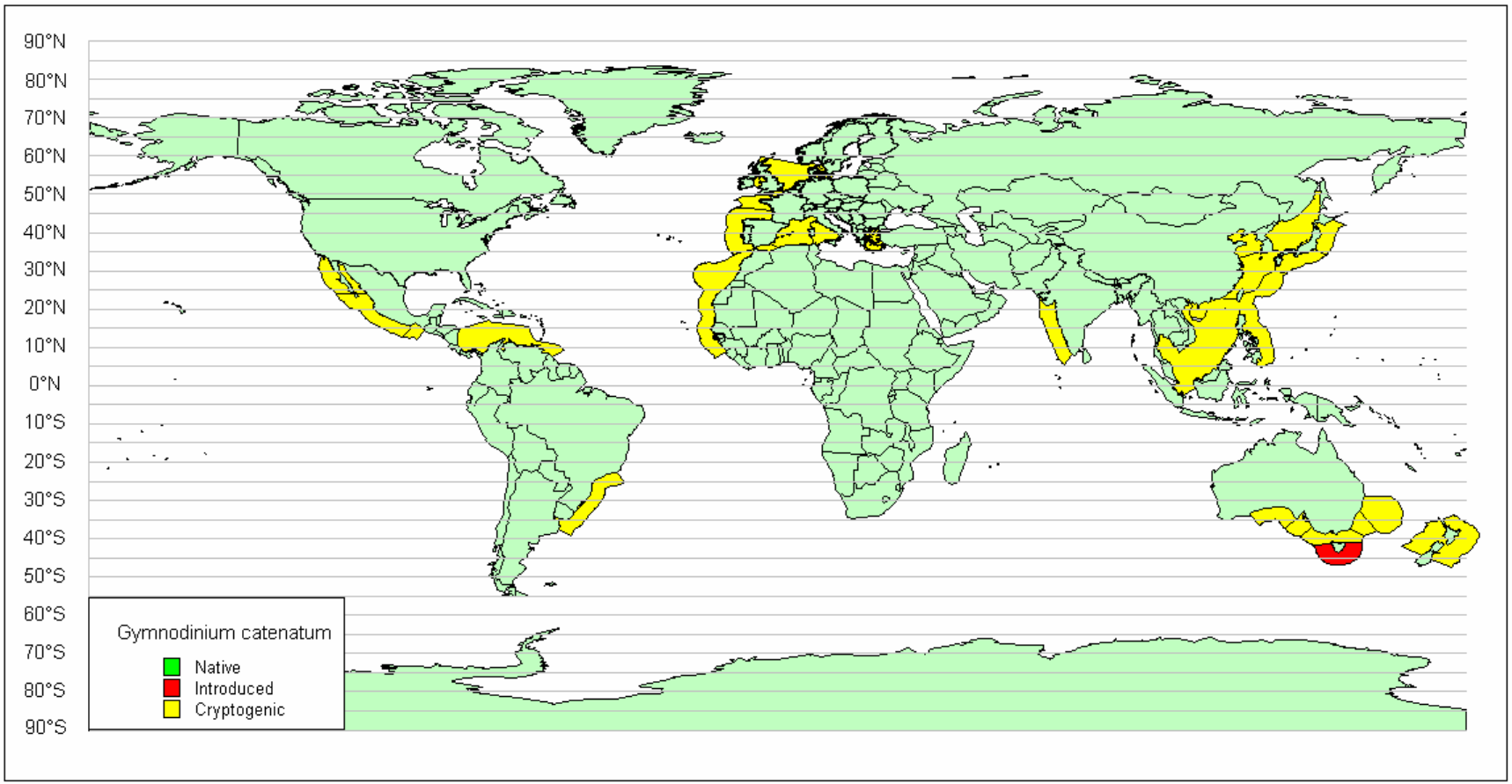


Table 3 Difference between the original and the amended proportion of *Gymnodinium catenatum* life-cycle predicted to be completed in 20 Australian ports by month of introduction

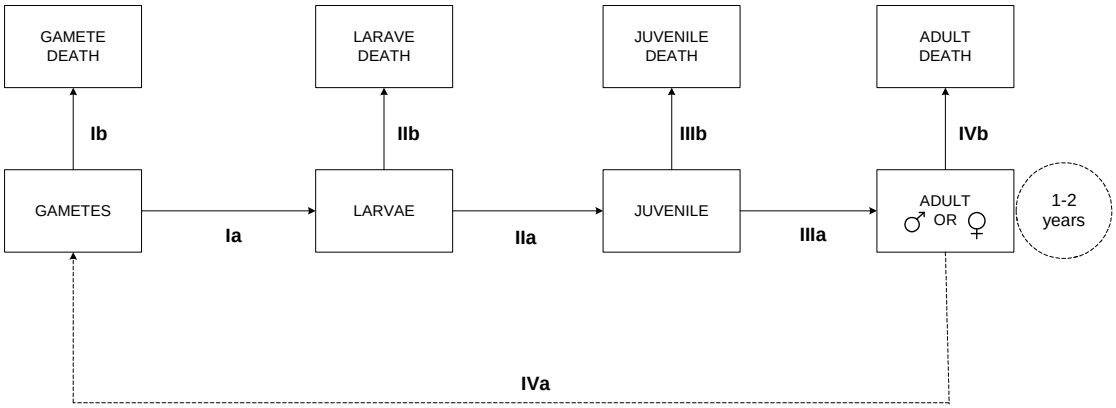
Port name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Abbot Point	-0.65	-0.63	-0.55	-0.05	0.30	0.34	0.33	0.20	-0.08	-0.38	-0.58	-0.64
Brisbane	-0.42	0.17	0.38	0.38	0.38	0.38	0.37	0.26	0.03	-0.17	-0.17	-0.33
Broome	-0.52	-0.52	-0.51	-0.45	-0.04	0.33	0.31	0.03	-0.28	-0.48	-0.52	-0.52
Burnie	-0.12	-0.32	-0.60	-0.88	-0.98	-1.00	-1.00	-1.00	-1.00	-0.98	-0.63	-0.15
Cape Flattery	-0.60	-0.60	-0.60	-0.58	-0.28	0.25	0.38	0.34	0.15	-0.17	-0.45	-0.57
Dampier	-0.57	-0.57	-0.52	-0.12	0.36	0.40	0.24	-0.05	-0.39	-0.54	-0.57	-0.57
Darwin	-0.53	-0.53	-0.53	-0.48	-0.31	-0.20	-0.29	-0.46	-0.53	-0.53	-0.53	-0.53
Esperance	0.00	0.00	0.00	-0.01	-0.01	-0.01	-0.03	-0.01	0.00	0.00	0.00	0.00
GrooteEylandt	-0.64	-0.64	-0.63	-0.50	-0.18	-0.05	-0.20	-0.45	-0.62	-0.64	-0.64	-0.64
Haypoint	-0.58	-0.58	-0.33	0.09	0.33	0.36	0.30	0.09	-0.18	-0.46	-0.56	-0.59
Hobart	-0.25	-0.58	-0.84	-0.99	-1.00	-1.00	-1.00	-1.00	-1.00	-0.99	-0.38	-0.05
Lucinda	-0.53	-0.52	-0.39	0.17	0.45	0.46	0.37	0.05	-0.29	-0.48	-0.53	-0.53
Mourilyan	-0.49	-0.49	-0.48	-0.25	0.37	0.45	0.37	0.11	-0.21	-0.46	-0.49	-0.49
Port Kembla	0.00	0.00	0.00	-0.01	-0.04	-0.03	-0.07	-0.12	-0.05	0.00	0.00	0.00
Portland	-0.06	-0.24	-0.56	-0.83	-0.99	-1.00	-1.00	-1.00	-0.88	-0.21	0.00	0.00
Port Stanvac	0.00	-0.03	-0.27	-0.54	-0.81	-0.93	-0.97	-0.97	-0.78	-0.20	0.00	0.00
Spring Bay	-0.18	-0.51	-0.83	-0.97	-1.00	-1.00	-1.00	-1.00	-1.00	-0.97	-0.43	-0.03
Thevenard	0.00	-0.09	-0.33	-0.69	-0.90	-0.98	-0.99	-0.92	-0.23	-0.01	0.00	0.00
Thursday Is.	-0.61	-0.61	-0.61	-0.58	-0.30	0.03	-0.13	-0.37	-0.52	-0.61	-0.61	-0.61
Townsville	-0.50	-0.48	-0.31	0.12	0.42	0.48	0.48	0.42	0.21	-0.10	-0.36	-0.50

3.2.6 *Musculista senhousia*

The independent reviewer of the life-cycle and potential distribution of *Musculista senhousia* was supportive of the data used in the original model. The reviewer also provided additional unpublished information that supported some, but not all, of the temperature tolerance and duration data in the original model. The life-cycle model has been amended in light of this new data (Figure 19). In particular the upper temperature tolerance limit for adult survival and spawning has been increased by a few degrees (see also Figure 20). The effect of these changes are summarised in Table 4. The slight increase in maximum temperature tolerance of adult survival and spawning has caused a slight increase in the proportion of the life-cycle completed in the mid-latitudinal regions of Australia.

The global IUCN bioregion distribution of *Musculista senhousia* is illustrated in Figure 21. The tropical to warm-temperate distribution of this species is reasonably well reflected in the predicted potential distribution in Australia, which is approximately bounded North of 37^0 South throughout the year (Figure 22). In the amended model, the southward limit of this species is controlled by the (relatively high) minimum temperature tolerance of *M. senhousia* larvae (15°C). This limit is supported by the data gathered for the original model and the additional information provided by the reviewer. This information, however, appears to be at odds with the northern, cold-temperate, limits of the species' native distribution (Figure 21). This native distribution is recorded to extend as far north as the southern end of the Sea of Okhotsk, approximately 50^0 North (Kulikova, 1978). In Australia, *M. senhousia* occurs in the Tamar River and Devonport at approximately 41^0 South. This information suggests that the minimum temperature tolerance of *M. senhousia* larvae could be lower than the values used in the original model and those provided by the reviewer. We propose to seek further information on the minimum temperature tolerance and distribution of *M. senhousia* in the next SLA, prior to the finalisation of the first operational draft of the ballast water risk tables

Figure 19 Amended life-cycle model for *Musculista senhousia*



Transition	Duration	Model	Temperature range	Temperature model
Ia	Few hours	< 1 day	15 to 31 deg C	Min ~ Uniform [15, 18]; Max ~ Uniform [28, 31]*
Ib	Few hours	<1 day	20> T > 26 deg C	Min > T > Max
IIa	14 to 25 days	Uniform [14, 25]	15 to 31 Deg C	Min ~ Uniform [15, 18]; Max ~ Uniform [28, 31]
IIb	Few hours	<1 day	15 > T > 31 deg C	Min > T > Max
IIIa	182 to 365 days	Uniform [182, 365]	no data	Min ~ Uniform [-5, 0]; Max ~ Uniform [32, 36]**
IIIb	Few hours	<1 day	0 > T > 35 deg C	Min > T > Max
IVa	Few hours	< 1 day	15 to 31 deg C	Min ~ Uniform [15, 18]; Max ~ Uniform [28, 31]
IVb	Few hours	< 1 day	-5 > T > 35 deg C	Min ~ Uniform [-5, 0]; Max ~ Uniform [32, 36]

*Inferred from larval data

**Inferred from adult data

Figure 20 Amended life-cycle variates of *Musculista senhousia* based on 1000 simulations of the life-cycle model

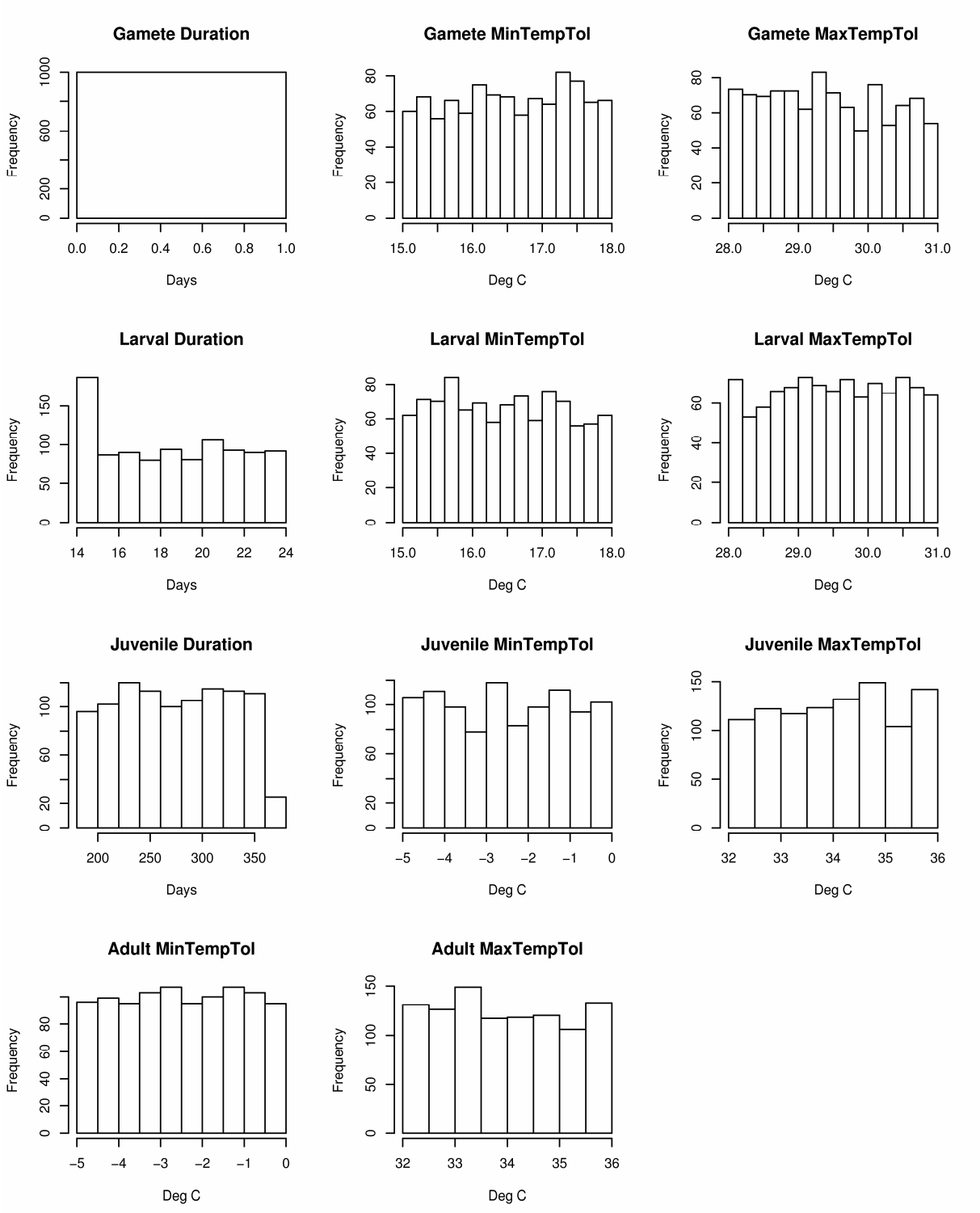


Table 4 Difference between the original and the amended proportion of *Musculista senhousia* life-cycle predicted to be completed in 20 Australian ports by month of introduction

Port name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Abbot Point	-0.02	-0.01	-0.02	-0.01	-0.02	-0.03	-0.04	-0.05	-0.06	-0.07	-0.07	-0.03
Brisbane	0.01	-0.01	0.00	0.00	0.00	0.00	-0.01	0.01	0.00	0.00	0.00	0.00
Broome	0.00	0.00	-0.02	-0.02	-0.05	-0.08	-0.11	-0.14	-0.18	-0.19	-0.14	-0.04
Burnie	0.00	-0.02	-0.04	-0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01
Cape Flattery	-0.01	0.00	-0.01	-0.03	-0.03	-0.04	-0.06	-0.07	-0.09	-0.10	-0.07	-0.03
Dampier	-0.05	-0.01	-0.01	-0.04	-0.06	-0.08	-0.11	-0.14	-0.16	-0.16	-0.07	-0.02
Darwin	-0.01	-0.01	-0.01	-0.04	-0.07	-0.11	-0.14	-0.18	-0.18	-0.13	-0.06	-0.02
Esperance	0.00	0.00	0.00	0.01	0.00	0.00	-0.01	0.01	-0.01	0.00	0.00	0.00
GrooteEylandt	-0.01	0.01	-0.02	-0.04	-0.09	-0.13	-0.16	-0.20	-0.20	-0.12	-0.05	-0.02
Haypoint	-0.03	-0.01	0.00	-0.01	-0.01	-0.01	-0.02	-0.02	-0.02	-0.02	-0.03	-0.03
Hobart	0.02	0.01	0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Lucinda	-0.02	-0.03	-0.01	-0.02	-0.03	-0.04	-0.06	-0.07	-0.09	-0.10	-0.10	-0.05
Mourilyan	-0.02	-0.03	0.00	0.00	-0.02	-0.02	-0.03	-0.04	-0.05	-0.06	-0.05	-0.04
Port Kembla	0.00	0.00	0.00	0.00	-0.01	0.01	-0.01	0.01	0.01	0.00	0.01	0.00
Portland	-0.01	-0.01	-0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Port Stanvac	0.00	0.00	0.01	0.00	-0.01	-0.01	0.00	0.00	0.00	0.00	-0.02	-0.01
Spring Bay	-0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.02
Thevenard	0.00	0.00	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Thursday Is.	-0.02	0.00	0.00	-0.01	-0.04	-0.06	-0.08	-0.10	-0.12	-0.13	-0.09	-0.04
Townsville	-0.03	0.00	-0.01	-0.01	-0.01	-0.01	-0.02	-0.03	-0.03	-0.04	-0.04	-0.04

Figure 21 Global IUCN bioregion distribution of *Musculista senhousia*

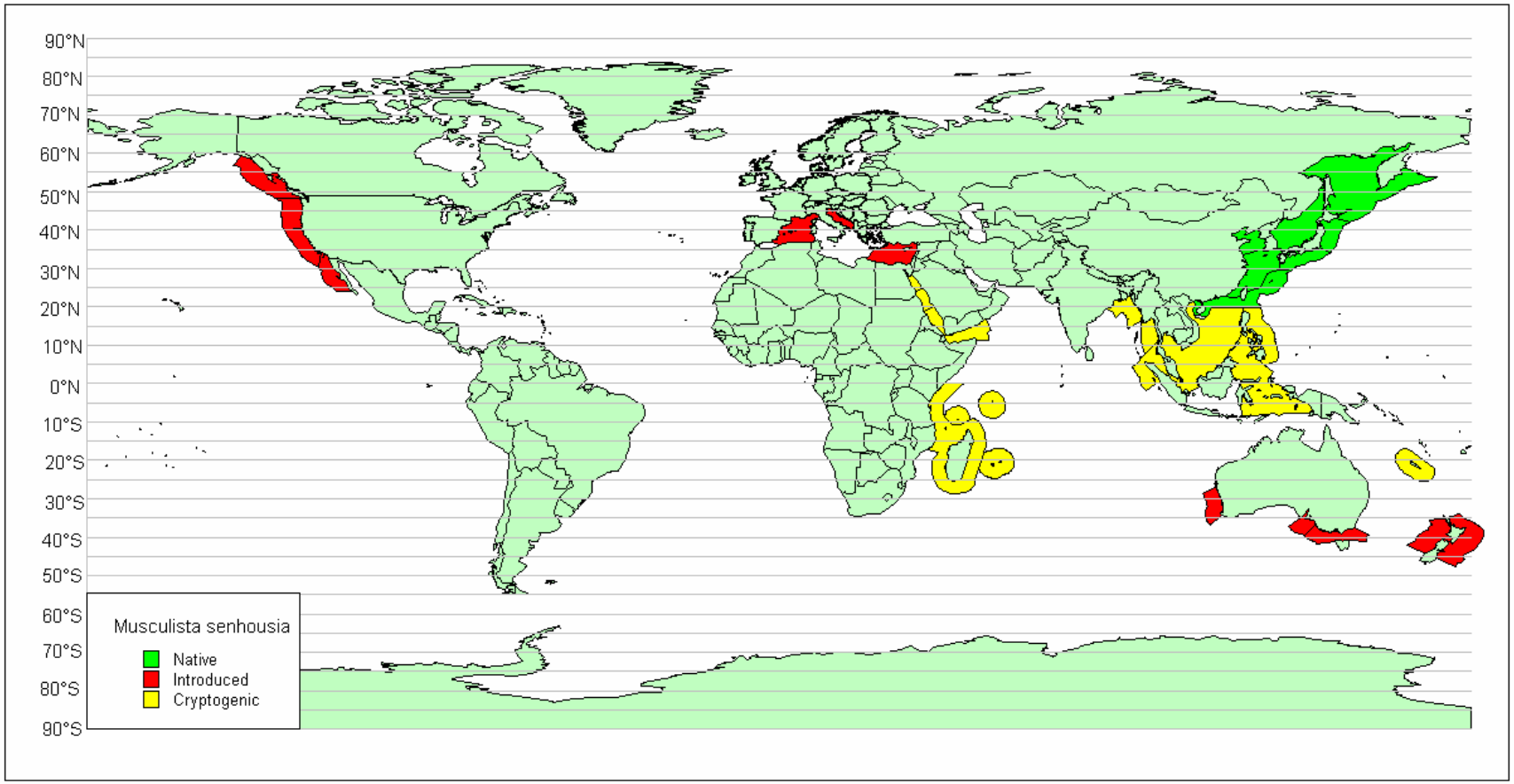
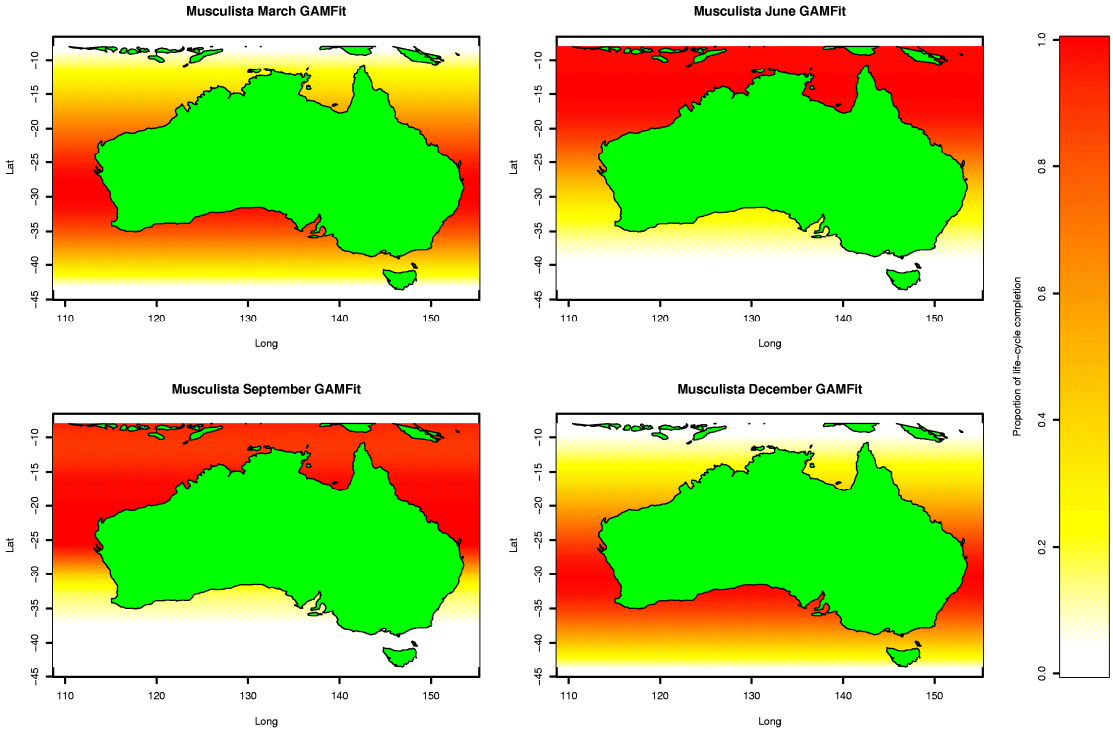


Figure 22 Predicted proportion *of Musculista senhousia* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)



3.2.7 Sabella spallanzanii

Two independent reviews of the life-cycle model and potential distribution of *Sabella spallanzanii* were completed. The reviewers' main points are:

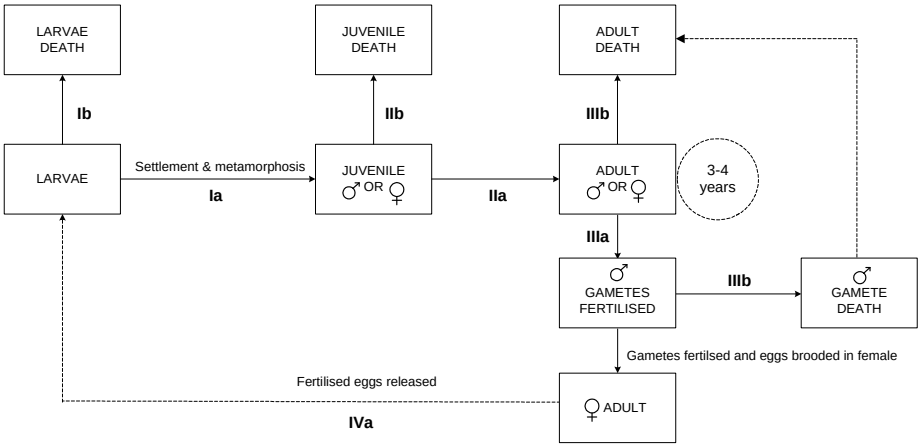
- the upper and lower limits of the larval, juvenile and adult temperature tolerances in the model are not supported by the (limited) available data;
- the lower limit on the larval duration, including settlement and metamorphosis may by too short.

We accept these points and have further followed the second reviewer's recommendations by comparing the temperature tolerance limits in the original model with ambient temperature limits in Cockburn Sound. Data held by CMAR records a maximum ambient temperature in Cockburn Sound of 24.6°C in January 1995, during which time viable *S. spallanzanii* juveniles, 10 to 15mm long, were collected. Examination of associated records suggests that maximum temperatures in Cockburn Sound vary between 20 – 25°C in summer. The second reviewer also provides unpublished data that suggests *S. spallanzanii* larvae are competent to settle in temperatures of 9 – 10°C and can grow at a rate of approximately 10mm per month in ambient temperatures between 9°C and 20°C.

On the basis of the reviewer's comments and this additional information we have amended the original model by increasing the lower limit of the larval duration from 10 to 15 days. Furthermore we have increased the upper limit of the larval temperature tolerance from 23°C to 30°C (Figure 23 and Figure 24). This represents an approximate 5°C rise above the summer ambient temperatures in Cockburn Sound. Similarly we propose to lower the upper limit of the larval temperature to 4°C. This represents an approximate 5°C decrease below the winter ambient temperatures in Port Phillip Bay. We recognise that this approach may be overly conservative but we consider it to be defensible in the absence of better data. The second reviewer recommends laboratory tolerance trials are conducted on the *S. spallanzanii* gametes and larvae. We support this recommendation.

The effect of these changes to the *S. spallanzanii* life-cycle model are summarised in Table 5. Unsurprisingly, the increase in the maximum temperature tolerance and the decrease in the minimum temperature tolerance has increased the prediction proportion of the life-cycle completed throughout Australia. The predicted potential distribution of *S. spallanzanii* in Australia, based on the amended model is summarised in Figure 25. This predicted range of life-cycle completion is broader than the *S. spallanzanii*'s global IUCN distribution (Figure 26) with the possible exception of the West African distribution and the single recorded incidence of *S. spallanzanii* from Indonesia. One of the reviewers notes that this observation comes from a labelled jar of specimens collected circa 1820 which has, apparently, never been independently verified. This record may therefore be an error. The West African bioregion (WA-I) stretches from Morocco to Sierra Leone, approximately 30 degrees of latitude. The original reference, however, simply states that *S. spallanzanii* is recorded from 'Western African including the Canary Islands' (Knight-Jones and Perkins, 1998). We are therefore unable to confirm the exact recorded locations but suspect that the southern extent of this apparent distribution may be an artefact of the IUCN bioregionalisation, rather than an accurate representation of this species temperature tolerance.

Figure 23 Amended life-cycle model of *Sabella spallanzanii*



Transition	Duration	Model	Temperature range	Temperature model
Ia	10 to 31 days	Uniform [15, 31]	2 to 30 deg C	Min ~ Uniform [2, 4]; Max ~ Uniform [25, 30]*
Ib	Few hours	<1 day	2 > T > 30	Min > T > Max
IIa	Within a year	Uniform[200, 300]#	no data	Min ~ Uniform [2, 4]; Max ~ Uniform [30, 32]**
IIb	Few hours	<1 day	2 > T > 32	Min > T > Max
IIIa	not applicable	< 1 day	2 to 30 deg C	Min ~ Uniform[2, 4]; Max ~ Uniform [30, 32]
IIIb	Few hours	<1 day	2 > T > 32	Min > T > Max
IVa	Within 24 hours	< 1 day	no data	Min ~ Uniform [2, 4]; Max ~ Uniform [25, 30]

*5 deg C added to ambient Summer temperatures in Cockburn Sound
**Inferred from adult data
#No specific data - thought to mature within a year

Figure 24 Amended life-cycle variates of *Sabella spallanzanii* based on 1000 simulations of the life-cycle model

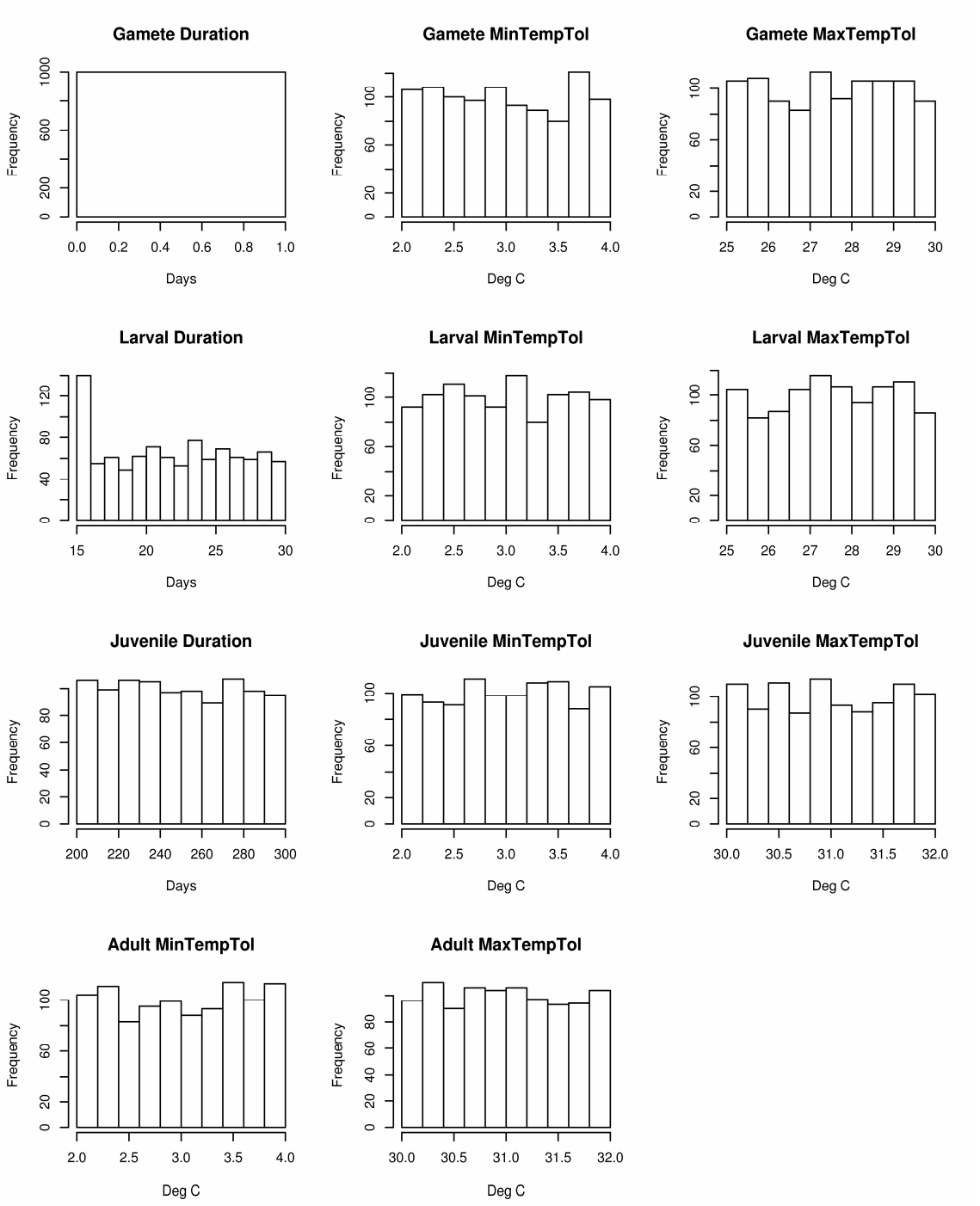


Table 5 Difference between the original and the amended proportion of *Sabella spallanzanii* life-cycle predicted to be completed in 20 Australian ports by month of introduction

Port name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Abbot Point	-0.09	-0.12	-0.34	-0.68	-0.90	-0.90	-0.84	-0.83	-0.79	-0.57	-0.27	-0.10
Brisbane	-0.43	-0.74	-0.96	-0.73	-0.30	-0.15	-0.18	-0.35	-0.75	-0.94	-0.72	-0.50
Broome	0.00	-0.01	-0.03	-0.20	-0.55	-0.70	-0.61	-0.52	-0.44	-0.29	-0.11	-0.03
Burnie	-0.11	-0.16	-0.10	-0.02	0.00	-0.03	-0.12	-0.18	-0.13	-0.03	0.00	-0.02
Cape Flattery	-0.04	-0.07	-0.23	-0.51	-0.81	-0.88	-0.86	-0.81	-0.67	-0.42	-0.18	-0.05
Dampier	-0.02	-0.04	-0.14	-0.46	-0.76	-0.72	-0.61	-0.59	-0.51	-0.29	-0.10	-0.04
Darwin	0.00	-0.01	-0.07	-0.19	-0.36	-0.45	-0.43	-0.33	-0.20	-0.09	-0.03	-0.01
Esperance	-0.59	-0.59	-0.47	-0.27	-0.08	0.00	0.00	0.00	-0.01	-0.11	-0.31	-0.49
GrooteEylandt	-0.01	-0.01	-0.07	-0.26	-0.49	-0.56	-0.50	-0.36	-0.19	-0.06	-0.01	0.00
Haypoint	-0.12	-0.25	-0.54	-0.88	-0.91	-0.72	-0.69	-0.81	-0.89	-0.71	-0.38	-0.18
Hobart	-0.07	-0.09	-0.02	0.00	-0.02	-0.18	-0.32	-0.31	-0.14	-0.01	0.00	-0.02
Lucinda	-0.04	-0.11	-0.30	-0.62	-0.87	-0.89	-0.82	-0.79	-0.69	-0.46	-0.18	-0.06
Mourilyan	-0.13	-0.08	-0.19	-0.42	-0.65	-0.80	-0.85	-0.82	-0.69	-0.48	-0.23	-0.10
Port Kembla	-0.94	-0.98	-0.98	-0.89	-0.59	-0.24	-0.06	-0.03	-0.07	-0.25	-0.59	-0.85
Portland	-0.20	-0.15	-0.06	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	-0.04	-0.15
Port Stanvac	-0.89	-0.91	-0.79	-0.42	-0.08	0.00	-0.02	-0.02	-0.01	-0.05	-0.31	-0.71
Spring Bay	-0.08	-0.08	-0.02	0.00	0.00	-0.07	-0.20	-0.21	-0.09	-0.01	0.00	-0.01
Thevenard	-0.95	-0.83	-0.60	-0.18	-0.01	0.00	0.00	0.00	0.00	-0.15	-0.57	-0.85
Thursday Is.	-0.01	-0.01	-0.06	-0.22	-0.48	-0.64	-0.67	-0.61	-0.45	-0.26	-0.10	-0.03
Townsville	-0.10	-0.14	-0.32	-0.62	-0.90	-0.90	-0.76	-0.77	-0.86	-0.82	-0.65	-0.36

Figure 25 Predicted proportion *of Sabella spallanzanii* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)

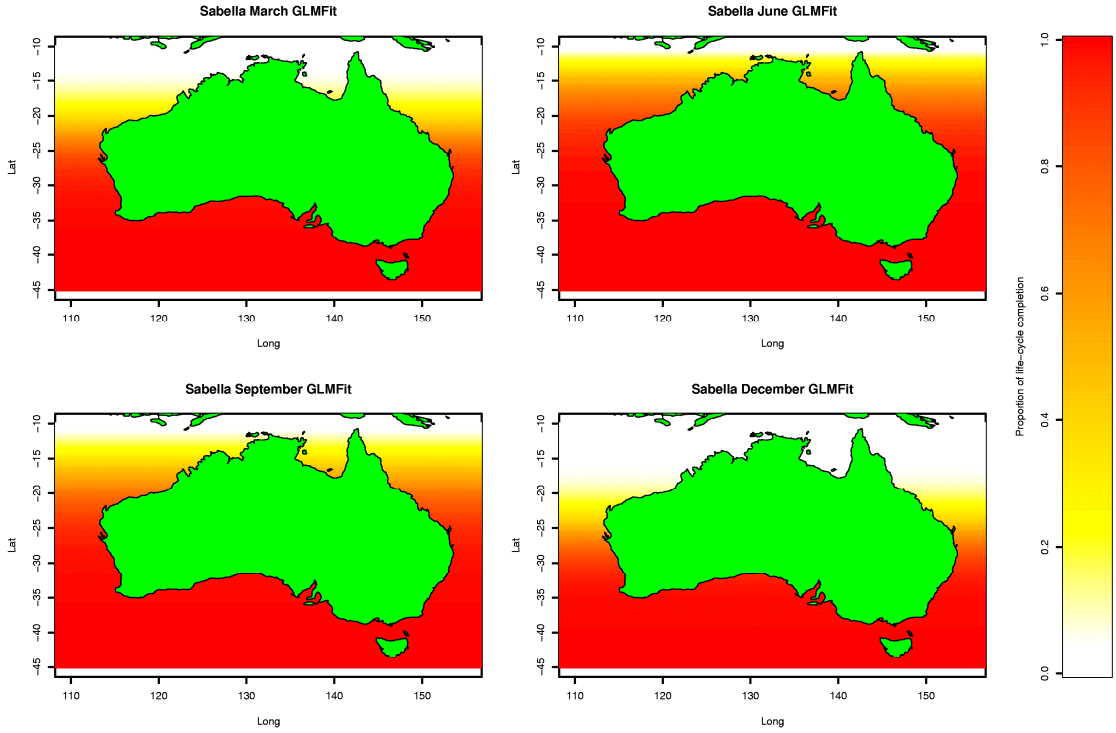
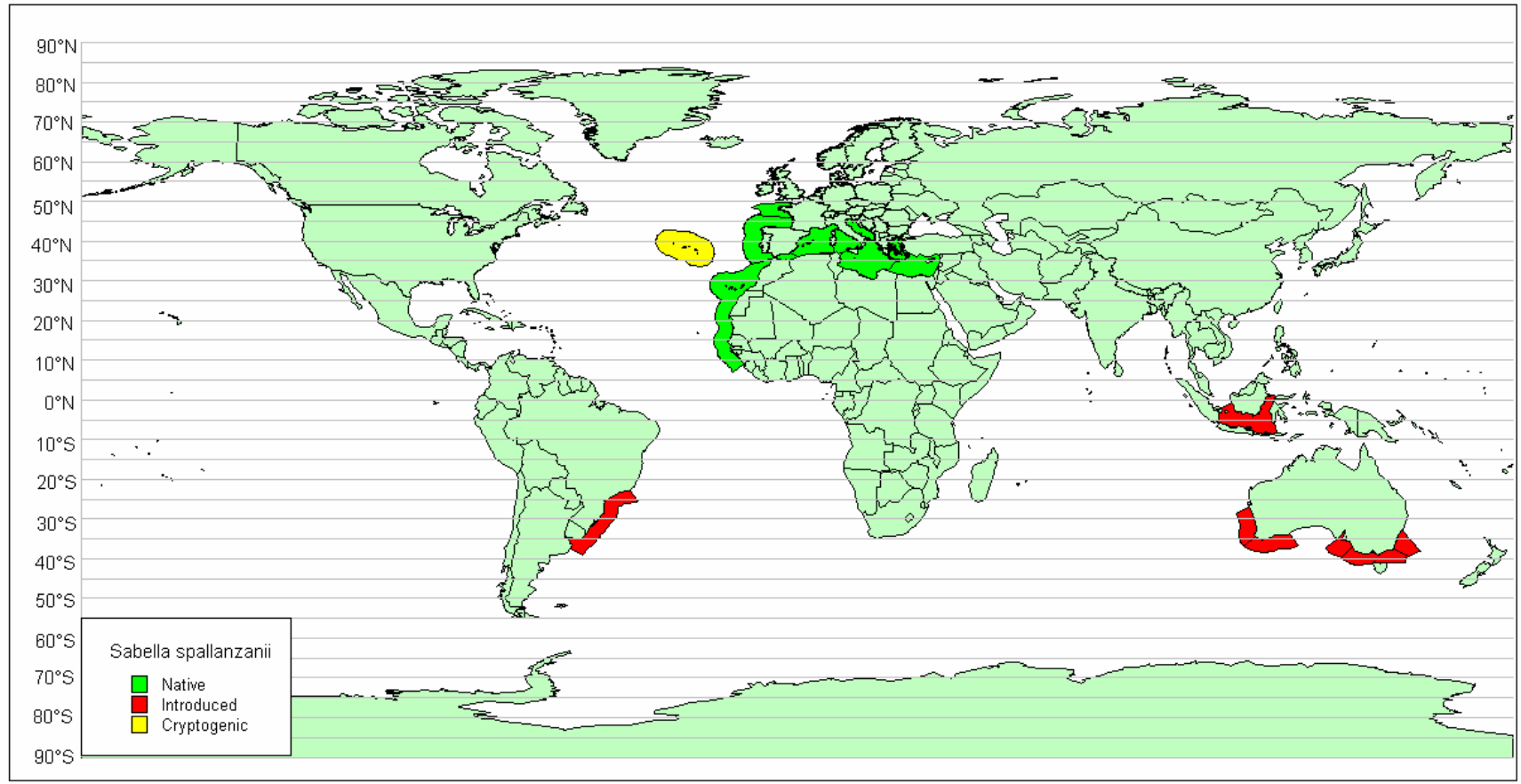


Figure 26 Global IUCN bioregion distribution of *Sabella spallanzanii*



3.2.8 *Undaria pinnatifida*

The independent reviewer of the *Undaria pinnatifida* model provided extensive comments on the life-cycle, life-stage duration and life-stage temperature tolerances of the original model. The reviewer's main points are:

- to amalgamate three of the original model life-stages (gametophyte maturation, sperm release/fertilisation and immature sporophyte) into a single life-stage and re-name "gametophyte cyst" to "dormant gametophyte"; and,

- change the duration and temperature tolerance of life-stage transitions Ia, Ib, IIIb and allow for continuous zoospore release via "sequential maturation" over a period of 30 days.

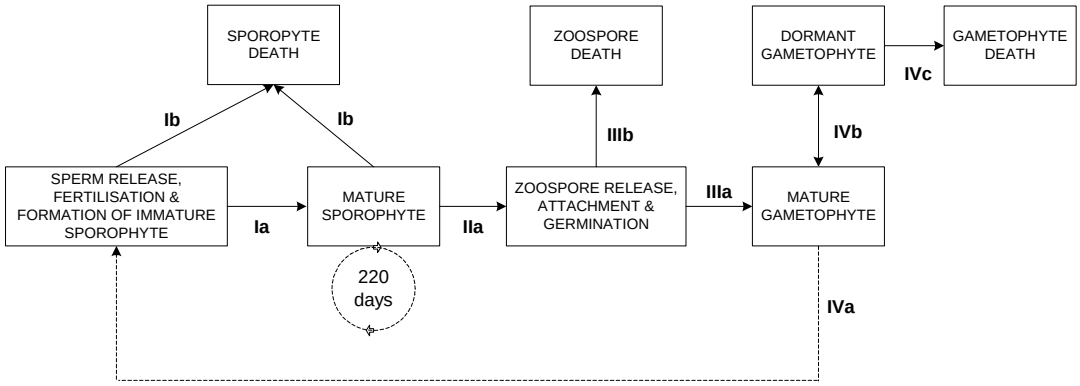
We have accepted most of the reviewer's comments and have made substantial changes to the *Undaria pinnatifida* life-cycle model (Figure 27). In particular we have amalgamated two of the life-stages into a single life-stage called "Gametophyte maturation, sperm release/fertilisation" and removed the reference to "Gametophyte cysts". Note, however, that we have retained the mature gametophyte life-stage. Without this the model could not allow for the possible formation of dormant gametophyte in warmer ports.

We have also amended the temperature tolerance for most life-stages of *Undaria pinnatifida*. In particular we have reduced the upper limit of the maximum temperature tolerance of the mature sporophyte from 28°C to 25°C and amended the statistical representation of this variable to allow for a greater probability mass at 23°C (Figure 28). Furthermore we have amended the upper limit of the temperature tolerance for zoospore release from 29°C to 25°C, the upper limit of the temperature tolerance of dormant gametophytes from 35°C to 30°C and the upper limit of gametophyte fertilisation to 24°C, above which the gametophytes are assumed to become dormant. The model already allows for sequential maturation by simulating introductions on each day of the year and does not therefore require any further amendment in this regard.

In the original model the lower limit on the time taken for sporophytes of *Undaria pinnatifida* to mature was set to 40 days. This has been reduced to 30 days in line with the reviewers' recommendations. Furthermore the time taken from zoospore release has been increased from less than a day (original model) to 3 – 5 days (modelled as uniform random variate). It is important to note that in the amended model, based on the reviewer's recommendations, *U. pinnatifida* can complete is life-cycle in a minimum of 48 days. The reviewer, however, suggests that the minimum duration of the *U. pinnatifida* life-cycle is about 2 – 3 months. The minimum life-cycle duration of a species is an important determinant of the probability that it will complete its life-cycle in a port. We note that the amended model is slightly conservative in this respect.

The overall effect of the changes to the life-cycle model of *Undaria pinnatifida* has been a slight decrease in the proportion of life-cycle completion in warm ports north of Port Kembla, for most of the year. The relatively high maximum temperature tolerance of the dormant gametophytes and the reduction in the minimum life-cycle duration, however, allow *U. pinnatifida* to complete its life-cycle in warm northern ports if introduced during the winter. Again we note that this may be slightly conservative.

Figure 27 Amended life-cycle model of *Undaria pinnatifida*



Transition	Duration	Models	Temperature range	Max/min temperature models
Ia	30 to 60 days	Uniform [30, 60]	0 to 25 deg C	Min ~ Uniform [0, 5]; Max ~ Pert [20, 23, 25]
Ib	Few hours	<1 day	0 > T > 25	Min > T > Max
IIa	Order of days	Uniform [3, 5]	Unknown	Min ~ Uniform [0,5]; Max ~ Uniform [20, 25]*
IIIa	About 14 days	Uniform [14, 30]	0 to 25 deg C	Min ~ Uniform [0, 5]; Max ~ Uniform [20, 25]
IIIb	Few hours	< 1 day	0 > T > 25	Min > T > Max
IVa	Unknown	1 day	0 < T < 24	Min > 0, Max < 24
IVb	Unknown	< 1 day	T > 24	Min < 0, Max > 24
IVc	Few hours	< 1 day	-5 > T > 30	Min ~ Uniform [-5, -3]; Max ~ Uniform [27, 30]

*Inferred from temperature range of germination

Figure 28 Amended life-cycle variates of *Undaria pinnatifida* based on 1000 simulations of the life-cycle model

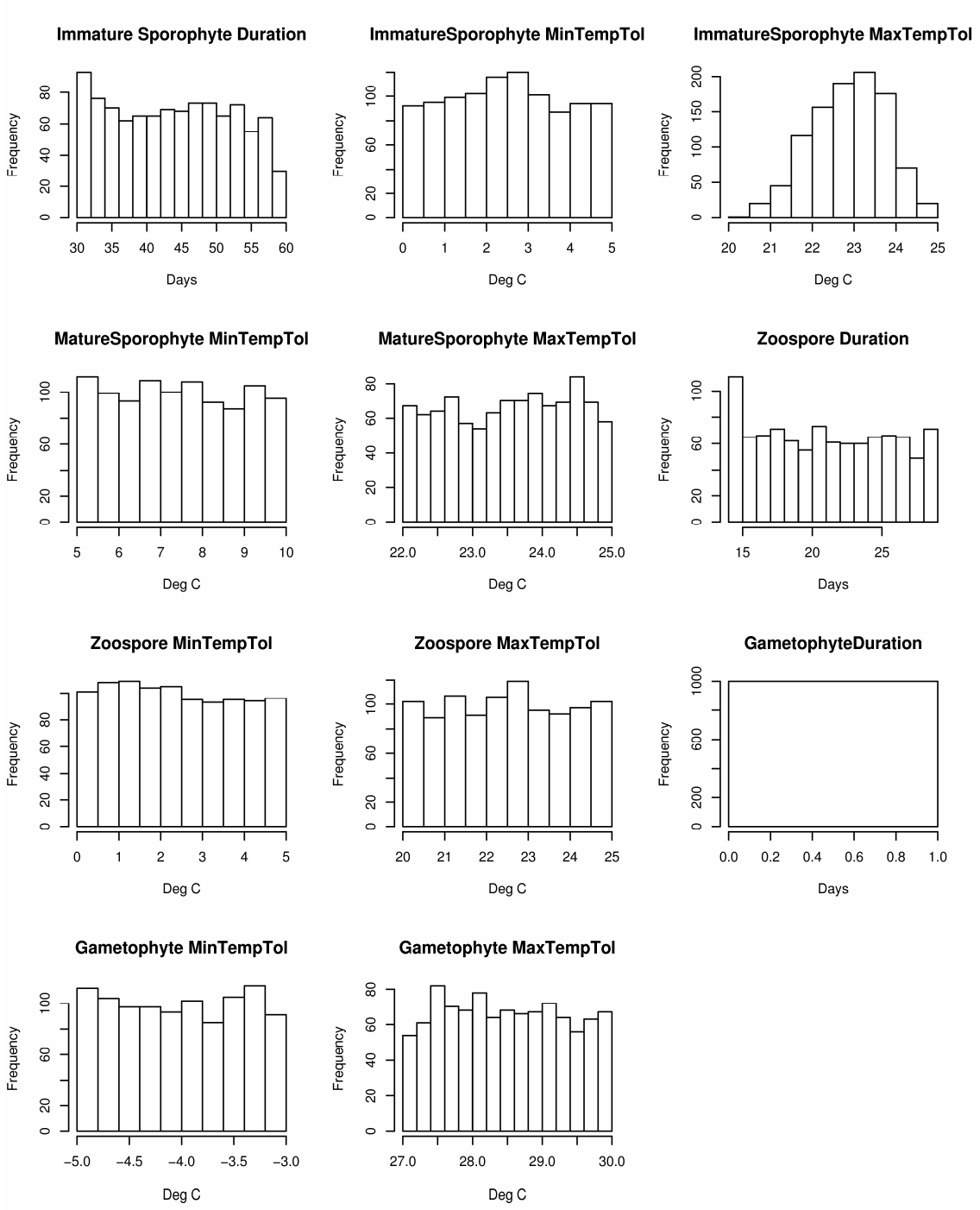


Figure 29 Predicted proportion *of Undaria pinnatifida* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)

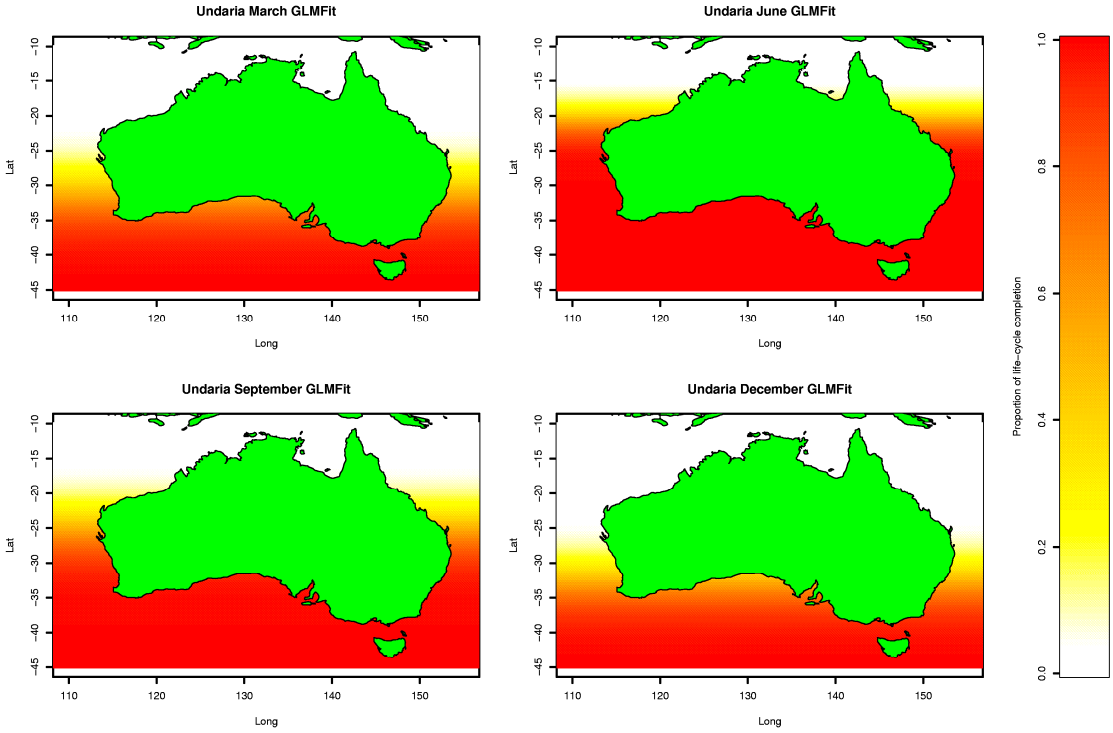
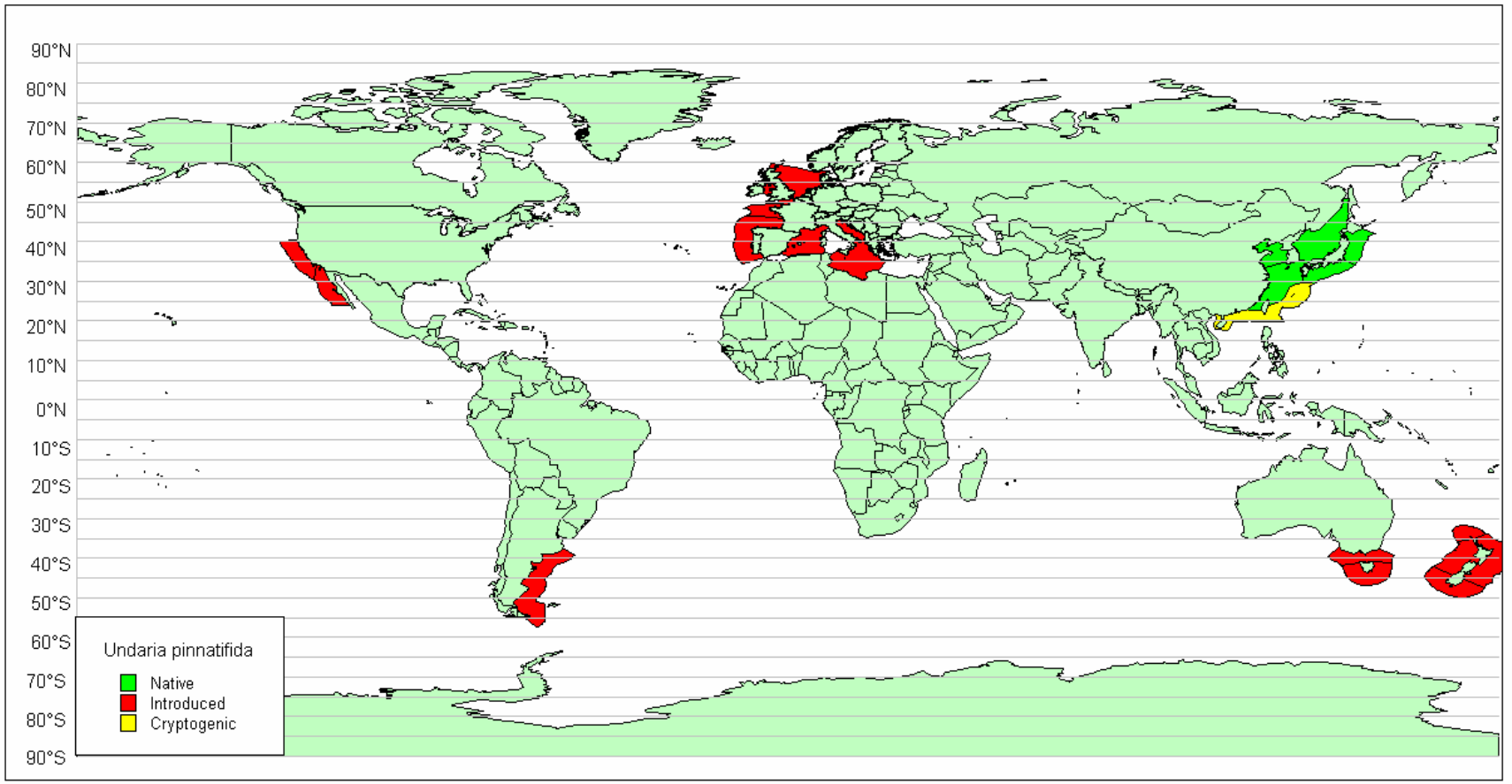


Figure 30 Global IUCN distribution of *Undaria pinnatifida*



The predicted potential distribution of *Undaria pinnatifida* in Australia, based on the amended model, is summarised in Figure 29. This compares well with its global IUCN bioregion distribution (Figure 30). In particular recorded instances of *U. pinnatifida* in the Mediterranean and south China sea suggest that this species may have some capacity to spread into warm-temperate ports.

3.2.9 Varicorbula gibba

Very little life-cycle information is currently available in the literature for *Varicorbula gibba*. Many of the life-stage specific values in the *V. gibba* model were therefore inferred from either other life-stages or from general bivalve development. The independent reviewer of the *V. gibba* life-cycle model and its predicted potential distribution in Australia notes that:

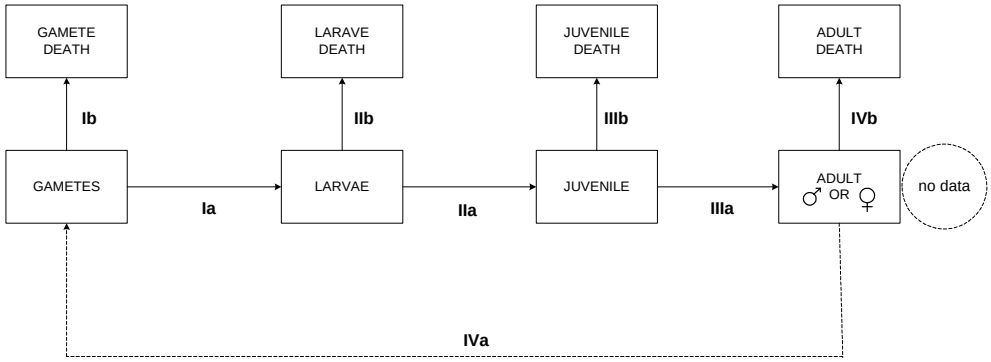
- *V. gibba* larvae must (by definition of the 'long pelagic life') remain in the water column for more than five weeks and must reach sexual maturity in less than a year;
- *V. gibba* is capable of completing its life-cycle in temperatures that range from -1 – 26°C; and,
- the predicted potential distribution in December and June appears to be in error.

In the apparent absence of any other information we propose to accept the reviewers recommendations with respect to the larval duration of *Varicorbula gibba* and have increased it in the new life-cycle model. The difficulty in this context, however, is that the maximum larval duration remains unspecified. In the absence of any species-specific information we have assumed that the upper limit of the larval duration is 70 days and the lower limit of the juvenile duration is 100 days (Figure 31 and Figure 32).

The adult growth and survival temperature tolerance of *Varicorbula gibba* in the original life-cycle model was assigned a range of -1 – 26°C, which was subsequently extended to all other life-stages due to the lack of any other life-stage specific information. This information, however, was drawn exclusively from measurements of ambient temperature in the native and introduced range of *V. gibba*. In the original model this range was extended above and below by two degrees Celsius to allow for uncertainty in the actual temperature tolerance of the species. The independent review has subsequently questioned this, maintaining the species can only survive within the range of -1 – 26°C. We have not, however, amended the model in this regard because the original approach is slightly conservative and maintains a level of uncertainty which we believe is appropriate in these circumstances.

Overall there has been very little change in the proportion of life-cycle completed in Australian ports following the post-review changes to the life-cycle mode of *Varicorbula gibba*. The predicted potential life-cycle completion of *V. gibba* in Australia is summarised in Figure 33. Note that the apparent error in the results noted by the reviewer has been corrected. This original error appears to have been caused by an error in the Visual Basic coding of the original model. This has now been corrected and re-coded in R. The global IUCN bioregion distribution of *V. gibba* is illustrated in Figure 34. This is narrower latitudinally than the amended model's predictions, with the possible exception of the West African record. Again, however, this is likely to be an artefact of the large IUCN bioregion in this area that extends through almost 30 degrees of latitude (i.e. from Morocco to Sierra Leone).

Figure 31 Amended life-cycle model of *Varicorbula gibba*



Transition	Duration	Model	Temperature range	Temperature model
Ia	no data	D = 1	no data	Min = Uniform [-3, -1]; Max ~ Uniform [26, 28]**
Ib	no data	<1 day	no data	Min > T > Max
IIa	Longer than 5 wks	Uniform[35, 70]*	no data	Min ~ Uniform [-3, -1]; Max ~ Uniform [26, 28]**
IIb	no data	<1 day	no data	Min > T > Max
IIIa	Less than a year	Uniform[100, 300]	-1 to 26 deg C	Min ~ Uniform[-3, -1]; Max ~ Uniform [26, 28]
IIIb	no data	<1 day	no data	Min > T > Max
IVa	no data	< 1 day	-1 to 26 deg C	Min = -1; Max = 26
IVb	no data	< 1 day	no data	Min > T > Max

*Inferred from general bivalve development and independant expert review

**Inferred from juvenile/adult data

Figure 32 Amended life-cycle variates of *Varicorbula gibba* based on 1000 simulations of the life-cycle model

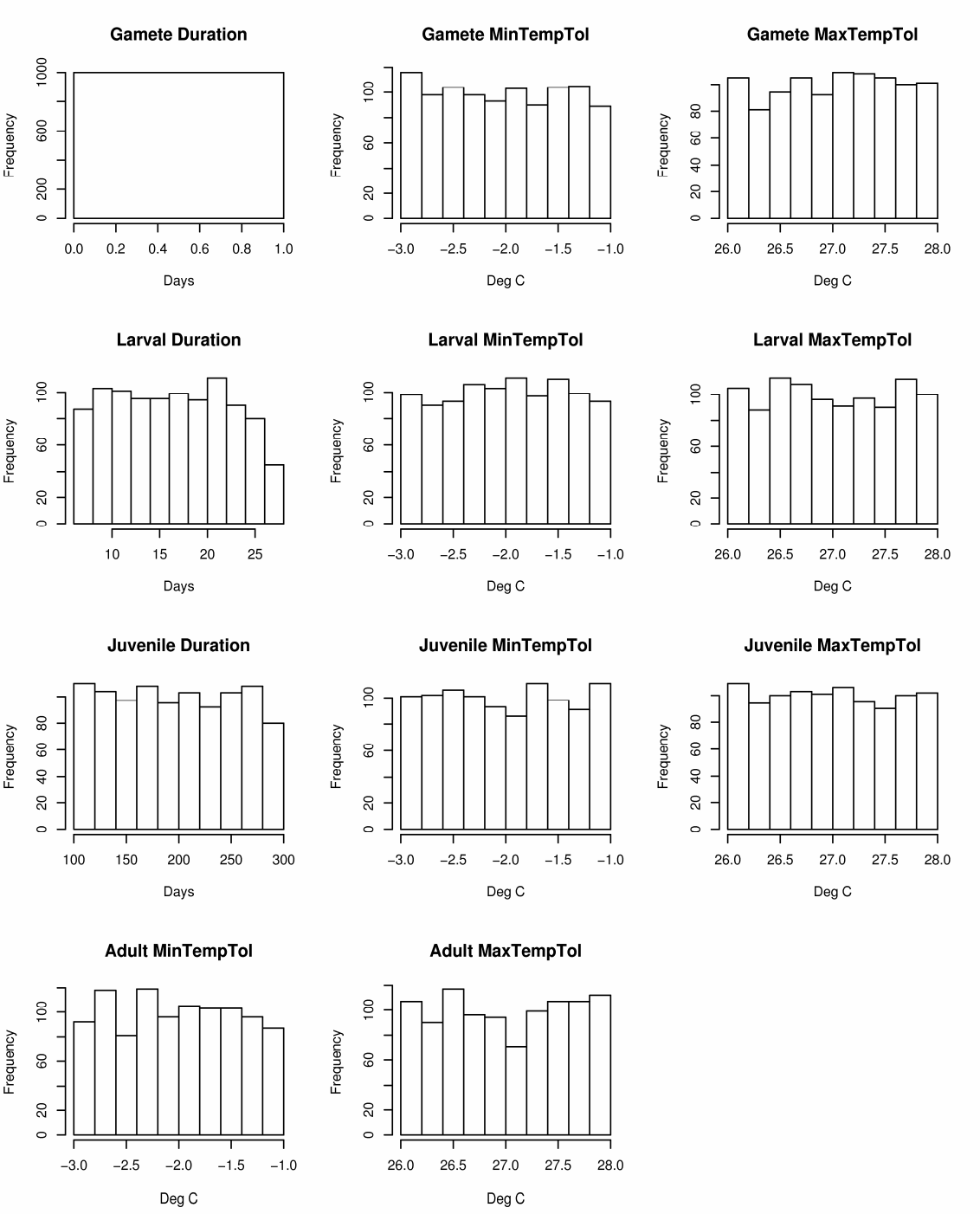


Figure 33 Predicted proportion *of Varicorbula gibba* life-cycle completion in Australia by month of introduction (based on temperature tolerance alone)

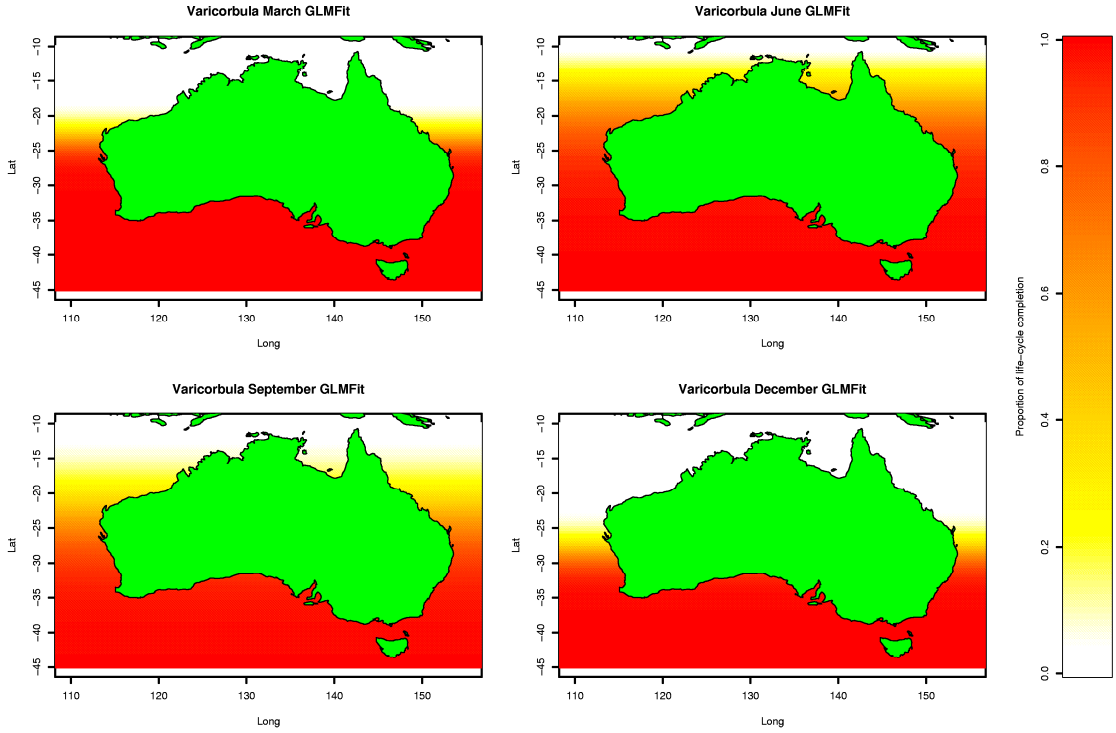
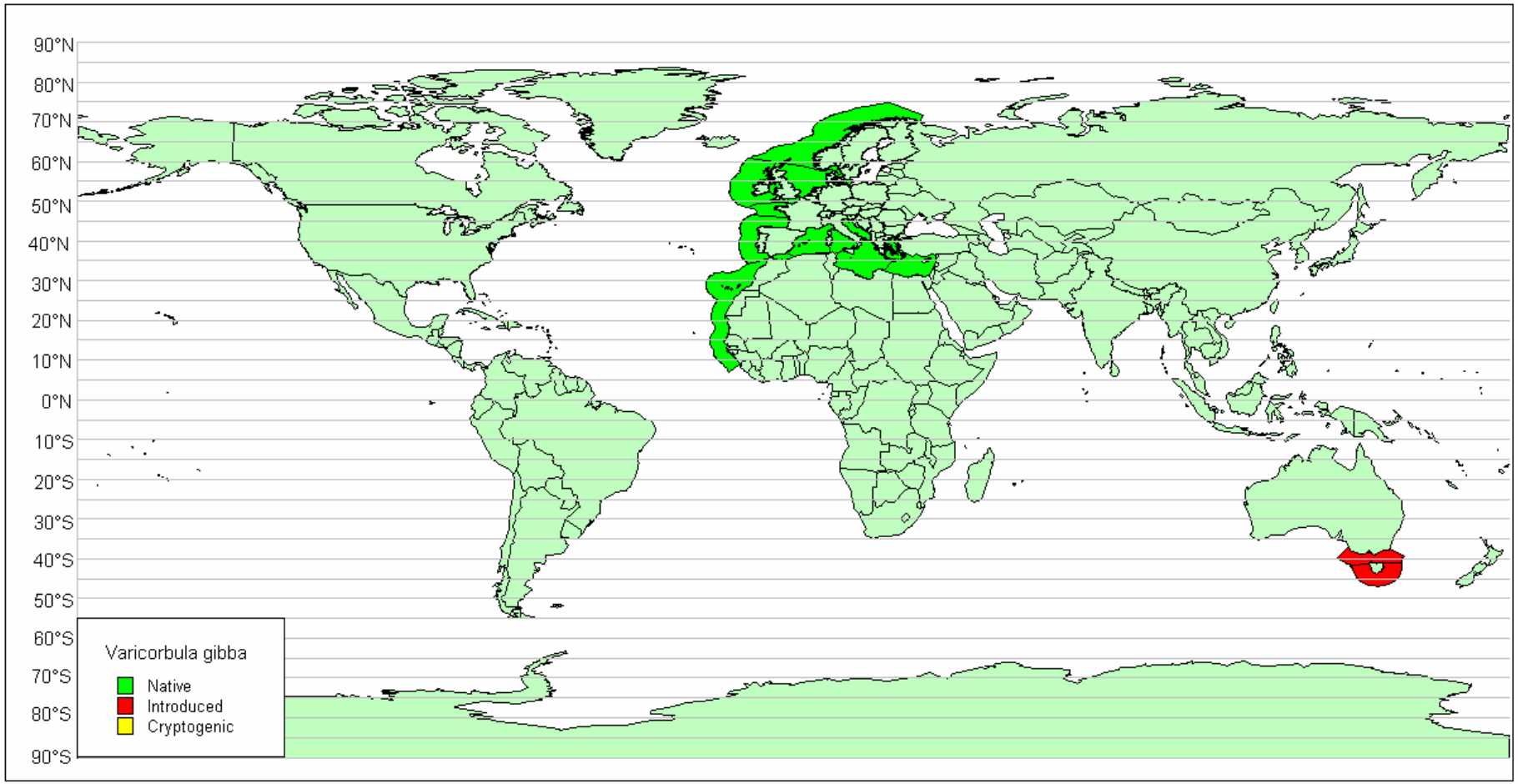


Figure 34 Global IUCN distribution of *Varicorbula gibba*



3.3 Life-cycle completion risk acceptance

The simulation model in Module D predicts the proportion of a species' life-cycle that will be completed in a recipient port for each month of the year that it is introduced. The simulation model captures variability in the important environmental (daily maximum temperature) and biological (life-stage duration and temperature tolerances) parameters, and (where appropriate) makes some allowance for knowledge uncertainty (incertitude) by providing generous bounds on the range of the probability distributions used to represent variability in these biological parameters. The model does not, however, provide *a priori* guidance on the overall proportion of life-cycle completion that should be considered acceptable risk – this is a policy decision.

Theoretically a species cannot become established in a new location unless it is able to complete all (i.e. 100%) of its life-cycle in that location. This limit is clearly high risk in terms of the assessment endpoint (survival in the recipient port) used in the DSS. Conversely species which can only complete a very small proportion (say 5%), or even none, of their life-cycle in a new location are clearly low risk. Managers must select a level of acceptable risk (risk cut-off) that lies on or between these limits. In making this selection managers must trade the comfort and environmental protection provided by a low level, against the benefits and better risk resolution provided by a higher level. This selection can be informed by the uncertainty in the risk prediction (i.e. the confidence limits on the proportion of life-cycle completed) and manager's belief in the accuracy of the prediction.

Figure 35 shows the results of the simulation model for *Asterias amurensis* (whose temperature tolerances we know a lot about) introduced into Townsville, whilst Figure 36 shows the results for *Sabella spallanzanii* (whose temperature tolerances we know relatively little about) introduced into Broome. These figures demonstrate two things:

- the 95% confidence limits (dashed lines) in both cases are small – i.e. variability and uncertainty has a relatively small effect on the mean predicted proportion of life-cycle completion; and,

- selecting a low proportion of life-cycle completion (say 5%) as high risk would significantly reduce the benefits and risk resolution of the DSS – i.e. vessels introducing *A. amurensis* or *S. spallanzanii* larvae into tropical ports would be deemed high risk for at least some part of the year.

Appendix B shows the overall effect on the results of the risk assessment of a high (0.80) versus a low (0.05) cut-off on the proportion of a species life-cycle completed for 7 target species – *Asterias amurensis*, *Carcinus maenas*, *Crassostrea gigas*, *Musculista senhousia*, *Undaria pinnatifda*, *Sabella spallanzanii* and *Varicorbula gibba* - for the busiest 30 ports in Australia based on their ballast water domestic export rank. The Appendix shows the number of months for which shipping routes are high risk for at least one species (donor ports marked with * are unsurveyed). The effect of the cut-off level on the risk assessment results is clearly significant, substantially increasing the number of months which vessels on routes between domestic ports would be deemed high risk for at least one species.

Figure 35 Mean proportion of *Asterias amurensis* life-cycle completion based on 1000 simulated introductions into Townsville

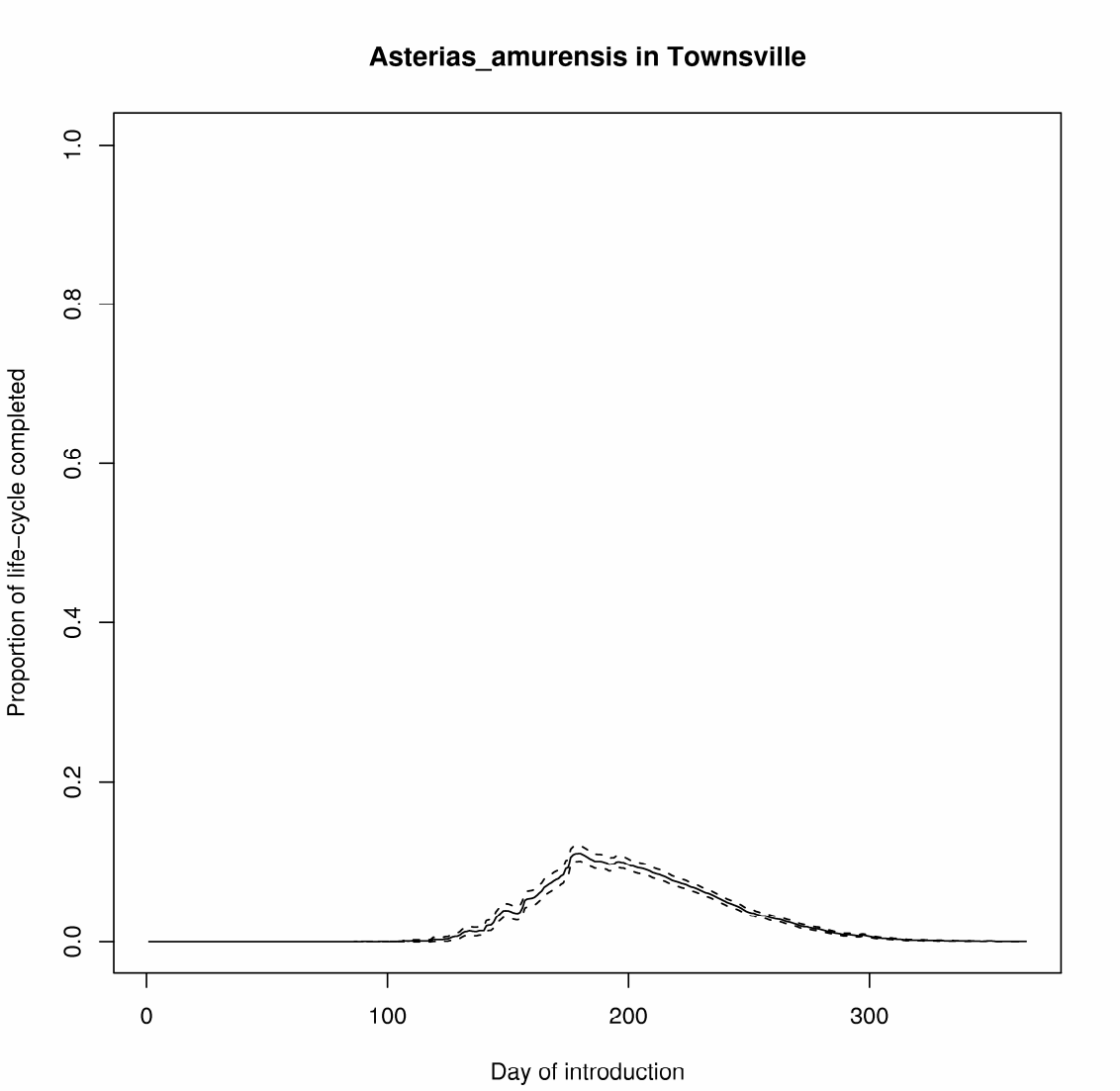
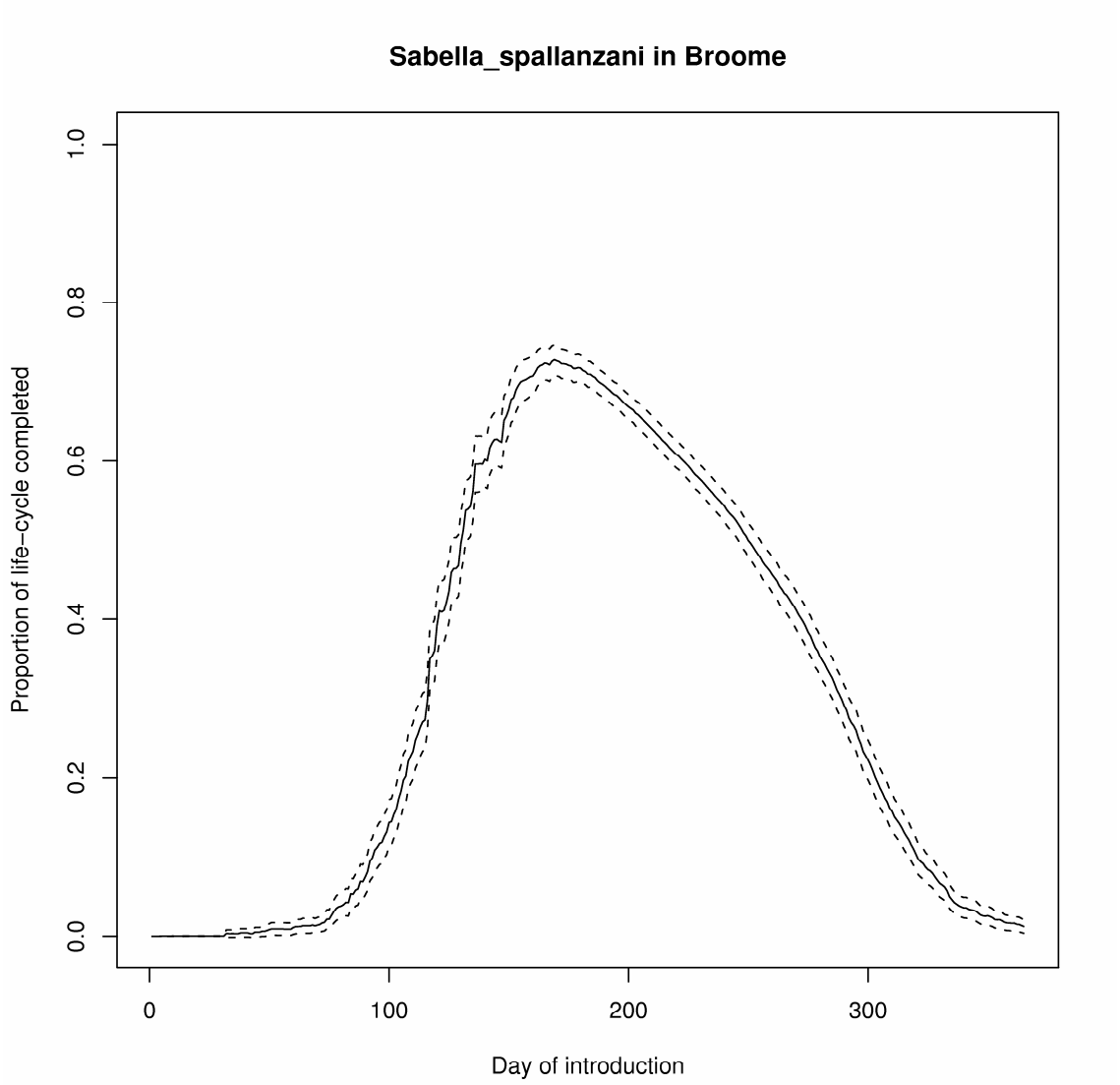


Figure 36 Mean proportion of *Sabella spallanzanii* life-cycle completion based on 1000 simulated introductions into Broome



Much of the SLA research effort over the last two years has been invested in improving the resolution of the DSS risk assessment, particularly Module D, in response to the BRS review of the risk assessment (Barry and Bugg, 2002). The Module has since been significantly improved and now provides demonstrable risk benefits. Moreover, the new model and the life-cycle information has been subject to a rigorous peer review process and its predictions, on the whole, have been compared favourably to the known native and introduced range of each of the target species (see Section 3.2 above). The outcomes of this process confirm that the modelling approach is reasonable and that the predictions are reasonably accurate.

In June 2004 NIMPCG agreed to a 0.05 cut-off based on early advice provided by CMAR. This is an extremely conservative value as it represents an average life-cycle completion of only 5% and is based only on temperature tolerance. It also does not account for other factors that would further reduce the probability of establishment in a new area, and it significantly reduces the benefits of the ballast water risk assessment. We therefore recommend that a much higher proportion of life-cycle completion – 80% – be used as the risk acceptance or "cut-off" value for the purposes of the DSS. This figure maintains some protection against knowledge uncertainty and also maintains good risk benefits.

4. MODULE C – JOURNEY SURVIVAL

4.1 Background

The density and life-expectancy of most species is known to exponentially decline in a ballast tank over the course of a vessel's journey (Hayes, 1998). On long journeys (ten days or more) the high rate of mortality observed in ballast water tanks will reduce the risk of biological invasion by substantially reducing the propagule pressure associated with ballast water discharges. The potential risk reduction gained by ballast tank mortality on short journeys, however, may be much less. Module C of the ballast water risk assessment aims to estimate the life-expectancy of species in the ballast tank based on empirical observations of journey survival. A simple Bayesian life-expectancy model has been developed, but to date it has only been applied to a limited set of observations for *Asterias amurensis* (Hayes, 1998).

In September 2005, CMAR personnel boarded the Bulk Carrier *MV Iron Sturt* to collect daily ballast water samples and provide information on the journey survival characteristics of *Asterias amurensis*, and the effects of ballast water exchange, over the course of a 12-day voyage. Delays in gaining permissions to board the vessel prevented us from gaining access to the vessel earlier in the larval season (a time more optimal for *A. amurensis* larvae in the plankton). Identification of asteroid larvae using morphological criteria is difficult due both to the morphological similarity of larvae across species, genera and families and the plasticity of asteroid larval morphology within species. Molecular techniques based on the genetic probe developed by Deagle *et al.*, (2003) were therefore used to detect presence/absence of the *Asterias* larvae.

4.2 Material and methods

4.2.1 Sample collection

Three replicate drop net samples were collected (approximately 4600 litres) from the Zinifex zinc smelter wharf (Risdon, Port of Hobart; Figure 37) on the 9th September 2005 (morning of the departure of the *MV Iron Sturt*) to obtain a baseline measurement of the presence/absence of *Asterias amurensis* larvae in the Derwent estuary at the time of ballast uptake. Onboard the vessel, the First officer assigned two ballast water tanks (4 Port Aft and After Peak) as suitable for investigation. Both ballast tanks were subsequently filled with seawater that morning. One tank (4 Port Aft) was to have no ballast water exchange during the trip, and could therefore act as a control, the other (After Peak) was assigned as a potential exchange tank.

Samples were taken from both ballast tanks, as close to every 24 hours as the ships operations would allow. Access to the 4 Port Aft tank was only possible for six days, whereas access to the After Peak tank continued throughout the twelve day journey. The tanks were accessed through deck manhole covers that were removed and replaced on each sampling occasion. A pneumatic driven diaphragm pump fitted with a 25 mm inlet and outlet hose was used to sample water from 1 − 2 metres below the water surface. Approximately 750 litres of ballast water were

filtered through a 100 µm net to obtain each sample. Three replicate samples were collected from each ballast tank at each sampling time. Samples were initially rinsed into a jar with seawater until all samples were collected. Samples were then rinsed through a small 100 µm net to remove water, fixed in SET (0.75 M NaCl, 5mM EDTA, 80mM Tris HCl, pH 7.8) buffered 80% ethanol fixative and held in a cool room until the trip was complete. Samples were then refrigerated in the laboratory until DNA extraction.

4.2.2 DNA extraction and PCR amplification

All the fixed drop net and pump samples were concentrated by vacuum filtration through a 5 µm pore-sized hydrophilic Durapore Filter (Millipore). The residue was briefly air-dried, weight measured, transferred to a 1.5 ml tube and DNA extracted using the DNeasy Plant mini Kit (QIAGEN) following suppliers instructions. DNA was retrieved in 200 µl elution buffer quantified using UV spectrophotometer (Beckman) and stored at 4 °C. All the plankton samples were diluted to get < 10 ng DNA before PCR amplification.

A two-step nested PCR was used for plankton samples to enhance the sensitivity of the test. Primary enrichment PCR was conducted using the universal primer pair ECOLa-F (Knott and Wray, 2000) and HCO-R (Folmer *et al.*, 1994). The secondary *Asterias* specific PCR was carried out using the 1/25th the volume of the primary reaction as template (Deagle *et al.*, 2003) A separate PCR reaction was carried out on all samples using universal 18S rDNA primers. Aerosol-resistant pipette tips were used with all PCR solutions and negative control reactions were performed with each PCR cocktail. Amplified products were electrophoresed on an 1.8% agarose gel and visualised on a Bio Rad XR Gel doc system.

4.3 Results

The *MV Iron Sturt* departed the Port of Hobart and took 12 days to complete an interstate circuit with stops at the ports of Geelong (VIC), Port Pirie (SA) and Burnie (TAS) before returning to Hobart via the east coast of Tasmania. The temperature increases recorded in the ballast water tanks reflect the warmer sea temperatures during and immediately after the transit through the Spencer Gulf, after which they cool due to conduction of temperature through the vessel's metal hull as the vessel proceeded south into cooler waters.

Water temperature in the 4 Port Aft tank increased from 12.5°C to a maximum of 14.9°C on day 6 – the last day that measurement was possible (Table 6), whereas the water temperature in the After Peak tank increased from 12.7°C to a maximum of 17.4°C (day 8) after which it declined to a minimum of 13.4°C on day 12 (Table 7) at the journey's end. Day 1 water temperatures are indicative of Port of Hobart ambient water temperature as samples were taken immediately after ballast uptake. It is possible that the larger volume of water in the 4 Port Aft tank (1230 m^3) contributed to the lower temperature maximum compared to After Peak tank (140 m^3), however, restricted access to the 4 Port Aft tank prevented a continuous comparison between the two tanks. The salinity measurements in both tanks remained stable throughout the journey.

Table 6 Results of the ballast water samples taken from the Derwent estuary and the 4 Port Aft ballast tank of the *MV Iron Sturt*

Location	Date	Time	No.	Wt (g)	DNA (ng/ul)	18S	Ast.	Temp (°C)	Sal (ppm)
Drop net	09-Sep-05	12:00	1	0.3382	145.97	1	0		
Drop net	09-Sep-05	12:00	2	0.3776	112.41	1	0		
Drop net	09-Sep-05	12:00	3	0.2765	93.52	1	1		
4 Port Aft	09-Sep-05	15:35	4	0.0306	4.871	1	1	12.5	39.8
4 Port Aft	09-Sep-05	15:35	5	0.0593	16.679	1	0	12.5	39.8
4 Port Aft	09-Sep-05	15:35	6	0.0225	4.734	1	0	12.5	39.8
4 Port Aft	10-Sep-05	14:35	10	0.2458	22.686	1	0	12.5	39.8
4 Port Aft	10-Sep-05	14:35	11	0.1675	3.525	1	0	12.5	39.8
4 Port Aft	10-Sep-05	14:35	12	0.4095	21.164	1	0	12.5	39.8
4 Port Aft	11-Sep-05	8:10	16	0.0313	0.147	1	0	13.3	40.1
4 Port Aft	11-Sep-05	8:10	17	0.0278	0.032	1	0	13.3	40.1
4 Port Aft	11-Sep-05	8:10	18	0.0083	0	1	0	13.3	40.1
4 Port Aft	13-Sep-05	8:30	22	0.0243	0.549	1	0	13.7	40.1
4 Port Aft	13-Sep-05	8:30	23	0.0329	5.686	1	0	13.7	40.1
4 Port Aft	13-Sep-05	8:30	24	0.1524	0.116	1	0	13.7	40.1
4 Port Aft	13-Sep-05	21:10	28	0.0432	0	1	0	14.6	40.1
4 Port Aft	13-Sep-05	21:10	29	0.0235	0	1	0	14.6	40.1
4 Port Aft	13-Sep-05	21:10	30	0.0934	0	1	0	14.6	40.1
4 Port Aft	14-Sep-05	15:00	34	0.021	0	1	0	14.9	40
4 Port Aft	14-Sep-05	15:00	35	0.0379	0	1	0	14.9	40
4 Port Aft	14-Sep-05	15:00	36	0.0369	0	1	0	14.9	40

Table 7 Results of the ballast water samples taken from the After Peak ballast tank of the *MV Iron Sturt*

Location	Date	Time	No.	Wt (g)	DNA (ng/ul)	18S	Ast.	Temp (°C)	Sal (ppm)
After Peak	09-Sep-05	18:15	7	0.0608	8.157	1	0	12.7	39.8
After Peak	09-Sep-05	18:15	8	0.0165	9.282	1	0	12.7	39.8
After Peak	09-Sep-05	18:15	9	0.1186	18.587	1	1	12.7	39.8
After Peak	10-Sep-05	13:30	13	0.0247	6.648	1	0	13.3	40.1
After Peak	10-Sep-05	13:30	14	0.0763	5.918	1	0	13.3	40.1
After Peak	10-Sep-05	13:30	15	0.0259	5.398	1	0	13.3	40.1
After Peak	11-Sep-05	9:10	19	0.0621	0	1	0	14.2	40
After Peak	11-Sep-05	9:10	20	0.0673	1.278	1	0	14.2	40
After Peak	11-Sep-05	9:10	21	0.0342	0	1	0	14.2	40
After Peak	13-Sep-05	9:35	25	0.1452	19.755	1	0	14.7	40.1
After Peak	13-Sep-05	9:35	26	0.0297	6.069	1	0	14.7	40.1
After Peak	13-Sep-05	9:35	27	0.0454	8.575	1	0	14.7	40.1
After Peak	14-Sep-05	8:30	31	0.2202	0.245	1	0	15.5	40
After Peak	14-Sep-05	8:30	32	0.16	0	1	0	15.5	40
After Peak	14-Sep-05	8:30	33	0.0284	0.988	1	0	15.5	40
After Peak	15-Sep-05	9:30	37	0.0378	0	1	0	16.4	40
After Peak	15-Sep-05	9:30	38	0.0576	1.312	1	0	16.4	40
After Peak	15-Sep-05	9:30	39	0.0385	0.682	1	0	16.4	40
After Peak	16-Sep-05	8:25	40	0.019	1.683	1	0	17.4	39.9
After Peak	16-Sep-05	8:25	41	0.0272	0.937	1	0	17.4	39.9
After Peak	16-Sep-05	8:25	42	0.0277	0.3	1	0	17.4	39.9
After Peak	17-Sep-05	10:45	43	0.0296	0.193	1	0	16.9	40
After Peak	17-Sep-05	10:45	44	0.0728	0.574	1	0	16.9	40
After Peak	17-Sep-05	10:45	45	0.0274	0.346	1	0	16.9	40
After Peak	18-Sep-05	13:30	46	0.1583	0.53	1	0	15.4	40
After Peak	18-Sep-05	13:30	47	0.0482	4.518	0	0	15.4	40
After Peak	18-Sep-05	13:30	48	0.2102	0.254	1	0	15.4	40
After Peak	19-Sep-05	8:35	49	0.0754	6.031	0	0	14.2	40
After Peak	19-Sep-05	8:35	50	0.05	5.327	0	0	14.2	40
After Peak	19-Sep-05	8:35	51	0.1123	8.036	0	0	14.2	40
After Peak	20-Sep-05	8:50	52	0.1139	2.126	1	0	13.4	40
After Peak	20-Sep-05	8:50	53	0.1125	1.952	1	0	13.4	40
After Peak	20-Sep-05	8:50	54	0.0988	3.47	1	0	13.4	40

Asterias specific genetic probes were applied to 54 samples: 3, 18 and 33 collected from, Zinifex zinc smelter wharf (Port water samples), 4 Port Aft and the After Peak ballast tanks respectively. Initial attempts to morphologically screen the samples for *Asterias amurensis* larvae were unsuccessful in all the samples analysed. The gene probes detected *Asterias* specific DNA in one out of three replicates of the Derwent samples. Similarly only one of the three ballast water samples collected from both the ballast tanks on first day of the voyage were positive for *Asterias* (i.e. one out of each of three replicates). All of the remaining samples gave negative results. All of the samples, however, tested positive against the 18S rDNA universal probes, indicating that the DNA obtained from these samples was adequate for gene probe testing, with the exception of one of, and all of, the three samples collected from the After Peak ballast tank on the 18th and 19th September respectively (Table 6 and Table 7).

The low frequency of positive samples in the Derwent samples is unexpected because the samples were taken towards the end of the peak *Asterias amurensis* spawning period. The peak spawning period of *A. amurensis* in the Derwent estuary is August to September, however, the initial onset of spawning can vary substantially from year to year - for example between August (2001) and April (2002) (Johnson *et al*., 2004). *A. amurensis* gametes and larvae are present in the estuary from the onset of spawning to December and occasionally January (see Section 5.2). The abundance of larvae in the estuary, however, varies significantly. Multiple spawning events create various peaks in abundance through the winter, during which time larvae are adverted into Storm Bay. Hence, the density of *A. amurensis* larvae during the latter part of the spawning season is highest in the lower reaches of the Derwent estuary (Johnson *et al.*, 2004). Furthermore larvae are flushed from the high estuary are very high rates during periods of river spates and this may explain the very low proportion of positive samples recorded from site of ballast uptake - the Zinifex zinc smelter wharf – in this study.

The number of ballast water samples that produced PCR positive results was also very low: only 2 of the 51 samples tested positive for *Asterias* larvae. It was therefore not possible to gain detailed insights into the journey survival characteristics of *Asterias amurensis* larvae based on this experimental data. Future journey survival experiments, however, will undoubtedly benefit from the field experience gained on this occasion and from the estimates of target larval density at the site of ballast uptake using recently developed real time molecular probes (Bax *et al.*, 2006). Additional trips planned for the next *A. amurensis* season in the winter of 2006, were cancelled after discussions with AGDAFF because the results of the journey survival models were not predicted to provide substantial risk reductions on short domestic journeys.

5. MODULE B – VESSEL INFECTION

5.1 Background

Module B calculates the probability that a vessel loading ballast water will be infected with a target species. Vessel infection dynamics depend on number of factors, notably the habitat of the target species through its various life-stages (e.g. holoplanktonic versus meroplanktonic), its size, the size of the mesh on the ballast pump, the depth of the port and the activity of vessels in and around the port (Hayes and Hewitt, 2001; Hayes, 2002). Many of these factors, however, are too complex to reliably predict and the Module is therefore operating in a binary fashion by assuming that all of the juvenile/adult life-stages of the target species are excluded from the ballast tank by size[4], and simply recording the presence or absence of the egg/gametes and larval life-stages of the target species in the plankton for each month of the year (Hayes and McEnnulty, 2002; Hayes and Sliwa, 2003).

Module B can potentially provide significant risk reductions, particularly for species which have a short larval duration. Information on the larval duration of most of the target species, however, is limited. In the absence of this information the Module conservatively assumes that gametes/larval life-stages are in the water column for six (e.g. winter/summer) or twelve months of the year. One of the objectives of the second SLA was therefore to collect additional literature and empirical information on the larval duration of the target species.

5.2 *Asterias amurensis*

Table 8 and Table 9 summarise recent records of *Asterias amurensis* in the plankton in Port Phillip Bay and Hobart. These records are collated from numerous sources, using traditional morphological and gonad indices methods, together with the more recent *Asterias* specific gene probe (Deagle *et al.*, 2003). The probe is significantly more sensitive than previous morphologically-based identification methods, and has significantly enhanced our understanding of the plankton dynamics of *A. amurensis*. These results suggest that gametes and larvae of *A. amurensis* are likely to be present in the water column from May to December in Port Phillip Bay, and potentially from April to January in the Derwent estuary.

In this context it is important to note that the probe developed by Deagle *et al.*, (2003) does not quantify the amount of DNA present in each sample. Positive samples may therefore reflect very low levels of gametes or larvae that may or may not pose a significant bioinvasion hazard. The absence of a strong positive signal in port and ballast tank samples taken in September 2005 in the Port of Hobart (see Section 4.3) attests to the presence of low concentrations of larvae that quickly die once taken onboard the vessel. This issue may be resolved further following the recent development of the real time *Asterias* probe (Bax *et al.*, 2006) that is capable of quantifying the amount of DNA in each sample, and by inference, the concentration of gametes or larvae.

[4] Excluding dinoflagellates that are conservatively assumed to be available for all months of the year.

Table 8 *Asterias amurensis* larval duration periods – Port Phillip Bay (reproduced from Hough and Dommisse, 2004)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1999[3,#]	0	0	0	0	0	0	1	1	1	1	1	1
2001[3,#]	0	0	0	0	0	1	1	1	1	1	1	1
2002[1,*]	0	0	0	0	1	1	1	1	1	1	1	0

Table 9 *Asterias amurensis* larval duration periods – Derwent (amended from Hough and Dommisse, 2004)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1993/4[5,#]	1	0	0	0	0	0	1	1	1	1	1	1
1995/7[4,$]	0	0	0	0	0	1	1	1	1	1	1	1
1999[2,&]	0	0	0	0	0	1	1	1	1	1	1	1
2001[1,2,*,&]	0	0	0	0	0	0	0	1	1	1	1	1
2002[1,2,*,$]	0	0	0	1	1	1	1	1	1	1	1	1
2004[6,*]	1	0	0	1	1	1	1	1	1	1	1	1

1 – Hough and Dommisse (2004); 2 – Johnson *et al.*, (2004); 3 – Parry and Cohen (2001); 4 – Sutton and Green (1999); 5 – Byrne *et al.*, (1997); 6 – Hayes *et al.*, (2004b).

* – Identification by gene probe; &– morphological identification, $ – morphological identification with gene probes used to verify a sample subset; # –gonad indices

5.3 *Crassostrea gigas*

5.3.1 Background

Crassostrea gigas is reported to spawn sometime during the summer months (Fabioux *et al.*, 2005, Chavez-Villalba *et al.*, 2003) and the larval phase is reported to last 15-30 days depending on temperature prior to settlement (NIMPIS, 2002; Mitchell *et al.*, 2000). Currently, however, there are no precise data about the spawning and larval duration of *C. gigas* in the Tasmanian ports of Burnie, Devonport and Hobart. Module B therefore simply assumes that *C. gigas* gametes or larvae are in the water column and available for potential entrainment by vessels that uptake ballast throughout the summer months from October to April inclusive. This relatively long larval duration may be over-conservative and unnecessarily penalise vessels travelling from infected Tasmanian ports to uninfected Victorian ports, particularly vessels travelling across Bass Strait from Burnie to Melbourne.

Logistical restraints prevented us from completing a systematic plankton survey in Burnie. This was, however, successfully achieved in the Port of Hobart. This survey employed molecular techniques based on the work of Patil *et al.*, (2005) to detect the presence/absence of *C. gigas* larvae in the plankton. It is difficult to identify bivalve larvae using morphological criteria

because of the morphological similarity of larvae across several species and the plasticity of bivalve larval morphology within species. Moreover, it is considered almost impossible to distinguish trochophore (and veliger) larvae of bivalves, even with the use of electron microscopy.

5.3.2 Methods

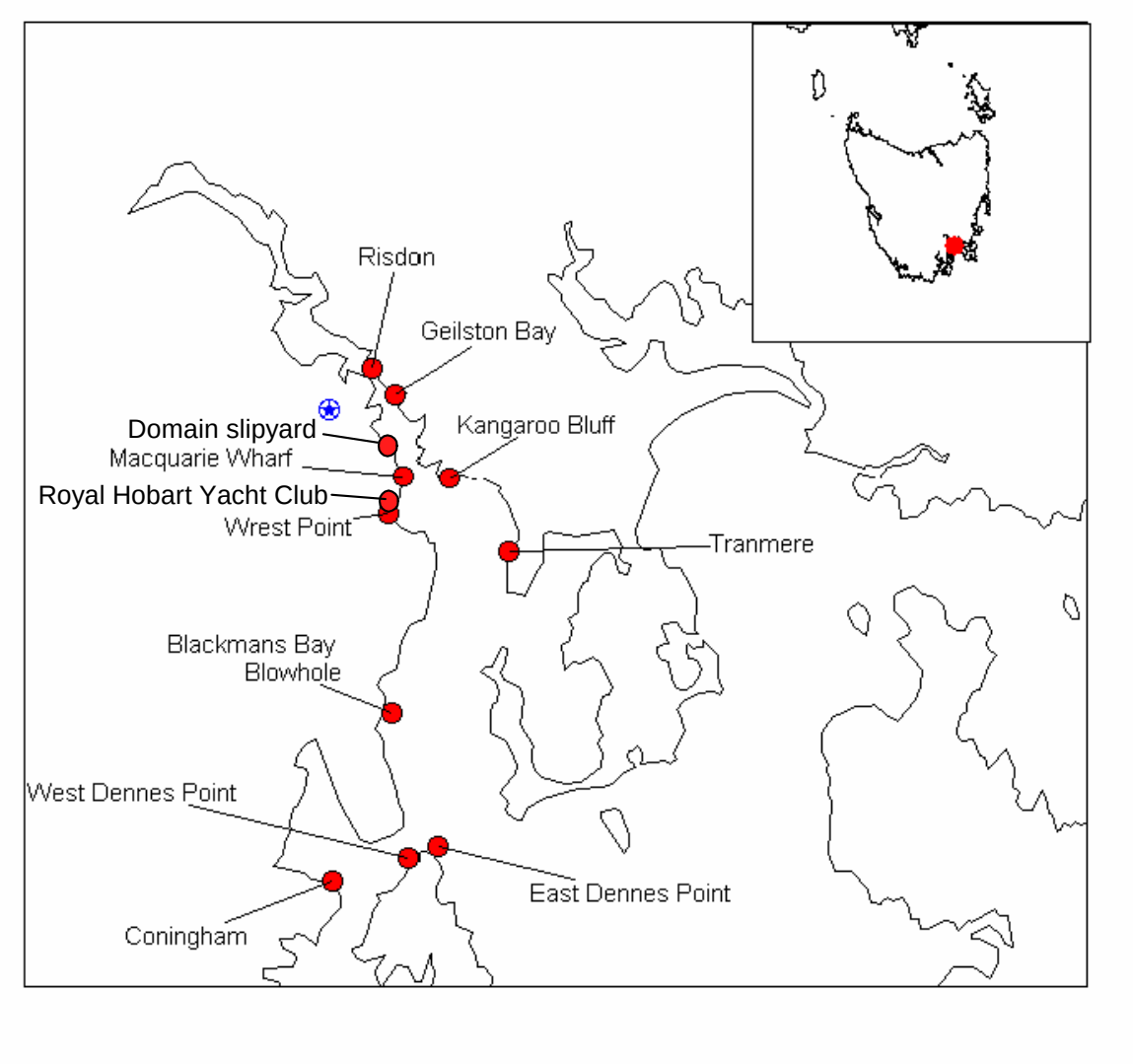
Plankton samples were collected from several sites in the Derwent Estuary (Figure 37). The Derwent Estuary is a relatively deep (up to 44 m) estuary that receives a consistently high freshwater input from the Derwent River. The middle and lower sections of the estuary are well mixed by tidal and wind-driven circulation, with large vertical movements of water. The upper reaches are highly stratified, characterised by a distinct salt wedge (Thomson and Godfrey 1985, Davies and Kalish, 1994).

Plankton samples collected by CMAR personnel during an earlier separate study (Hayes *et al.*, 2004b) were analysed using the *Crassostrea gigas* probe to provide further insights in the larval duration of *C. gigas* in the Derwent estuary and to assist in the sample design for this study. These samples were collected within the Port of Hobart by pumping seawater through 100 µm mesh plankton net on 34 occasions between August 2003 and September 2004. Significant *C. gigas* beds are known to exist in the vicinity of both of the sample locations (Hobart Royal Yacht Club and Hobart Domain slipway). This, in conjunction with ready availability of samples, prompted the prior analysis to provide a snapshot on which to base a more detailed sampling regime.

The detailed sampling regime for this study was designed to collect larval *Crassostrea gigas* from locations where adult specimens were known to exist (Mitchell *et al.*, 2000; Aquenal, 2002) over a period that was expected to span the duration of the known spawning season. Consideration was also given to the proximity of the sampling site to active ports as well to estuarine flow dynamics. In all ten sample stations were selected, from Risdon (Zinifex zinc smelter) in the upper reaches of the estuary, down to the estuary mouth (East Dennes point), representing a mix of open channel and embayment locations (Figure 37). Plankton samples were collected at fortnightly intervals from December 2005 to April 2006.

At each sampling station, plankton were filtered from the water column with a drop-net. The drop net had a mouth diameter of 0.7 metres (opening of 0.39 m^2), a mesh size of 100 µm and was fitted with a choke collar so that filtering only occurred on the descent. The net was deployed from the side of the vessel and allowed to sample the water column down to approximately 9 metres depth, filtering ~3.5 m^3 of seawater. Samples were all preserved with 95% reagent grade ethanol immediately after collection. Three replicate samples were collected at each sampling station. The nets and cod-end were cleaned between sampling stations by soaking in a freshwater bath for 10 – 20 minutes. Water temperature and salinity profile data was collected at each station on each occasion with a ‘Platypus’ submersible data logger (model PSW-CTD02) lowered down to 9 metres depth.

Figure 37 *Crassostrea gigas* plankton sample locations in the Derwent estuary, Tasmania.



Collected samples were processed in the laboratory to remove larger particles and fixed in SET (0.75 M NaCl, 5mM EDTA, 80mM Tris HCl, pH 7.8) buffered 80% ethanol fixative. Samples were kept at 4°C until DNA extraction. All pre-processed and fixed plankton samples were concentrated by vacuum filtration through a 5 µm pore-sized hydrophilic Durapore Filter (Millipore). The residue was briefly air-dried, weight measured, transferred to a 50 ml tube and DNA extracted using the DNeasy Plant maxi Kit (QIAGEN) following suppliers instructions. DNA was retrieved in 1500 µl elution buffer quantified using UV spectrophotometer (Beckman) and stored at 4 °C. All the plankton samples were diluted to get 5-7 ng DNA before PCR amplification.

A two-step nested PCR was used for plankton samples to enhance the sensitivity of the test. Primary enrichment PCR was conducted using mitochondrial COI universal primer pair LCO-F and HCO-R (Folmer *et al.,* 1994). The secondary *C. gigas* specific PCR was carried out using the 1/25th the volume of the primary reaction as the template (Patil *et al.*, 2005). A separate PCR reaction was carried out on all plankton samples using universal 18S rDNA primers. Aerosol-resistant pipette tips were used with all PCR solutions and negative control reactions were performed with each PCR cocktail. Amplified products were electrophoresed on a 1.8% agarose gel and visualised on a Bio Rad XR Gel doc system.

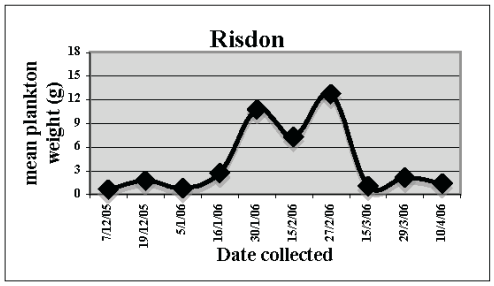
5.3.3 Results

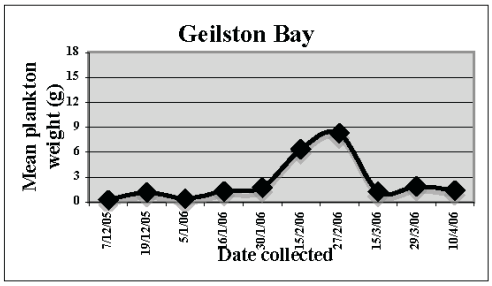
A total of 102 plankton samples from the earlier CMAR study were subject to genetic analysis. The results (Appendix C) indicate that *Crassostrea gigas* gametes and/or larvae are predominately present in the water column between February and March. The high frequency of positive results in February and March suggest the peak spawning may occur in these months. Two samples collected in the last week of September 2004, however, also returned positive for *C. gigas*. All positive September samples were sequenced to confirm that positive signatures were indeed that of *C. gigas*. The positive results in September suggest a broader spawning period than industry reports based on adult reproductive condition. Note, however, that samples taken at the same location (Royal Hobart Yacht Club) in the same month the previous year did not return positive results. This indicates that patchy, low concentrations of *C. gigas* larvae may occur around the margins of the main summer spawning period.

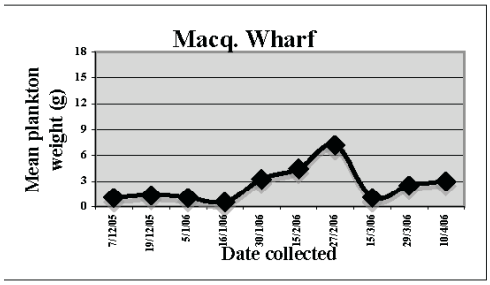
A further 300 samples were collected from ten locations in the Derwent River estuary between the 7th of December 2005 and the 10th of April 2006 (Appendix C). The temperature and salinity profiles of the sample locations located towards the mouth of the estuary were relatively stable and uniform (from the surface to the bottom) with fairly consistent temperatures of ~16°C and salinities of ~34 ppt. The sample locations in the upper reaches of the estuary, particularly Risdon, exhibited pronounced salinity stratification with depth. The surface salinity was as low as 18 ppt, with 34 ppt only occurring below 3 metres (December 2005 data). Water surface temperatures reached a maximum of 18°C in the upper estuary during January and February 2006, with a difference of ~2°C between the surface and the deepest point sampled (~9 metres depth).

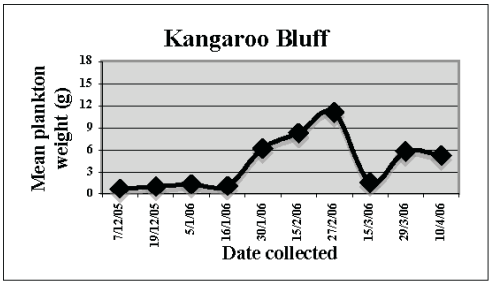
The planktonic biomass collected from the various sampling stations exhibits significant variation both between stations as well as between different time points (Figure 38). There is a general increase in biomass over the course of the summer across all stations associated with increased primary productivity with onset of spring-summer warming of the water.

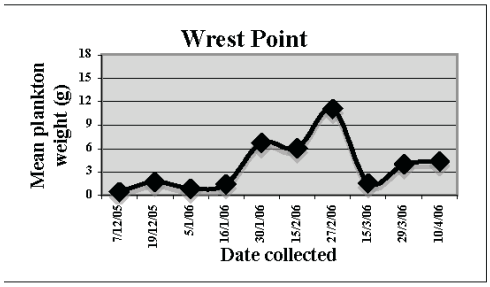
Figure 38 Biomass (as weight wet) of plankton samples collected at ten locations in the Derwent estuary between December 2005 and April 2006

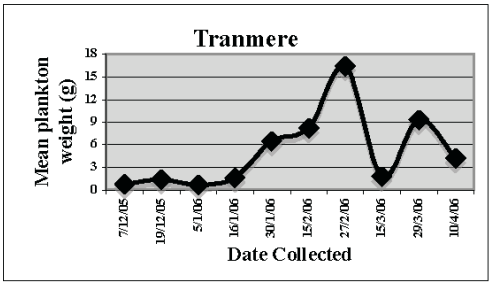


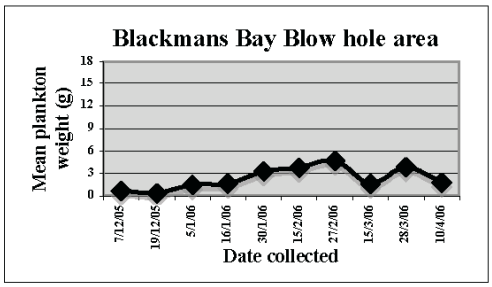


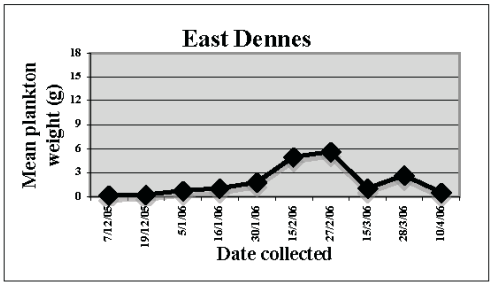


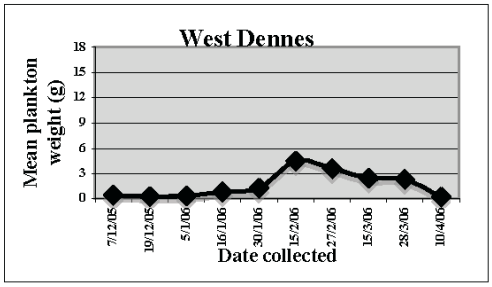












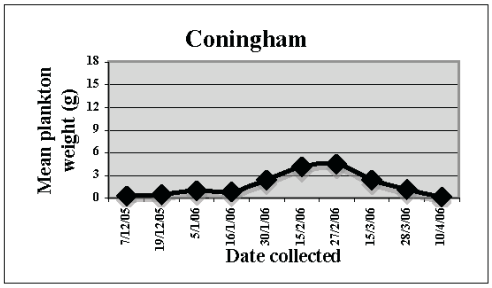


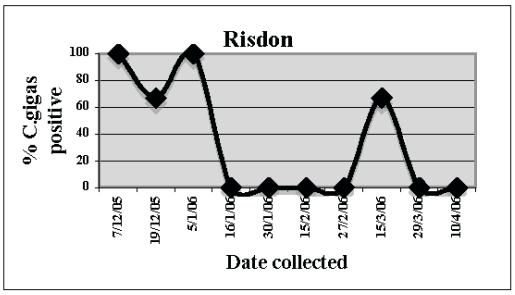
Figure 39 summarises the proportion of *Crassostrea gigas* positive samples in the ten sample locations. With the exception of two sites, East Dennes Point and Coningham, *C. gigas* gametes or larvae were already present in the Derwent estuary by the time the first samples were taken (7th December). The delayed occurrence of gametes/larvae at East Dennes or Coningham may be due to non-synchronous spawning of adult populations in the estuary and/or subtle differences in hydrodynamic conditions such as slower warming of the water or greater water movement due to proximity to Storm Bay.

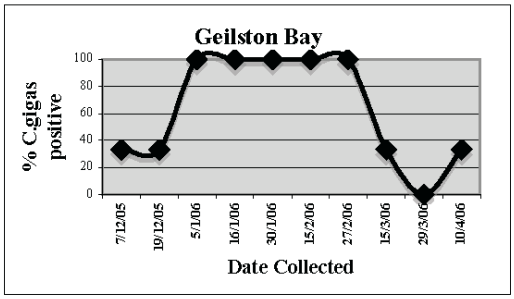
The frequency of *Crassostrea gigas* positive samples tends to reach a maximum through the months of January to March at all sites except Risdon. Risdon appears atypical in this context because there are no *C. gigas* positive samples in the last half of January and February. It should be noted, however, that all the control (18s) samples from Risdon were negative on the 16th January and 30th January, and 2 of the 3 controls were negative on the 15th February and 27th February (see Appendix C). Negative control samples indicate that the sample is not suitable for genetic analysis and hence the results cannot be relied upon. This may have been caused by large amounts of humic and/or metal contamination in the Risdon samples.

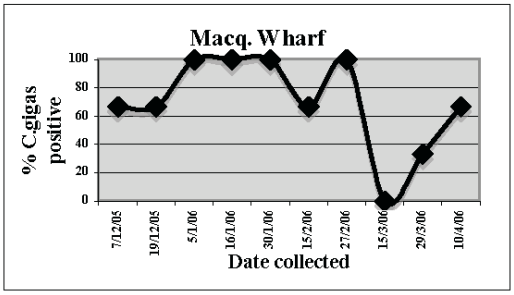
Crassostrea gigas is known to spawn in both Boreal and Austral summer months. The larval period varies from ~10 − 30 days depending on water temperature (see section 3.2.4). The peak spawning period in New South Wales is thought to be December to February, but *C. gigas* larvae can be found in the water column from October through to March (*pers. comm.* John Nell, NSW Fisheries). In Tasmania *C. gigas* is thought to spawn no earlier than November and no later than March (*pers. comm.* Col Dyke, 12.12.05). The results obtained during this study suggest that spawning peaks during January and February but may start towards the end of September and extend beyond March. At the Coningham station, for example, the frequency of positive samples was very high throughout March and April, indicating that spawning in at least some parts of the Derwent estuary was occurring towards the end of March, and possibly into April. Note that the two positive samples obtained in the last week of September from the Royal Hobart Yacht Club represent a significant extension on the previously assumed start date for *C. gigas* spawning in the Derwent.

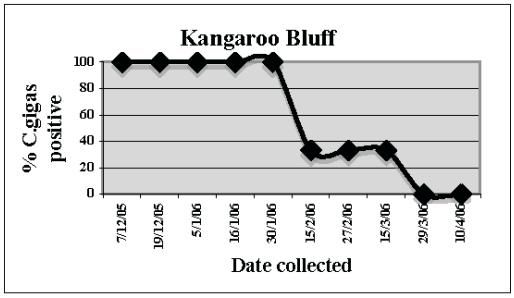
The DSS vessel infection database currently records the plankton period of *C. gigas* to start in October and end in April. The results of this study, together with the personnel communications, suggest that the start date may be a week too late and the end date may be a month too early for the Derwent as a whole, but a month too late for the site of ballast uptake in the Port of Hobart (Risdon). Further sampling from September to November, and from April to May is clearly needed to resolve this issue further. In the meantime, we recommend that the plankton period of *C. gigas* in the DSS database be amended for NSW ports but remain unchanged for the Port of Hobart. Further sampling would also benefit from use of real time probes to quantify the amount of *C. gigas* DNA in positive samples. As noted above for *Asterias amurensis*, very small levels of DNA can provide a positive result but pose little if any translocation risk.

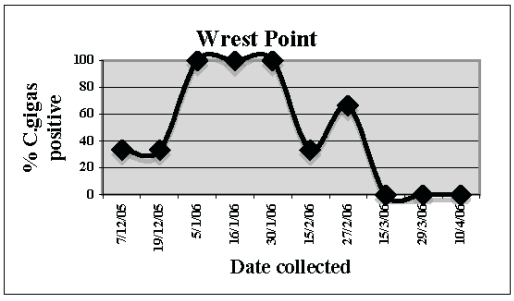
Figure 39 Proportion of plankton samples that tested positive for *Crassostrea gigas* from ten stations in the Derwent estuary

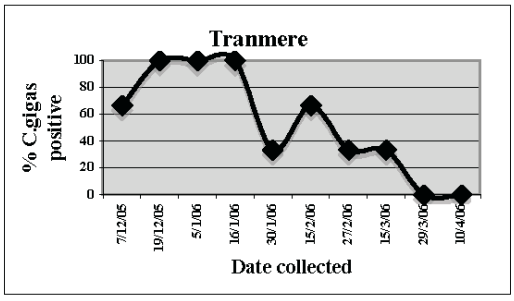


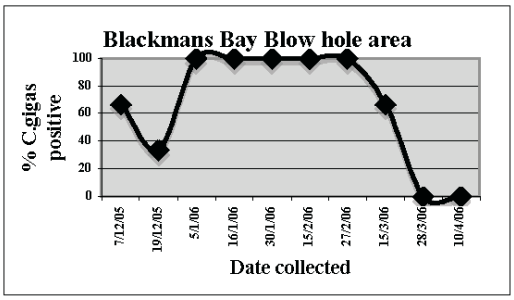


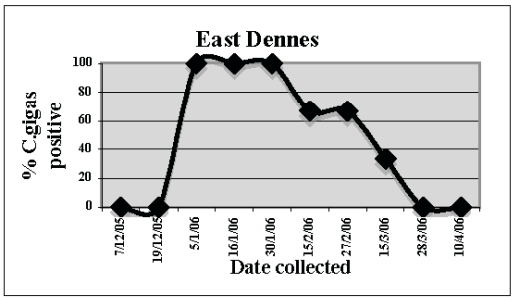


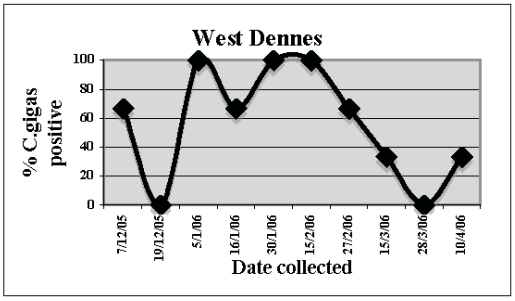


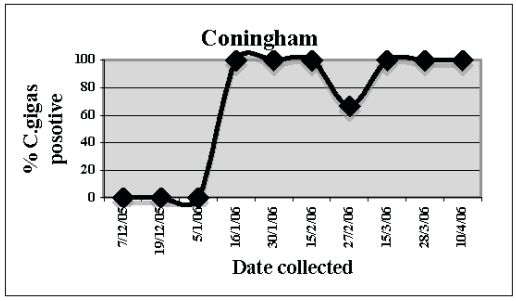












6. MODULE A – PORT INFECTION

6.1 Background

The probability that the donor port and recipient ports are infected with a target species forms an important component of the ballast water risk assessment. In the table-based approach to the DSS, the probability of target-species infection for a port surveyed to the relevant baseline standard (Hewitt and Martin, 1996; 2001), but found to be free of that target species, is set to an arbitrarily low level (e.g. 0.05). Whereas the infection probability of ports that have not been surveyed to the relevant baseline standard is set equal to the probability that the target species concerned could survive there (refer to Figure 12, Hayes and Sliwa, 2003)

In June 2005, CMAR completed an analysis and integration of all existing port survey information (McEnnulty *et al.*, 2005). The project report notes that 42 ports have been surveyed in Australia, at least once since 1995, but only 39 have been surveyed using accredited port survey protocols. Data was subsequently entered into the National Port Survey Database (NPSD) in a standardised format for 36 ports: Abbot Point, Adelaide, Albany, Botany Bay, Brisbane, Bunbury, Burnie, Cairns, Darwin, Devonport, Eden, Esperance, Fremantle, Geelong, Geraldton, Gladstone, Gove, Hastings, Hay Point, Hobart, Karumba, Lady Barron, Launceston, Lucinda, Mackay, Melbourne, Mourilyan, Newcastle, Port Hedland, Port Kembla, Port Lincoln, Port Walcott (dinoflagellates and phytoplankton only), Portland, Sydney, Townsville and Weipa.

Since the completion of the Hastings project (Patil *et al.*, 2004) there has been a growing awareness of the potential problems associated with the port baseline surveys, particularly for species such as *Crassostrea gigas*, *Carcinus maenas* and the toxic dinoflagellates: *Alexandrium catenella*, *A. minutum*, *A. tamarense* and *Gymnodinium catenatum*, culminating in the development of a new national monitoring strategy that incorporates statistically sound sampling protocols (Hayes *et al.*, 2005b, Anon, 2006). During this period a number of changes were incorporated into the DSS databases leading to a series of discrepancies between the NPSD (held at CMAR) and the DSS databases (held at DAFF). These discrepancies are summarised below together with additional information relevant to the port infection status information currently held in the DSS.

6.2 Port infection status by species

6.2.1 Alexandrium catenella

Genetic analyses suggest that all Australian populations of *Alexandrium catenella* have been introduced from Japan, or other areas of temperate Asia, and are toxic (de Salas *et al.*, 2001). Table 10 compares those ports recorded as infected with *A. catenella* in the DSS database with the equivalent NPSD records, together with other possible cases of infection. All DSS and NPSD records match except for Triabunna, Botany Bay and Port Kembla where *A. catenella* is present in the DSS but were not recorded in the port surveys. In the Port of Hobart survey *A.*

catenella could not be distinguished from *Alexandrium tamarense* (in Hobart and Triabunna). *A. catenella*, however, was identified in Triabunna in 1997 (de Salas *et al.,* 2001) and is recorded as present in the DSS. The port survey reports sparse findings of *A. tamarense* like cysts which may have been *A. catenella*. Germination attempts and culturing experiments to confirm the identifications were unsuccessful. To date, *A. catenella* has not been documented in the Derwent Estuary and only the non-toxic *A. tamarense* genotype has been cultured from the nearby D'Entrecasteaux Channel (*pers. comm.* G. Hallegraeff, UTAS, 11.07.06).

Table 10 Recorded presence of *Alexandrium catenella* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
South Australia	Adelaide	1	1	(1, 2)
New South Wales	Botany Bay	0	1	*Alexandrium* sp. "catenella type" (3, 8)
New South Wales	Eden	0*	1	(4)
Victoria	Flinders (Western Port)	0	0	Found in Flinders Bight (5)
Victoria	Geelong	0	0	Found in Port Phillip Bay
Victoria	Melbourne	1	1	(6, 8)
New South Wales	Newcastle	1	1	(1, 7)
New South Wales	Port Hacking	0	0	(8)
New South Wales	Port Kembla	0	1	*Alexandrium* sp. "catenella type" (3)
New South Wales	Sydney	0	0	(8)
Australia	Triabunna	0	1	Id not confirmed in Hobart port survey

*present in the port survey report and NIMPIS mapping but missing from the NPSD database

References: 1. Hallegraeff *et al.* (1998); 2. Cohen *et al.* (2001b); 3. Pollard and Pethebridge (2002a); 4. Hewitt *et al.* (1997b); 5. Parry and Cohen (2001); 6. Cohen *et al.* (2001a); 7. Hewitt *et al.* (1998); 8. Hallegraeff *et al.* (1988)

Alexandrium catenella has been recorded from NSW from Woolooware Bay (Botany Bay), Sydney Harbour, the Lower Hawkesbury River Estuary, and Batemans Bay (Hallegraeff *et al.*, 1991; Hallegraeff *et al.*, 1998; de Salas *et al.*, 2001) but may be synonymous with *Gonyaulax conjuncta* which was recorded much earlier at Port Hacking (Wood, 1954). Strong circumstantial evidence suggests that *A. catenella* has been present in Sydney coastal waters for at least 60 years based on mussel toxicity reports in Batemans Bay in February 1935 (Hallegraeff *et al.*, 1998) and in Port Hacking in the 1940's (Hallegraeff *et al.*, 1998). *A. catenella* was not recorded in the Sydney port survey (AMBS, 2002) and is recorded in the DSS as absent from Sydney.

In the Botany Bay port survey low levels of *Alexandrium* sp. "catenella type" cysts were detected in sediment samples. The dinoflagellate cores from the Botany Bay survey, however, were not examined until a year after sampling (*pers. comm.* Steve Brett 13/07/06), and these cysts could not be germinated because of the poor condition of the sediment cores[5]. No motile *Alexandrium* cells were recorded from the phytoplankton net samples (Pollard and Pethebridge,

[5] Unequivocal determination of cyst species, requires germination of healthy, viable cysts

2002a). *Alexandrium* sp. "catenella type" cysts were also detected in sediment samples taken during the Port Kembla port survey (Pollard and Pethebridge, 2002b). The DSS currently considers *A. catenella* to be present in Botany Bay and Port Kembla but it is recorded as absent in the NPSD due to the uncertainty with the identifications. We recommend that the infection status of these ports is confirmed as soon as possible.

Alexandrium catenella is known to occur in Port Phillip Bay. The first records of *A. catenella* in Australia are from the Brighton Marina, St. Kilda Marina, Hobsons Bay, Station Pier, Williamstown, Altona and Werribee (Hallegraeff *et al.*, 1988; Hallegraeff *et al.*, 1991; Sonneman and Hill, 1997). *A. catenella* has also been recorded in the phytoplankton of Port Phillip Bay since 1987 and is thought to be widely distributed throughout the Bay but is highly seasonal, occurring only in summer and autumn and (Parry and Cohen, 2001). Unsurprisingly *A. catenella* cysts were discovered in the Melbourne port survey (Cohen *et al.*, 2001a). The DSS reflects the results of the port surveys and simply records *A. catenella* as present in Melbourne but absent in Geelong (Cohen *et al.*, 2001a; Currie *et al.*, 1998). If this species is to be managed in the DSS, Melbourne and Geelong should both be considered as infected with this species. *A. catenella* has been recorded twice in Western Port (between 1987 and 1996) as part of the monitoring program for the Flinders Bight shellfish growing areas (Parry and Cohen, 2001) but was not recorded in the Port of Hastings survey (Currie and Crookes, 1997) and hence is considered absent in the DSS and the NPSD.

Alexandrium catenella was discovered in the Adelaide port survey and has also been recorded by the South Australian Shellfish Quality Assurance Program in low numbers during routine shellfish monitoring programs in localities other than Adelaide (*pers. comm.* Ken Lee, SASQAP, PIRSA 06.06.06). The location of these reports has not yet been followed up and may include other port areas.

6.2.2 Alexandrium minutum

Alexandrium minutum occurs in Australia as two different genotypes both of which are toxic. Further research regarding Indian Ocean strains is required, however, before the origins of Australian populations can be confirmed. The two genotypes are currently believed to be a non-native genotype (European clade) present in southern Western Australia and South Australia; and, a native genotype (West Pacific clade) present in eastern and south-eastern Australia (New South Wales and Victoria). The Pacific clade is genetically identical to samples from New Zealand and Taiwan (de Salas *et al.*, 2001; *pers. comm.* G. Hallegraeff , UTAS, 01.03.06).

Table 11 compares those ports recorded as infected with *Alexandrium minutum* in the DSS database with the equivalent NPSD records, together with other possible cases of infection. All DSS and NPSD records match except for Melbourne. *A. minutum* was not recorded in the port survey of Geelong or Port Melbourne (Currie *et al.*, 1998; Cohen *et al.*, 2001a) but is considered present in Melbourne by the DSS but not Geelong. *A. minutum* cells were found in phytoplankton blooms throughout Port Phillip Bay in 1988 and 1994, but these blooms are highly seasonal and only occur during spring and summer (Parry and Cohen, 2001). *A. minutum* cysts have not been germinated from Port Phillip Bay (*pers. comm.* Steve Brett, in Parry and Cohen, 2001). *A. minutum* was collected in Western Port in July 2001 by Parry and Cohen (2001) but it is not recorded as present in the Port of Hastings in the DSS because it was not recorded in the Port of Hastings survey (Currie and Crookes, 1997).

The non-native genotype of *Alexandrium minutum* was recorded in the Swan River in 1983 (de Salas *et al.*, 2001). *A. minutum* was not, however, detected in the Port of Fremantle survey (Hewitt *et al.*, 2000), *A. minutum* is a small cyst and easily overlooked and it seems more likely than not (based on its occurrence in the Swan River) that *A. minutum* is actually present in the port (*pers. comm.* G. Hallegraeff, UTAS, 11.07.06).

Table 11 Recorded presence of *Alexandrium minutum* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
South Australia	Adelaide	1	1	Non-native, Outer Harbour (3), Port River (4,5)
Western Australia	Bunbury	1	1	Non-native (1,2)
Western Australia	Fremantle	0	0	Non-native, Swan River , Fremantle (8)
Victoria	Geelong	0	1	Native* (6)
Australia	Hobart	0	0	*A. minutum* like cells, unconfirmed id (10)
Victoria	Melbourne	0	1	Native* (6)
New South Wales	Newcastle	1	1	Native (7)
South Australia	Port Giles	0	0	Non-native*, Coobowie and Stansbury
New South Wales	Port Kembla	0	0	Native, Shoalhaven. nr. Port Kembla (8)
South Australia	Port Lincoln	0	0	Non-native* (9)
South Australia	Thevenard	0	0	Non-native* (9)
Victoria	Western Port	0	0	Native* (6)

References: 1. Hallegraeff and Hosja (1993); 2. Hewitt *et al.*, (1997a); 3. Cohen *et al.*, (2001b); 4. Hallegraeff *et al.*, (1988); 5. Hallegraeff *et al.*, (1991); 6. Parry and Cohen (2001); 7. Hewitt *et al.*, (1998); 8. de Salas *et al.*, (2001); 9. EPA (2003); 10. Aquenal Pty Ltd, (2002)

* Assumed origin, samples not examined by UTAS to determine toxicity and genotype

Alexandrium minutum has been recorded in the Port of Adelaide, the Gulf of St Vincent and Spencer Gulf. The 2003 State of the Environment Report for South Australia (EPA, 2003) states that *A. minutum* is present in Port River (Adelaide), Port Lincoln and Thevenard, and in a number of other locations, namely: American River, Penneshaw, Ballast Head, Kinscote, Coffin Bay, Franklin Harbour and Streaky Bay. The South Australian Shellfish monitoring Program (SASQAP) has also detected low numbers of *A. minutum* in various shellfish growing locations in St Vincent Gulf and Streaky Bay (*pers. comm.* Ken Lee, SASQAP 06.06.06). The precise location of these records has not been followed up. The Port Lincoln, Streaky Bay and St Vincent Gulf populations recorded by SASQAP are likely to be the non-native strain given their proximity to the non-native Adelaide population. *A. minutum* was not recorded in the Port Lincoln survey (Hewitt *et al.*, 1997c) and is therefore considered to be absent from this port in the DSS.

The distribution of *Alexandrium minutum* in Australia is uncertain. *A. minutum* like cells were recorded in the Derwent Estuary during the Port of Hobart survey. This identification cannot be confirmed, however, without further molecular and culture analysis (Aquenal Pty Ltd, 2002).

Indeed the presence of *A. minutum* in Australia has not yet been confirmed by cultures (*pers. comm.* G. Hallegraeff , UTAS, 11.07.06). Furthermore, there are at least three species that can be confused for *A. minutum* cultures if the identification is not confirmed by genetic sequencing or Scanning Electron Microscope (SEM): *A. tamutum*, *A. angustitabulatum* and *A. camurascutulum*. The non-toxic *A. tamutum* has been reported from the Derwent River and is thought to be widespread in Australia and New Zealand (*pers. comm.* Miguel de Salas, UTAS, 02.07.06). The native genotype of *Alexandrium minutum* is present in Newcastle and Shoalhaven (de Salas *et al.*, 2001; Hewitt *et al.*, 1998). *A. minutum* was not recorded in the Port Kembla survey (Pollard and Pethebridge, 2002b) but it is possible that the Shoalhaven population extends into and includes the Port Kembla region to the north.

6.2.3 Alexandrium tamarense

Alexandrium tamarense is very difficult to identify because it has many different genotypes, all morphologically identical, that differ in their toxicity (*pers. comm.* Miguel de Salas, UTAS 07.03.05). *A. tamarense* is found in Japan (toxic), North America (toxic), Europe (non-toxic), New Zealand and Australia. The only record of the toxic genotype of *A. tamerense* in Australia is from Triabunna (Australia). It was collected in the 1990's but was not recorded during surveys conducted ten years earlier (de Salas *et al.*, 2001). The toxic genotype, however, was not subsequently collected during the Port of Hobart survey (which included Triabunna) in 2002. The port survey found sparse *A. tamarense* like cysts (some of which may have been *A. catenella*) but was unable to germinate the cysts and could not therefore confirm the identification (Aquenal Pty Ltd, 2002). A non-toxic *A. tamarense* ribotype has been recorded from northern and south-eastern Australia (Hallegraeff *et al*., 1991). This Australian ribotype is now known to be widespread in southern Australia (Port MacDonnell to Cape Jaffa) and has bloomed regularly in the last few years, but the blooms have always been non-toxic (*pers. comm.* M. de Salas, UTAS 07.03.05). The non-toxic Australian samples have a unique genetic fingerprint not found elsewhere in the world that suggests they may be native (Scholin *et al.*, 1995; M. de Salas unpub. data – as cited in Aquenal Pty Ltd, 2002)

With the exception of the single toxic Triabunna strain (not sequenced and since lost in culture), a strong case can be made to remove *Alexandrium tamarense* from the DSS on the grounds that the genotypes present in Australia are non-toxic. It is also important to note that very few workers could confidently discriminate between *A. tamarense* and *A. catenella* from field samples. Cyst germination experiments or molecular probes, that have now been developed by the University of Tasmania (see MacKenzie *et al.*, 2004), are needed to discriminate between the cysts of these two species. Table 12 compares those ports recorded as infected with *Alexandrium tamarense* in the DSS database with the equivalent NPSD records, together with other possible cases of infection. There is a considerable mis-match between the DSS and NPSD records.

The DSS currently records three Tasmania ports as uninfected by *A. tamarense* (Burnie, Launceston and Hobart) and seven ports as infected, despite the fact that in all cases the strains in question are thought to be native and non-toxic (see above). This confused scenario reflects the taxonomic uncertainty associated with specimens of *A. tamarense* collected in the port surveys. The Burnie port survey, for example, notes: "*Analyses were performed on cysts collected at Burnie to determine their genetic and toxic status. Genetic analyses confirmed that populations of A. tamarense at Burnie belong to the genetic 'ribotype' previously recorded in*

North West Bay and the Tamar River and are likely to be native. Toxin analyses confirmed that, like the strains present in North West Bay and the Tamar River, populations at Burnie are non-toxic." (Aquenal Pty Ltd, 2004). The specimens of *A. tamarense* collected from the port were therefore deemed to be native, non-toxic strains hence subsequently recorded as absent from the port for the purposes of the DSS (*pers. comm.* Karina McLachlan, AGDAFF 16.03.05).

Table 12 Recorded presence of *Alexandrium tamarense* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
South Australia	Adelaide	1	1	Outer Harbour (4)
Australia	Beauty Point	1	0	Incl. in Port of Launceston survey
Australia	Bell Bay	1	0	Incl. in Port of Launceston survey (7)
Western Australia	Bunbury	0#	1	*Alexandrium cf. tamarense* (1, 2)
Australia	Burnie	1	0	(8)
Australia	Devonport	0	1	*Alexandrium cf. tamarense* (9)
Western Australia	Fremantle	0*	1	(3)
Victoria	Geelong	0	0	Found in Port Phillip Bay (4, 7)
Australia	Hobart	0	0	*A. tamarense* like cells (13)
Western Australia	Kwinana	0*	1	Included in Port of Fremantle survey
Australia	Launceston	1	0	(10)
Australia	Margate	0	0	(7)
Victoria	Melbourne	1	1	(5)
Victoria	Portland	1	1	
South Australia	Port Macdonnell	0	0	(7)
Australia	Triabunna	1	1	One record of toxic strain (11)
Victoria	Western Port	0	0	(12)

References: 1. Hallegraeff and Hosja (1993); 2. Hewitt *et al.*, (1997a); 3. Hewitt *et al.*, (2000); 4. Cohen *et al.*, (2001b); 5. Cohen *et al.*, (2001a); 6. Parry *et al.*, (1997); 7. Hallegraeff *et al.*, (1991); 8. Aquenal Pty Ltd (2004); 9. Martin *et al.*, (1996); 10. Aquenal Pty Ltd (2001); 11. de Salas *et al.*, (2001); 12. Parry and Cohen (2001); 13. Aquenal Pty Ltd (2002)

The port surveys for Bunbury and Devonport (Hewitt *et al.*, 1997a; Martin *et al.*, 1996) discovered *Alexandrium cf. tamarense* specimens which were subsequently not included in the NPSD as a definite species. The Devonport specimens have since been confirmed to be the non-toxic native ribotype, whereas the origin and toxicity of the Bunbury specimens has not (to date) been confirmed. In addition, *A. tamarense* was recorded from the Mersey River – Devonport and Tamar River – Bell Bay in 1987 (Bolch and Hallegraeff, 1990; Hallegraeff *et al.*, 1991) and hence is included in the DSS.

Alexandrium tamarense has been collected from the Port of Adelaide (Cohen *et al.*, 2001b) and the non-toxic strain has also been found as part of the shellfish monitoring in South Australian

waters (*pers. comm.* Ken Lee, SASQAP, PIRSA). The precise location of these reports, however, has not yet been followed up and may include port areas. *A. tamarense* was collected in the Port of Melbourne survey (Cohen *et al.*, 2001a) but was not collected in the Port of Geelong survey (Currie *et al.*, 1998). *A. tamarense* cells, however, were found in seasonal (autumn and summer) phytoplankton blooms throughout Port Phillip Bay every year between 1991 and 1996 (Hallegraeff *et al.*, 1991; Steve Brett, *pers. comm.* in Parry and Cohen, 2001). *A. tamarense* was collected in Western Port in July 2001 by Parry and Cohen (2001) but the DSS does not record it as present in this port because it was not recorded in the Port of Hastings survey in 1997 (Currie and Crookes, 1997). *A. tamerense* has also been recorded on three occasions, between September 1987 and December 1996, in the Flinders Bight shellfish growing area and is thought to have a self-sustaining population in Western Port (Parry and Cohen, 2001).

6.2.4 Asterias amurensis

Table 13 compares those ports recorded as infected with *Asterias amurensis* in the DSS database with the equivalent NPSD records, together with other possible cases of infection. All DSS and NPSD records match except for Geelong. *A. amurensis* was not found in the Geelong port survey in October 1997 despite being established in Port Phillip Bay at this time and present in and around the Port of Melbourne (Currie *et al.*, 1998). *A. amurensis* has since been confirmed to be present in Geelong, although its density here is less than in Port of Melbourne (*pers comm.* Jeff Ross, TAFI, 06.06.2006).

One possible case of a (new) port infection was the discovery in November 2003 of a dead *Asterias amurensis* adult at the Warneet boat ramp in northwestern Western Port. The discovery of a single dead specimen was not considered sufficient evidence to warrant changes to the DSS database because the Warneet boat ramp is used for launching and retrieving boats (and gear) that have been used in Port Phillip Bay where the seastar is abundant. In this case it seems more likely that specimen was simply dislodged or discarded from a boat that was infected in Port Phillip Bay.

Table 13 Recorded presence of *Asterias amurensis* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments
Victoria	Hastings (Western Port)	0	0	Dead individual discovered 27.11.03
Australia	Hobart	1	1	
Victoria	Geelong	0	1	
Victoria	Melbourne	1	1	
Australia	Triabunna (Spring bay)	1	1	Incl. in the Hobart port survey

6.2.5 Carcinus maenas

Table 14 compares those ports recorded as infected with *Carcinus maenas* in the DSS database with the equivalent NPSD records, together with other cases of possible port infection that are not currently recorded in either database. *C. maenas* was not recorded in either the Burnie or Hobart port surveys but it is known to be present in both of these localities based on previous trap surveys (Aquenal Pty Ltd, 2004), the scientific literature (Aquenal Pty Ltd, 2002) and subsequent collections in the Port of Hobart (CMAR *unpub. data*).

Carcinus maenas was recorded in the Eden port survey but was not recorded in the baseline surveys of Sydney, Botany Bay or Port Kembla. Two specimens of *C. maenas* were collected from Sydney in 1891 and 1936, suggesting that it has been present in Sydney for at least as long as it has been known from Port Phillip Bay (Ahyong, 2005). Furthermore *C. maenas* is recorded as being "regularly sighted" in the littoral zone at Kyeemagh in Botany Bay between 1977 and 1987 (Ahyong, 2005). New records were also obtained from the coastal estuaries/lakes of NSW: Burras Lake, Burril Lake, Lake Conjola and Jervis Bay in December 1992 and November 1997, and Narrawallee Inlet in November 1985 (Figure 40) (Ahyong, 2005). These records suggest that the current DSS records for Sydney and Port Botany are incorrect and further suggest that the record for Port Kembla, located between Botany Bay and Eden, may also be suspect. Finally, the 2003 State of the Environment Report for South Australia (EPA, 2003) lists *C. maenas* as present in Port Stanvac (not surveyed to date). We recommend that the infection status of all of these ports is confirmed as soon as possible.

6.2.6 Crassostrea gigas

Table 15 compares those ports recorded as infected with *Crassostrea gigas* in the DSS database with the equivalent NPSD records, together with other cases of possible port infection that are not currently recorded in either database. The results of the Hastings project (Patil *et al.*, 2004) provided the first strong evidence of a systematic failure of the port baseline surveys to detect *C. gigas* in a number of New South Wales ports, namely: Botany Bay, Eden, Newcastle, Sydney and Port Kembla. This issue was subsequently investigated by John Nell (NSW Fisheries). Dr. Nell confirmed that *C. gigas* make up around 30, 60 and 90% of all oysters in Quibray Bay, Woolooware Bay and the Georges River respectively (Botany Bay) and that *C. gigas* occur in the Hunter River (Newcastle Harbour) and on the foreshore of Sydney Harbour (*pers. comm.* John Nell, NSW Fisheries, 28.04.04). Dr. Nell was unable to comment on the situation in Port Kembla but in light of the Hastings study and *C. gigas* distribution along the New South Wales coast it seems highly likely that the oyster is present in this port as well. The DSS database was changed to reflect this situation in June 2004, whilst the NPSD continues to reflect the results of the surveys themselves.

Table 14 Recorded presence of *Carcinus maenas* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
South Australia	Adelaide	1	1	
Australia	Beauty Point	1	1	Incl. in the Launceston port survey
Australia	Bell Bay	1	1	Incl. in the Launceston port survey
New South Wales	Botany Bay (Port Botany)	0	0	Regular sightings since 1977 (1)
Australia	Burnie	0	1	
Australia	Devonport	1	1	
New South Wales	Eden (Twofold Bay)	1	1	
Victoria	Geelong	1	1	
Victoria	Hastings (Western Port)	1	1	
Australia	Hobart	0	1	
Australia	Launceston	1	1	
Victoria	Melbourne	1	1	
New South Wales	Port Kembla	0	0	
South Australia	Port Stanvac	NA	0	(2)
New South Wales	Sydney (Port Jackson)	0	0	2 specimens collected in 1891 & 1936 (1)
Australia	Triabunna (Spring Bay)	0	1	Incl. in the Hobart port survey

References: 1. Ahyong (2005); 2. EPA (2003)

It is important to note that the infection status of a number of other Australian ports remains unresolved. In a general survey of Western Port, Cohen *et al.*, (2000) notes that "a small number of *Crassostrea gigas* have been found in Western Port and the population of this species may not yet be self-sustaining, as its population density is much lower (<5%) than the density at which this exotic species is typically found in infested areas." The authors of the survey therefore concluded that the species is actually absent for Hastings, as currently reflected in the NPSD. The opinions of survey authors on the likelihood of establishment, however, are clearly not a sufficient basis for the purposes of the DSS. We recommend that this issue is resolved as soon as possible. It is also pertinent to note in this context that feral populations of *C. gigas* were discovered in Anderson Inlet in 1985 (Coleman and Hickman, 1986).

Crassostrea gigas is farmed in 24 locations around the South Australian coast (Figure 41) This includes sites adjacent to the ports of Thevenard (Ceduna), Port Augusta and Port Giles (Coobowie). None of these ports have been surveyed. Whilst *C. gigas* is not thought likely to become feral in South Australia (*pers. comm.* Ken Lee, SASQAP, 06.06.06) it would seem prudent to double-check the status of these ports for populations of *C. gigas* prior to the implementation of the DSS.

Figure 40 Distribution and dates of recorded sightings (green stars) of *Carcinus maenas* in New South Wales (based on Ahyong, 2005)

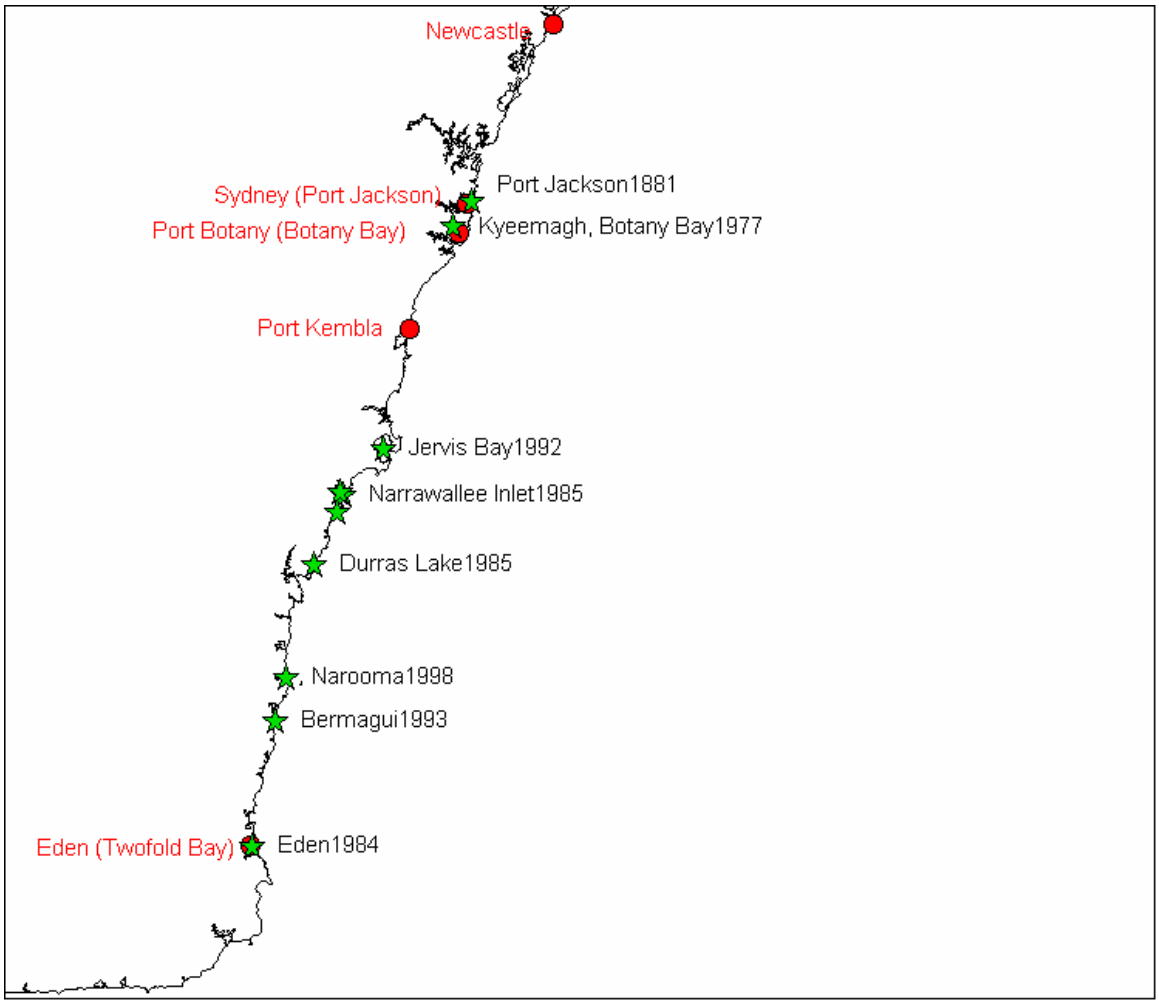
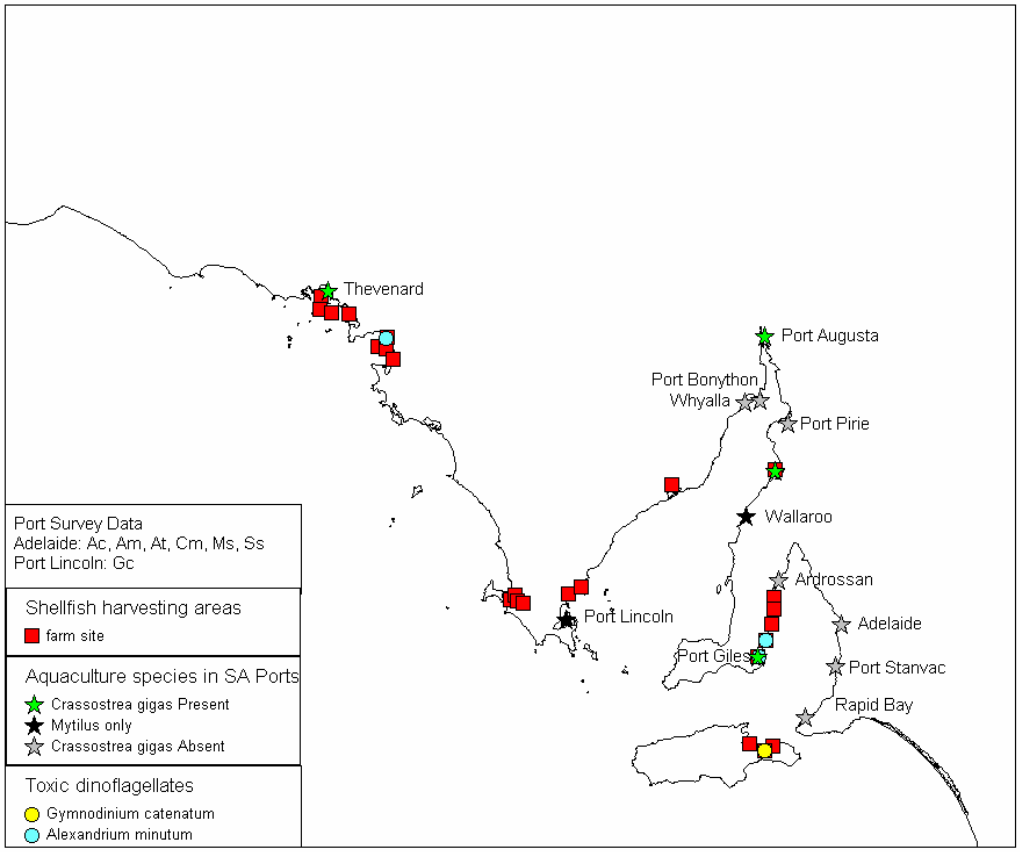


Table 15 Recorded presence of *Crassostrea gigas* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
Australia	Beauty Point	1	1	Incl. in the Launceston port survey
Australia	Bell Bay	1	1	Incl. in the Launceston port survey
New South Wales	Botany Bay (Port Botany)	0	1	
Australia	Burnie	1	1	
Australia	Devonport	1	1	
New South Wales	Eden (Twofold Bay)	0	1	
Victoria	Hastings (Western Port)	0	0	
Australia	Hobart	1	1	
Australia	Launceston	1	1	
New South Wales	Newcastle	0	1	(*pers. comm.* Dr Nell)
South Australia	Port Augusta	NA	0	Commercial *C. gigas* farm site
South Australia	Port Giles	NA	0	Commercial *C. gigas* farm site
New South Wales	Port Kembla	0	1	
South Australia	Port Lincoln	0	1	
New South Wales	Sydney (Port Jackson)	0	1	(*pers. comm.* Dr Nell)
South Australia	Thevenard	NA	0	Commercial *C. gigas* farm site
Australia	Triabunna (Spring Bay)	1	1	Incl. in the Hobart port survey

Figure 41 Map of *Crassostrea gigas* farm and hatchery locations in South Australia including locations of commercial ports (based on information from the PIRSA website and Ken Lee, SASQAP)



6.2.7 *Gymnodinium catenatum*

The global distribution of *Gymnodinium catenatum* consists of two ecophenotypes: a warm-temperate type and a tropical type. All Australian records of *G. catenatum* are thought to only be the warm-temperate ecophenotype. The tropical ecophenotypes is not thought to be present in Australian waters. It is important to note, however, that there is currently no morphological or genetic way to distinguish between the two ecophenotypes (*pers. comm.* G. Hallegraeff, UTAS, 19.05.06). The current risk tables for *G. catenatum* are based on life-cycle information drawn from the warm-temperate ecophenotype.

Table 16 compares those ports recorded as infected with *Gymnodinium catenatum* in the DSS database with the equivalent NPSD records, together with other possible cases of infection. All DSS and NPSD records match. *G. catenatum* has been recorded in Portland as cysts in sediments (Sonneman and Hill, 1997) but was not recorded in the port survey (Parry *et al.,* 1997) and is absent from the DSS. Additional information which may affect the DSS records is discussed below.

Table 16 Recorded presence of *Gymnodinium catenatum* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
Tasmania	Hobart	1	1	Warm-temperate ecotype
Victoria	Portland	0	0	Cysts in sediments (1)
Victoria	Port Campbell	0	0	Cysts in sediments (1)
Tasmania	Port Huon	0	0	(2)
South Australia	Port Lincoln	1	1	Warm-temperate ecotype
Victoria	Port Welshpool	0	0	Cysts in sediments (1)
Tasmania	Triabunna	1	1	Warm-temperate ecotype, incl. in the Hobart port survey
Victoria	Western Port (Bass River)	0	0	Cysts in sediments (1)

References: 1. Sonneman and Hill (1997); 2. McMinn *et al.*, (1997).

Gymnodinium catenatum has been collected from the Queenscliff marina as cysts in sediments (Sonneman and Hill, 1997), but was not detected in the surveys of the Port of Geelong (Currie *et al.*, 1998) or Port Melbourne (Cohen *et al.*, 2001a), and is currently considered to be absent from these ports in the DSS and NPSD. These databases also record *G. catenatum* as absent from Western Port because it was not recorded in the Port of Hastings port survey (Currie and Crookes, 1997) or in subsequent surveys of Western Port (Cohen *et al.*, 2000; Parry and Cohen, 2001). *G. catenatum* cysts, however, have been collected from sediments in the Bass River which enters Western Port on the eastern side of the bay (Sonneman and Hill, 1997). *G. catenatum* cysts have also been recorded in coastal Victorian sediments at Apollo Bay, Lorne, Warrnambool, Port Campbell and Port Welshpool (McMinn *et al.*, 1997; Sonneman and Hill, 1997).

In South Australia, *Gymnodinium catenatum* has been recorded (in very low numbers) in localities close to Port Lincoln (such as American River, Nepean Bay and Kangaroo Island) during routine shellfish monitoring programmes by the South Australian Shellfish Quality Assurance Program (PIRSA website, accessed 17/10/2005). Precise location data for these (and previous) records has not been followed up but may include port areas.

The DSS does not currently record *Gymnodinium catenatum* as present in any of the New South Wales ports because it was not detected in any of the New South Wales port surveys (Newcastle, Sydney, Botany Bay, Port Kembla or Eden) . It has, however, been recorded from the Lower Hawkesbury River Estuary (Hallegraeff, 1998; Bolch and Reynolds, 2002). *G. catenatum* cysts were also detected last year in routine shellfish monitoring along the southern New South Wales coast (from just north of Eden northwards) together with blooms in the Hawkesbury River (*pers. comm.* Steve Brett , MicroAlgal Services, 13.07.06). Again precise location data for these records has not been followed up but may include port areas.

6.2.8 Musculista senhousia

Table 17 compares those ports recorded as infected with *Musculista senhousia* in the DSS database with the equivalent NPSD records, together with other cases of possible port infection that are not currently recorded in either database. All DSS and NPSD records agree except for Devonport. *M. senhousia* was not discovered during the Devonport port survey (Martin *et al.*, 1996) and the current record in the NPSD appears to be an error.

Table 17 Recorded presence of *Musculista senhousia* in the NPSD and DSS databases

State	Port name	NPSD	DSS	Comments (References)
South Australia	Adelaide	1	1	
Australia	Bell Bay	1	1	Incl. in the Launceston port survey
Australia	Beauty Point	1	1	Incl. in the Launceston port survey
Australia	Devonport	1	0	
Western Australia	Fremantle	1	1	
Victoria	Geelong	1	1	
Victoria	Hastings	1	1	
Western Australia	Kwinana	1	1	Incl. in the Fremantle port survey
Australia	Launceston	1	1	
Victoria	Melbourne	1	1	
Victoria	Portland	1	1	

6.2.9 Sabella spallanzanii

Table 18 compares those ports recorded as infected with *Sabella spallanzanii* in the DSS database with the equivalent NPSD records, together with other cases of possible port infection

that are not currently recorded in either database. All DSS and NPSD records agree. The port baseline survey of Hastings did not detect *S. spallanzanii* (Currie and Crookes, 1997). *S. spallanzanii* has been detected on mussel farms at Flinders in the past but was eradicated. A subsequent survey for exotic species by Parry and Cohen (2001) concluded that *S. spallanzanii* is not self-sustaining in Western Port. A further survey of Flinders Pier, conducted in February 2004 following a possible sighting, found only native *Sabellastarte* (*pers. comm.* Michaela Dommisse, DSE, 20.06.06).

Table 18 Recorded presence of *Sabella spallanzanii* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
South Australia	Adelaide	1	1	
Western Australia	Albany	1	1	
Western Australia	Bunbury	1	1	
Australia	Devonport	1	1	
New South Wales	Eden (Twofold Bay)	1	1	
South Australia	Esperance	1	1	
Western Australia	Fremantle	1	1	
Victoria	Geelong	1	1	
Victoria	Hastings	0	0	
Western Australia	Kwinana	1	1	Incl. in the Fremantle port survey
Victoria	Melbourne	1	1	

6.2.10 Undaria pinnatifida

Table 19 compares those ports recorded as infected with *Undaria pinnatifida* in the DSS database with the equivalent NPSD records, together with other cases of possible port infection that are not currently recorded in either database. A survey for exotic species in Western Port discusses the presence and eradication of *U. pinnatifida* from Flinders in December 2000 (Parry and Cohen, 2001). The site was re-surveyed in January and May 2001 and found to be free of *U. pinnatifida*. The survey of Flinders pier in February 2004 (following the possible sighting of *Sabella spallanzanii* – see above) also noted the absence of *U. pinnatifida* although it should be noted that this is not the correct time of year to detect sporophytes. The Geelong port survey (October 1997) did not detect *Undaria pinnatifida* (Currie *et al.,* 1998). It has, however, been confirmed from the Port of Melbourne (Cohen *et al.*, 2001), and from boat yards on the western arm of Port Phillip Bay (Hayes *et al.*, 2005a). It therefore seems very likely that *U. pinnatifida* is in Geelong and the DSS database has been amended to reflect this.

Table 19 Recorded presence of *Undaria pinnatifida* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)

Victoria	Geelong	0	1	
Victoria	Hastings (Western Port)	0	0	
Australia	Hobart	1	1	
Victoria	Melbourne	1	1	
Australia	Triabunna (Spring Bay)	1	1	Incl. in the Hobart port survey

6.2.11 *Varicorbula gibba*

Table 20 compares those ports recorded as infected with *Varicorbula gibba* in the DSS database with the equivalent NPSD records, together with other cases of possible port infection that are not currently recorded in either database. The issue of *V. gibba* in Burnie, Hastings and Esperance highlight some of the difficulties associated with reported sightings of species in a port in the absence of a clear (statistical) protocol for both the surveys and the information that is generated by them. Dead specimens of *V. gibba* were detected during the Burnie port survey however, no live individuals were found (Aquenal Pty Ltd, 2004). The port was subsequently deemed infected in the NPSD database, and deemed present for uptake but absent for discharge for the purposes of the DSS (*pers. comm.* Karina McLachlan, AGDAFF, 16.03.05). Note, however, that the risk table algorithms simply reflect this species as present in Burnie.

Table 20 Recorded presence of *Varicorbula gibba* in the NPSD and DSS databases and other possible cases of port infection

State	Port name	NPSD	DSS	Comments (References)
Australia	Burnie	1	1	
Australia	Devonport	1	1	
South Australia	Esperance	0	0	*Corbula cf. gibba* detected in the port
Victoria	Geelong	1	1	
Victoria	Hastings (Western Port)	0	1	
Australia	Hobart	1	1	
Victoria	Melbourne	1	1	
Victoria	Portland	1	1	

A single (dead) *V. gibba* valve was found in Hastings in 1997. No live specimens, however, have since been found despite intensive sampling (Currie and Crookes, 1997; Cohen *et al.*, 2000). The DSS database records this species as present in the port. We recommend that this issue is resolved as soon as possible. Specimens of juvenile *Corbula cf. gibba* specimens were detected during the Esperance port survey but could not be identified further (Campbell, 2003). CMAR recommended that the port be re-surveyed to confirm the presence or absence of *V. gibba* in the port but to date this has not (to our knowledge) occurred.

7. ADDITIONAL WORK ITEMS

7.1 Monitoring strategy

During the course of the second SLA, CMAR personnel were instrumental in the inception and assisted in the development, of the Marine Pest Monitoring Manual (Anon., 2006). The results of the Hastings Demonstration project (Patil *et al.*, 2004) clearly demonstrated that target species were present in ports that were surveyed but declared free of these species (see also Section 6). The monitoring manual was developed in response to these findings and subsequent publications that demonstrated the need, and methods, for more statistical rigour in port surveys (Hayes *et al.*, 2005b). In June 2005 CMAR personnel attended a technical workshop to assist in the development of the monitoring manual, and have continued to support its implementation through the design and implementation of the Adelaide trial.

7.2 NIMPCG/CCIMPE activities

Over the course of this SLA, CMAR personnel have attended eleven meetings of the National Introduced Marine Pest Coordinating Group (NIMPCG), together with various meetings and teleconferences of the Coordinating Committee on Introduced Marine Pest Emergencies (CCIMPE). We have provided advice and supported the review of the CCIMPE trigger list and provided extensive comments on various drafts of the IMO Ballast Water Convention G7 guidelines on risk assessment.

8. CONCLUSIONS AND RECOMMENDATIONS

The vast majority of the research effort funded under the second SLA has been directed towards the probability that the donor port is infected with a target species (Module A), the probability that a vessel becomes infected during ballast uptake (Module B) and probability that the target species will complete its life-cycle in a recipient port (Module D). In the context of domestic ballast water management, where journey durations are short, these three modules are thought to provide the best risk reduction returns for research investment.

Confirming the infection status of donor ports is critical to the sensitivity and specificity of the ballast water risk assessment. During the course of this SLA we identified a number of discrepancies between the DSS port infection database (that supports Module A), the NPSD and other information sources. It is important that these discrepancies are eliminated prior to the implementation of the domestic ballast water DSS, and in this context we recommend that:

- *Carcinus maenas* be listed as present in Sydney and Port Botany, and that the presence or absence of *C. maenas* in Port Kembla and Port Stanvac be confirmed as soon as possible;

- *Crassostrea gigas* be listed as present in Hastings unless the absence of this species here can be verified by a systematic survey of Western Port, and that the presence or absence of *C. gigas* in Thevenard, Port Augusta and Port Giles be confirmed as soon as reasonably practicable. Information on the location of *C. gigas* farms close to commercial ports, and the possible existence of feral populations in the proximity of ports and ballast uptake areas is investigated by the relevant state authorities;

- the presence or absence of *Varicorbula gibba* in Burnie, Hastings and Esperance be confirmed as soon as possible.

The discrepancies between the DSS, NPSD and other information sources are particularly acute for the dinoflagellates *Alexandrium catenella, A. tamarense. A. minutum*, and *Gymnodinium catenatum*. The problems associated with theses dinoflagellates are largely due to taxonomic uncertainty associated with toxic/non-toxic and native/non-native strains, ecotypes, genotypes or ribotypes of these species – and the range of nomenclature, as well as the difficulties of identifying these species accurately. All of these species have several different genotypes that differ in their toxicity, but are all morphologically identical and can only be discriminated by toxicity testing and/or genetic analysis.

Genetic analyses suggest that all Australian populations of *Alexandrium catenella* have been introduced from Japan, or other areas of temperate Asia (de Salas *et al.* 2001), and are toxic. The evidence collated during this SLA suggests that *Alexandrium tamarense* is not toxic in Australian waters and should therefore be removed from the DSS target list. The toxic form of *A. tamarense* detected in Triabunna in the 1990s has not been detected since. *A. minutum* appears to exist in Australia as two genotypes: one native to Australia and the Western Pacific (currently restricted to south-eastern Australia) and the other non-native (currently restricted to South Australia and southern Western Australia). Both types are toxic. The DSS database does not currently distinguish between *A. minutum* genotypes, but rather adopts a cautionary stance treating all records of this species as non-native. Finally, only one (the warm temperate)

ecophenotype of *Gymnodinium catenatum* is thought to be currently present in Australia. Again both types are toxic.

The national shellfish monitoring program currently monitors shellfish growing areas for the presence of toxic dinoflagellates. This program provides an opportunity to develop a more detailed picture of the distributions of these toxic dinoflagellates around Australia. For example, *Gymnodinium catenatum* bloomed in coastal New South Wales last year (*pers. comm.*, Steve Brett, Microalgal Services, 13.07.06) and the three *Alexandrium* species and *G. catenatum* have all been recorded in South Australian waters in proximity to several ports (*pers. comm.* Ken Lee, SASQAP, 06.06.06). To date none of this information is used in the DSS port infection database.

It is important that the port infection issues associated with toxic strains of dinoflagellates are resolved if the DSS is used to manage the potential transfer of these strains between domestic Australian ports. If the DSS is used in this manner we recommend that:

- *Alexandrium catenella* be listed as present in Geelong and Hastings, the status of all other ports that the DSS database currently records as infected with *A. catenella*, with the exception of Adelaide and Melbourne, be confirmed as soon as possible;

- the origins of the native strains of *Alexandrium minutum* be confirmed and the implications of potentially managing toxic strains of native dinoflagellates within the DSS be given further consideration;

- *Gymnodinium catenatum* be listed as present in Portland, Port Campbell, Port Welshpool, the presence or absence of *G. catenatum* in Hastings be confirmed as soon as possible, and genetic methods to reliably distinguish between the two ecophenotypes of this species are developed and implemented to confirm the absence of tropical ecophenotypes in Australian ports; and,

- site specific information from the national and state shellfish monitoring programs is gathered and used in the DSS port infection database.

The recent advent of genetic probes provides a much more sensitive and (often) more reliable mechanism to identify the presence/absence, or quantify the level, of gametes and larvae in the water column. The use of these types of probes, together with more traditional techniques, has provided a much more detailed picture of the planktonic duration of *Asterias amurensis* in the Derwent estuary and Port Phillip Bay. In the Derwent estuary gametes and larvae of *A. amurensis* are now known to be present in the water column from April through to January, whereas in Port Phillip Bay the planktonic duration appears to be two months shorter lasting from May to December. We recommend that the vessel infection database used to support Module B is updated to reflect this information.

During the course of this SLA, CMAR staff applied a presence/absence probe for *Crassostrea gigas* to plankton samples collected from the Derwent estuary between December and April. The results of this analysis indicate that *C. gigas* gametes or larvae were already present in the Derwent by December, and were still detectable during April. The pattern of positive samples, however, varied quite significantly across the ten sample sites. At Risdon for example, where vessels uptake and discharge ballast water, *C. gigas* positive samples were not detected beyond the end of March.

The DSS vessel infection database currently records the plankton period of *Crassostrea gigas* to start in October and end in April. The field samples, together with the other information sources collected over the course of this SLA suggest that:

- in the Derwent estuary as a whole, *C. gigas* gametes and larvae may be present in the water column from the end of September through to the end of April, and possibly into May;

- at the site of ballast uptake in the Port of Hobart (Risdon) *C. gigas* gametes and larvae were present in the water column from November to the end of March; and,

- in New South Wales, *C. gigas* gametes and larvae may be present in the water column from October through to March.

We recommend that the DSS vessel infection database for ports in NSW be amended to reflect this information, but remain unchanged for the Port of Hobart for the moment. We also recommend that all subsequent use of genetic probes to determine the plankton period of target species use real time probes that are capable of quantifying the amount of DNA in positive samples. The limited analysis of journey survival dynamics conducted during this SLA indicates that presence/absence probes are capable of detecting very low levels of gametes and/or larvae that are very unlikely to pose a bio-invasion risk when translocated by ballast water.

Module D of the ballast water risk assessment has been significantly improved during the course of this SLA. The resolution of this Module and the risk benefits that it returns, however, are determined by the proportion of the species life-cycle that is considered to pose a bio-invasion risk. The risk reduction benefits of the new Module are maximised if management agencies are prepared to accept a reasonably high proportion, for example 80%, as a risk cut-off value for this Module. If a very low proportion is used – for example if the completion of 5% of a species life-cycle is considered to pose a bioinvasion risk – then this significantly reduces the risk reduction benefits provided by the Module.

The risk assessment approach, and data used to support this approach, in Module D has been subject to a rigorous independent review. Furthermore, the potential latitudinal distribution of the target species in Australia, particularly for sub-tidal species, compares favourably with the known native and invaded range of most of the target species. The approach and predictions of the new Module seem to be sufficiently reliable to allow a reasonably high risk cut-off. We therefore recommend that management authorities adopt an 80% risk cut-off value for the purposes of the ballast water DSS.

The review of the risk assessment approach and species life-cycle data that supports Module D identified a number of issues and limitations. Most of these have been resolved. There are, however, a number of outstanding issues:

- there is a lack of life-stage specific data for *Varicorbula gibba* and *Sabella spallanzanii* – the conservative approach currently adopted for these species is leading to risk overestimates that could be rectified with additional data;

- the minimum temperature tolerance of *Musculista senhousia* appears to be too high and does not accord with the latitudinal limits of its native and invaded range; and,

- the residuals of the Broome time series model shows periodic autocorrelation at low time lags, suggesting that this model could be improved slightly.

The next SLA will address and improve the Broome time series model prior to the implementation of the ballast water risk tables. We also propose to check for any new literature regarding the life-stage specific temperature tolerances of *Varicorbula gibba* and *Sabella spallazanii*. It is important to note, however, that CMAR has conducted extensive literature reviews during the course the last two SLA's and we are very unlikely to discover significant new sources of information for these two species in the literature. We therefore recommend that national and state authorities gather additional tolerance information for these species using experimental approaches.

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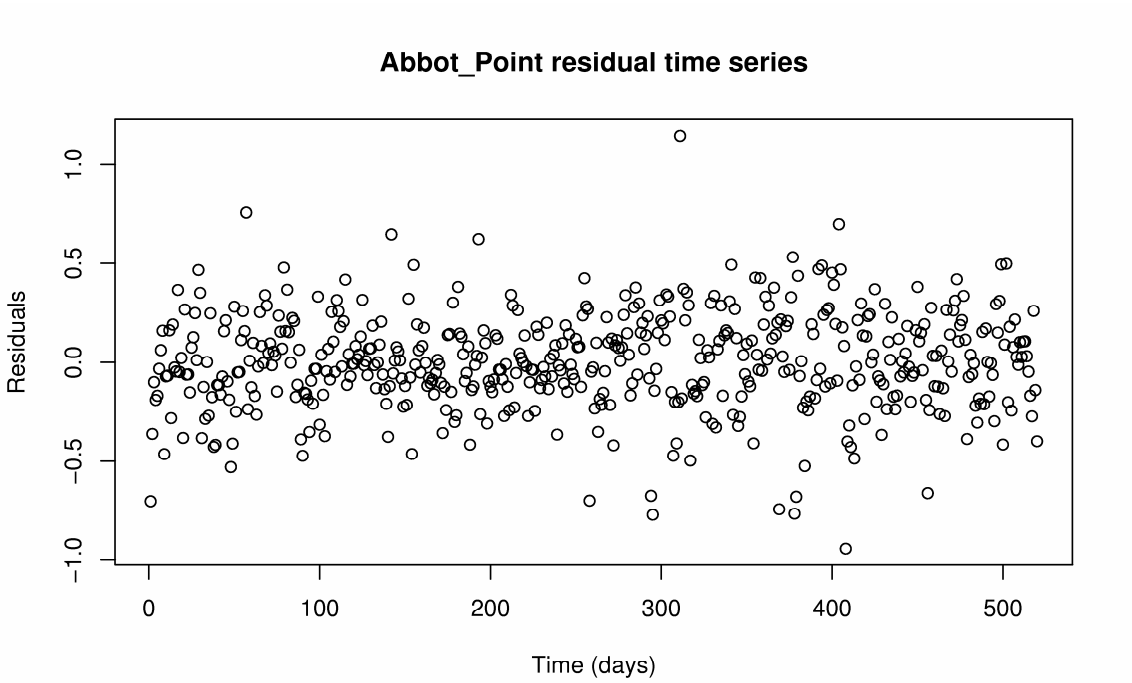
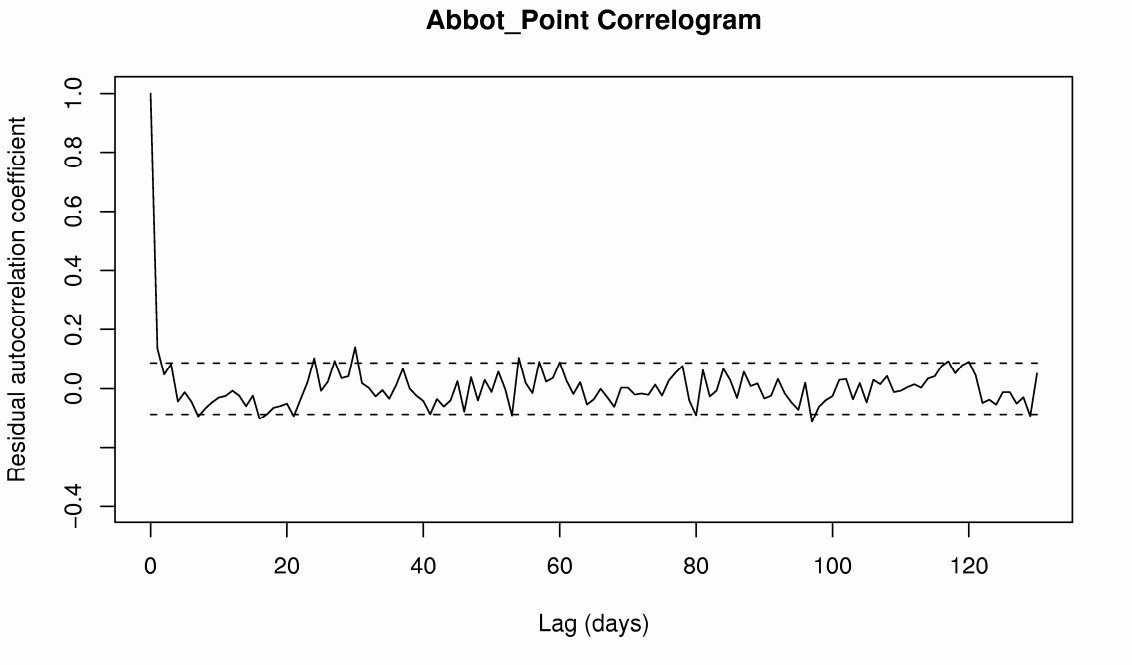
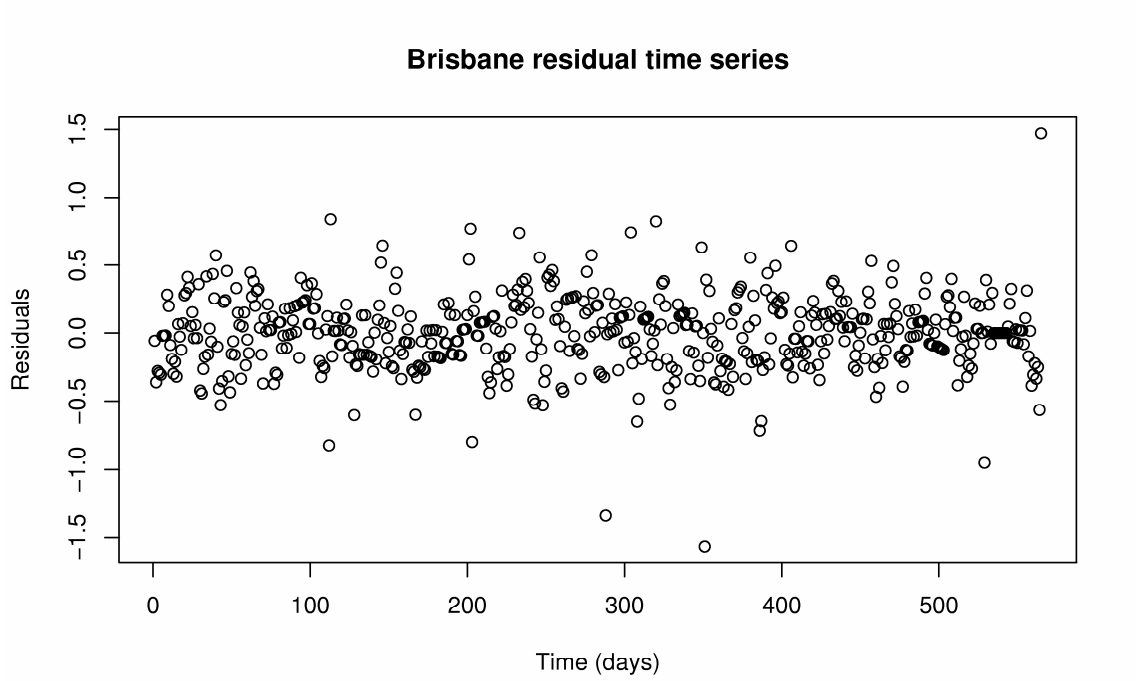
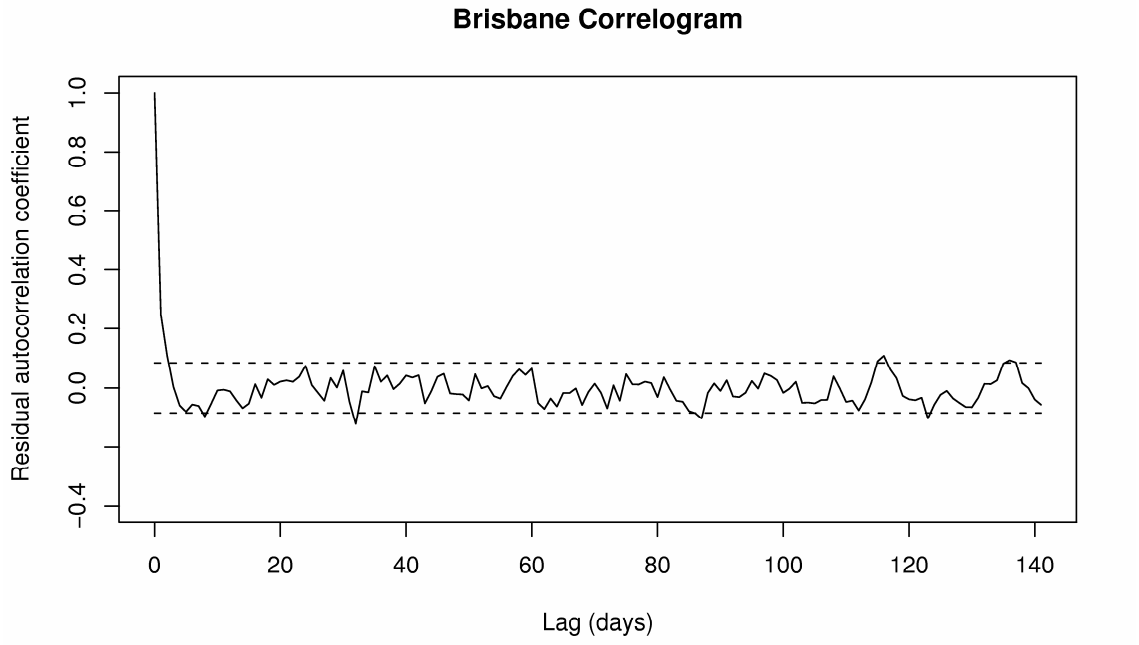
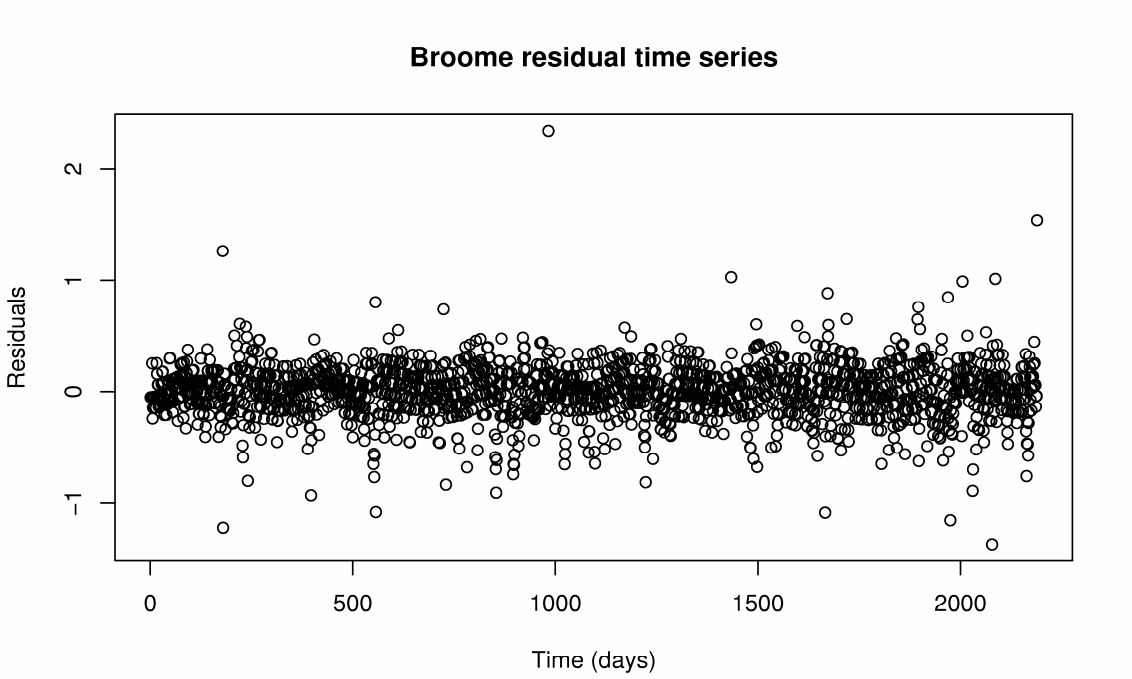
APPENDIX A RESIDUALS AND CORRELOGRAMS

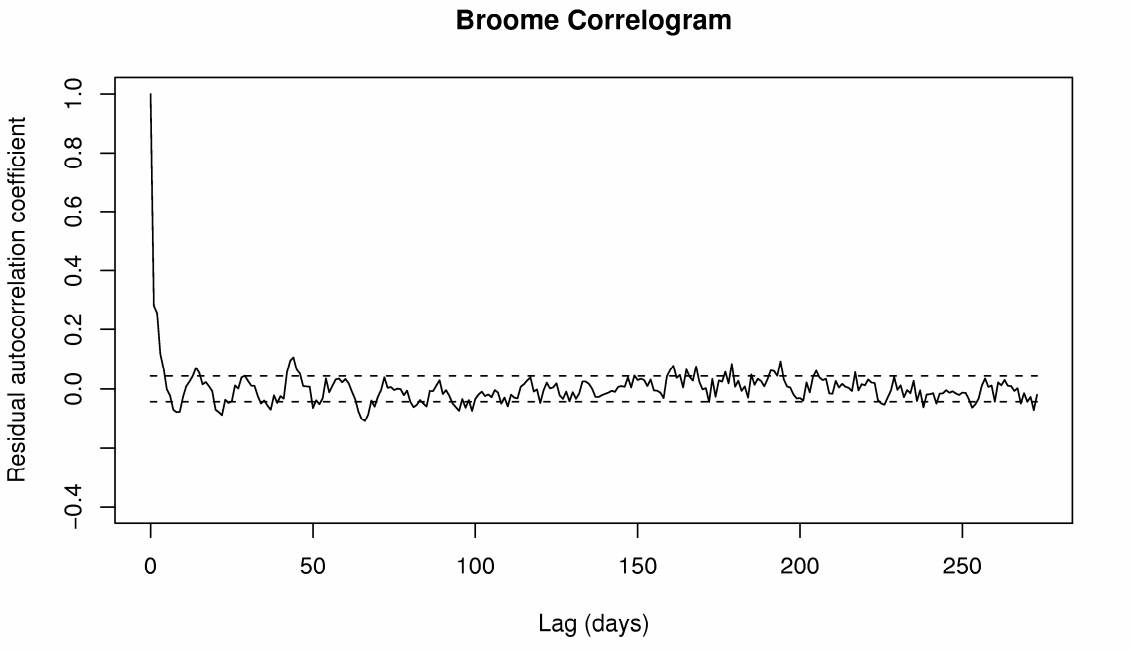


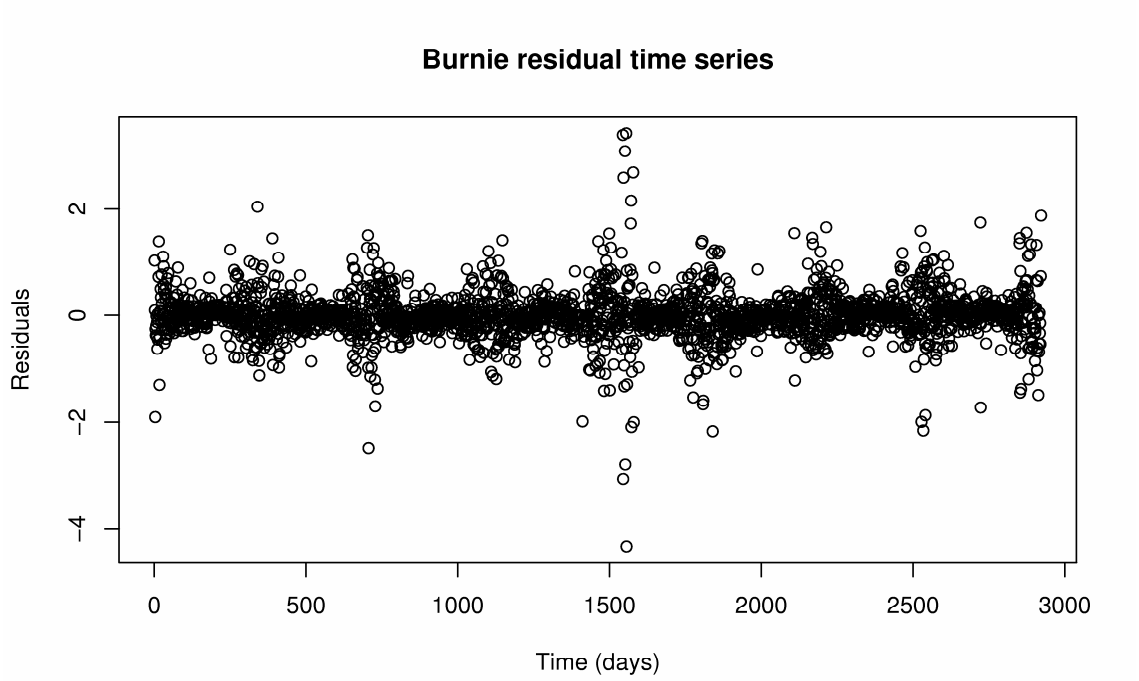


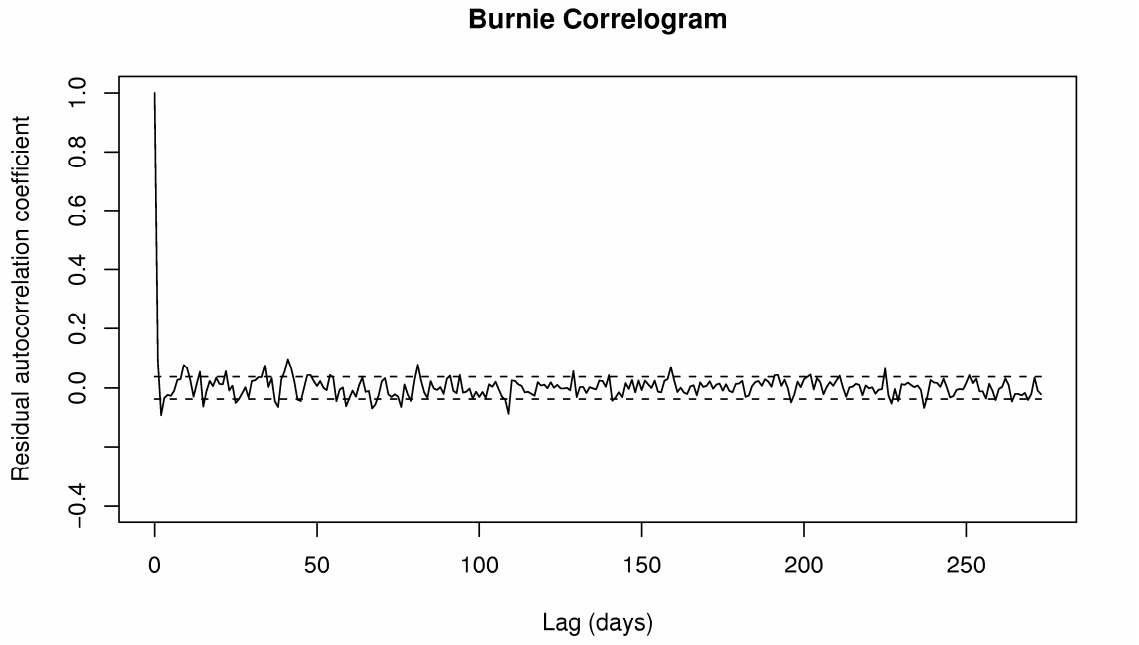

Brisbane residual time series
Residuals
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
0
100
200
300
400
500
Time (days)

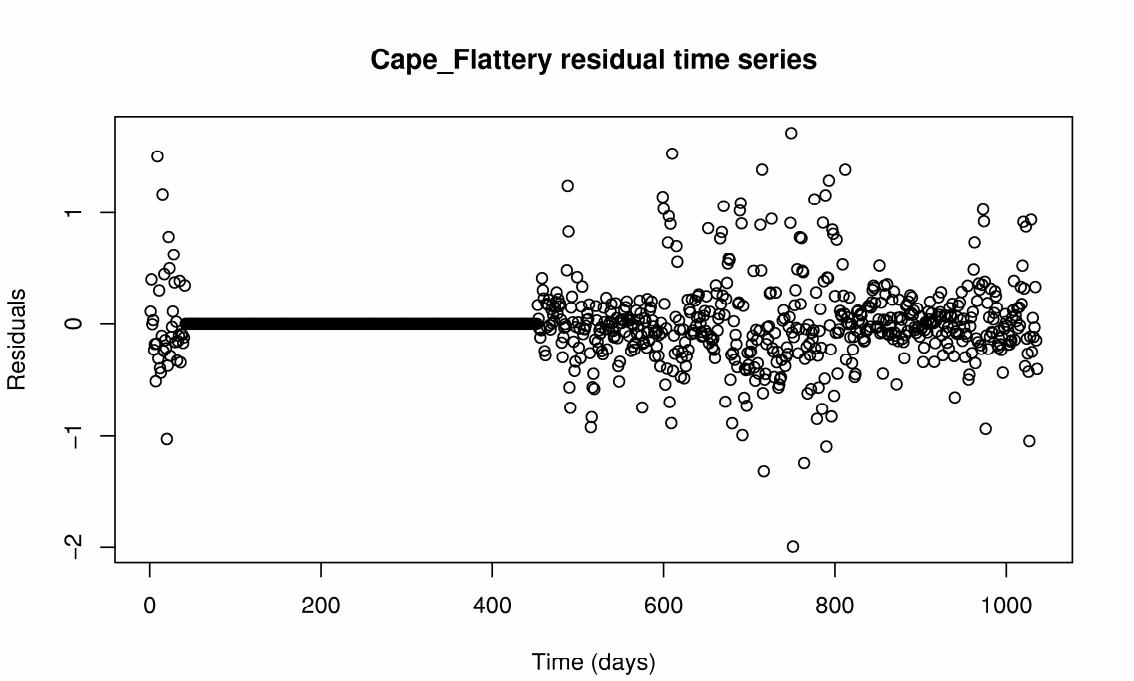

Brisbane Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
20
40
60
80
100
120
140
Lag (days)

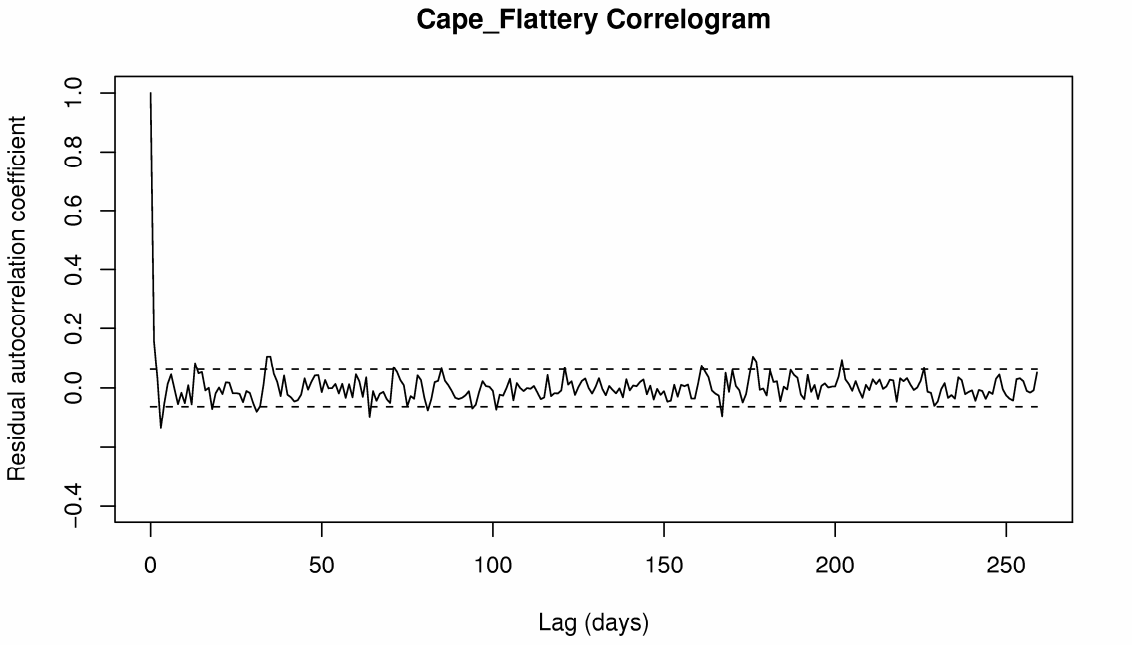

Broome residual time series
Residuals
2
1
0
−1
0
500
1000
1500
2000
Time (days)

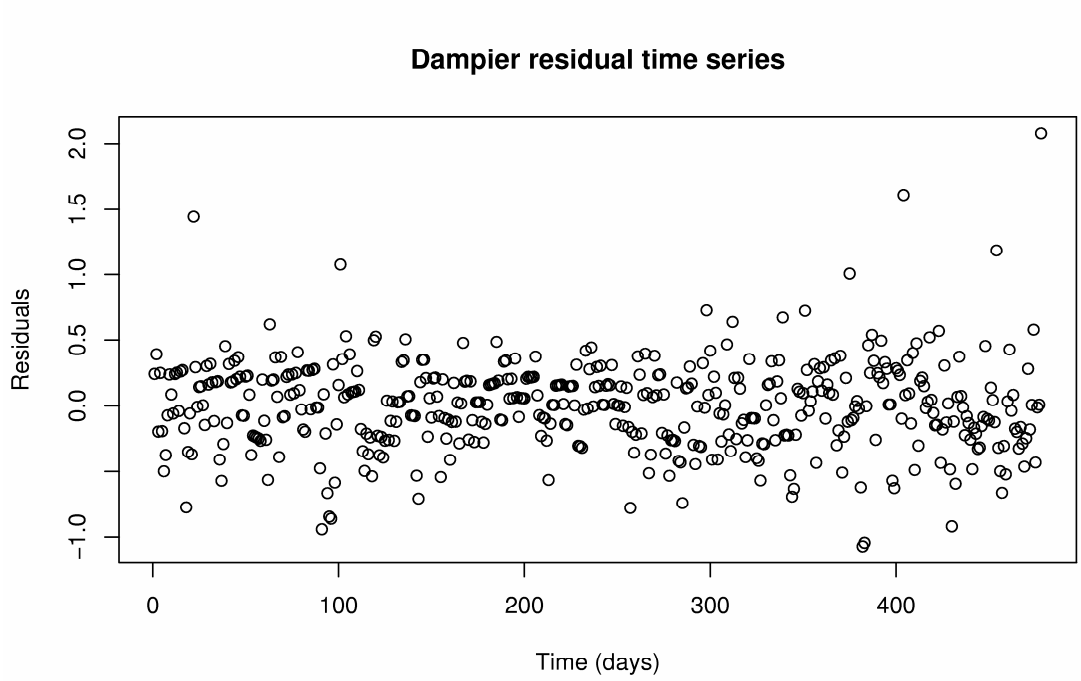

Broome Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

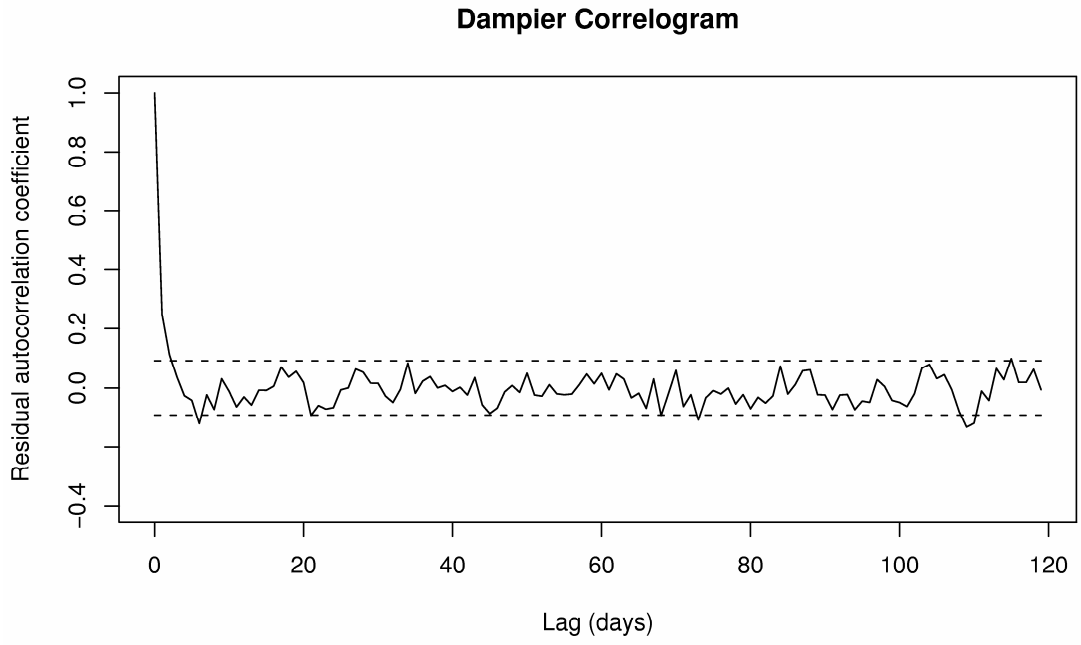

Burnie residual time series
Residuals
2
0
−2
−4
0
500
1000
1500
2000
2500
3000
Time (days)

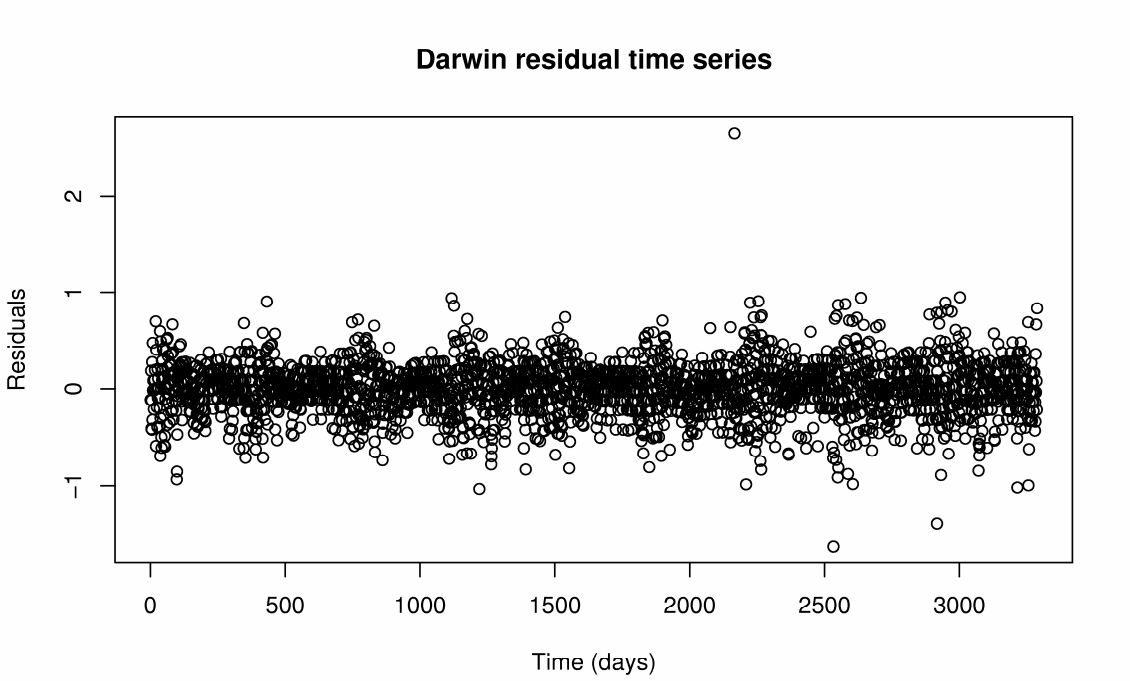

Burnie Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

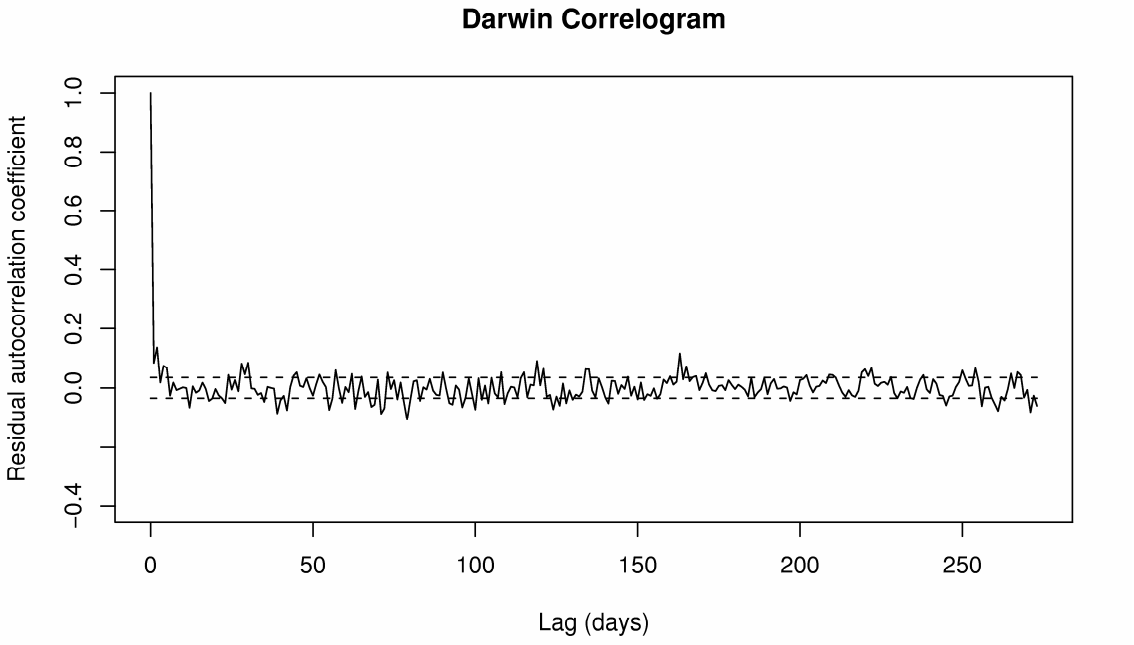

Cape_Flattery residual time series
Residuals
1
0
−1
−2
0
200
400
600
800
1000
Time (days)

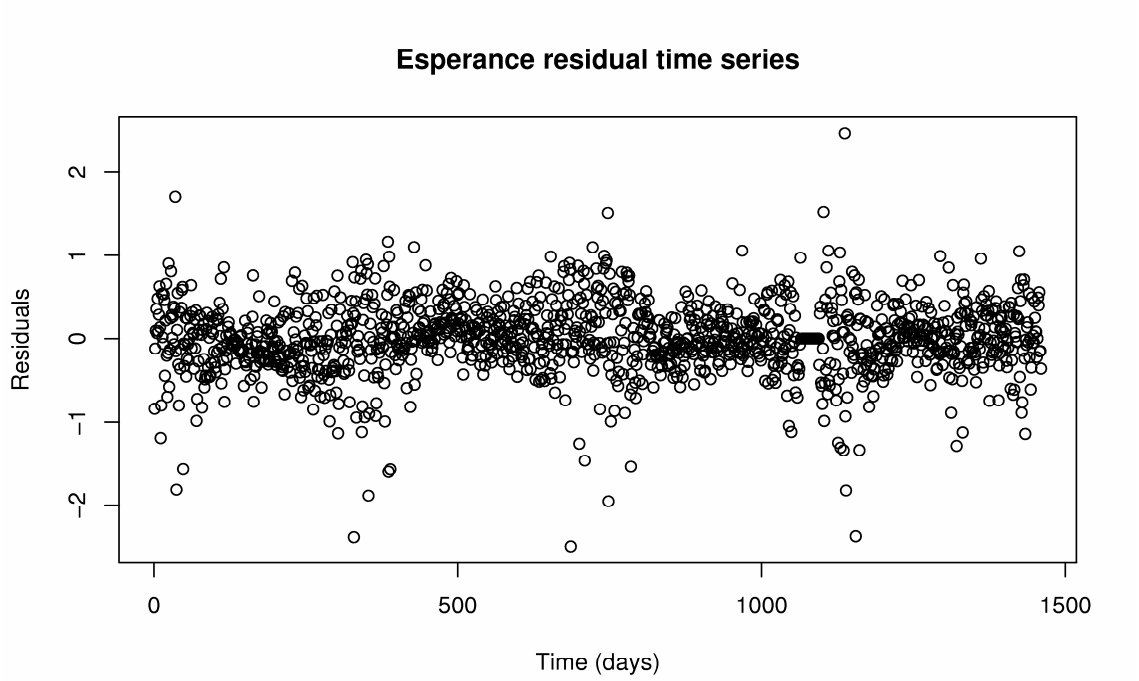

Cape_Flattery Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

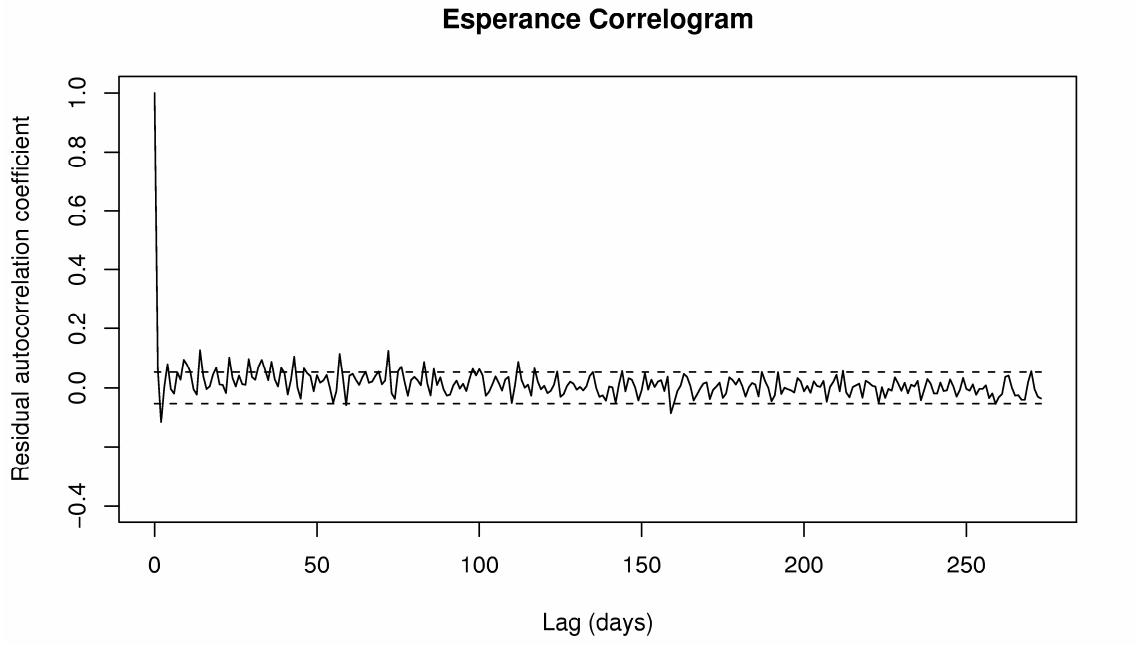

Dampier residual time series
Residuals
2.0
1.5
1.0
0.5
0.0
−1.0
0
100
200
300
400
Time (days)

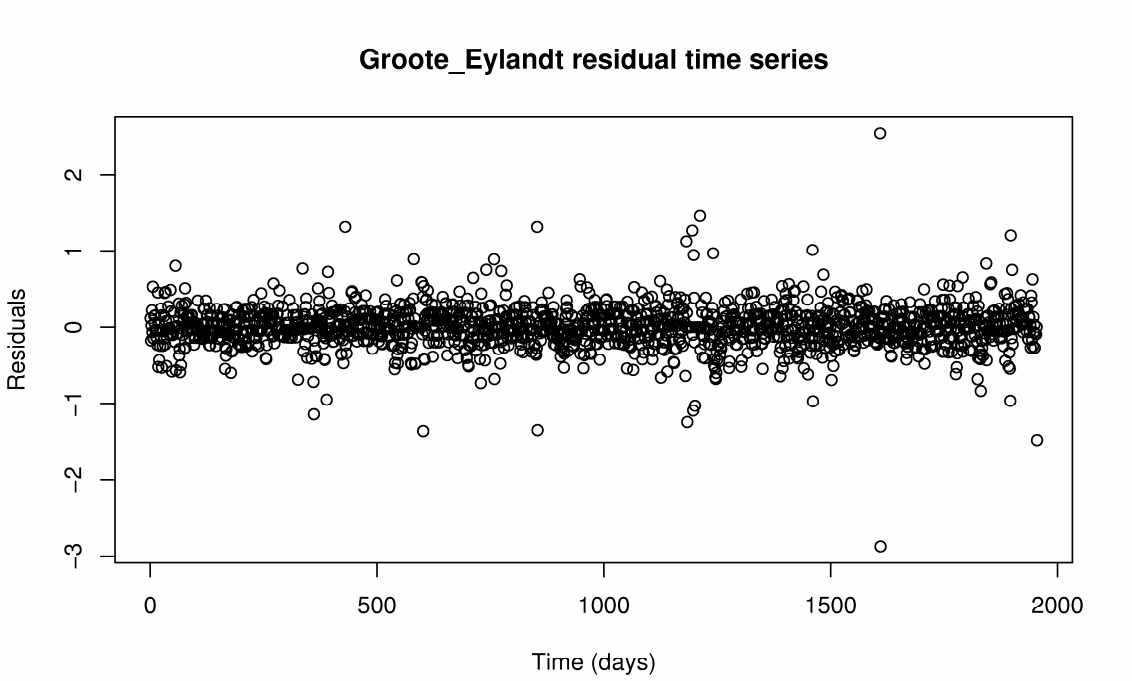

Dampier Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
20
40
60
80
100
120
Lag (days)

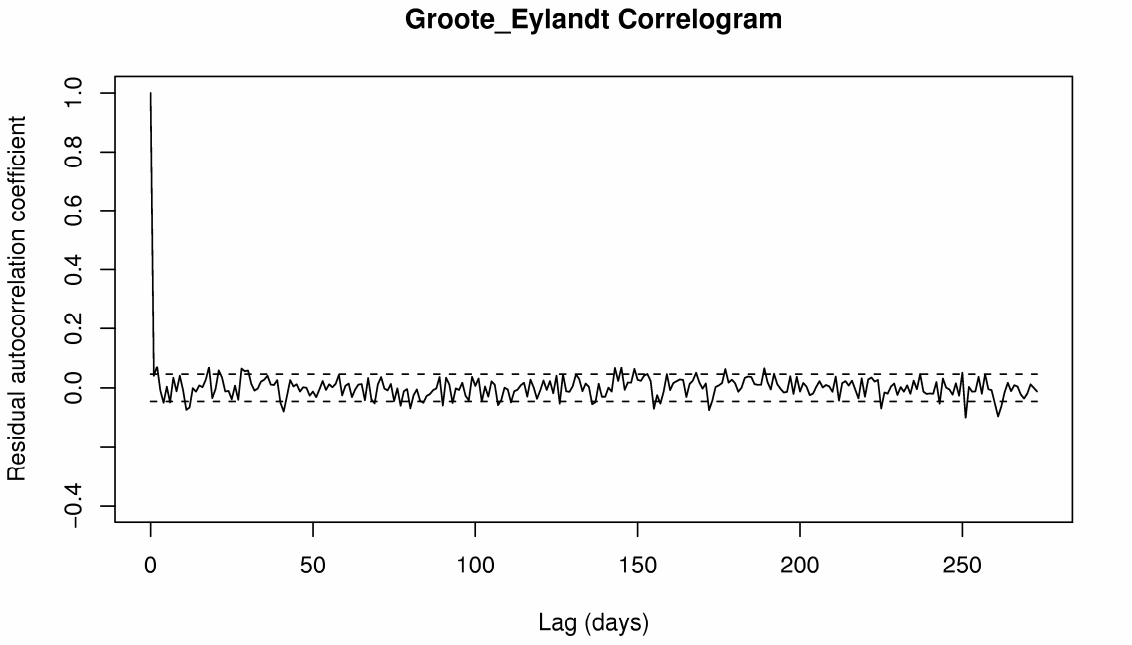

Darwin residual time series
Residuals
2
1
0
−1
0
500
1000
1500
2000
2500
3000
Time (days)

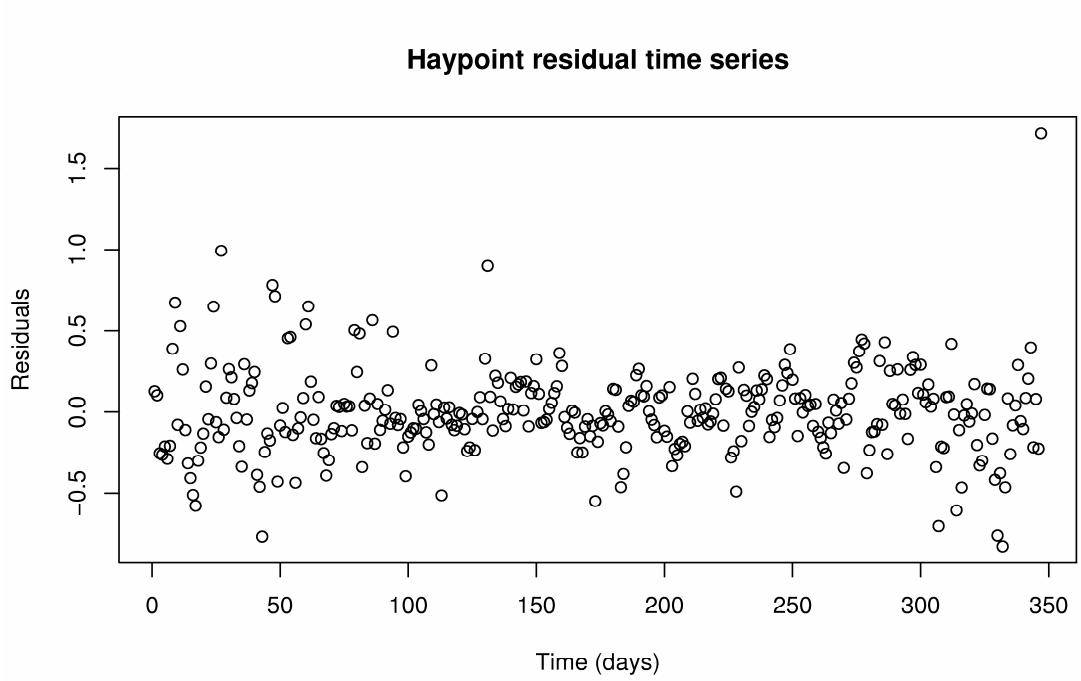

Darwin Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

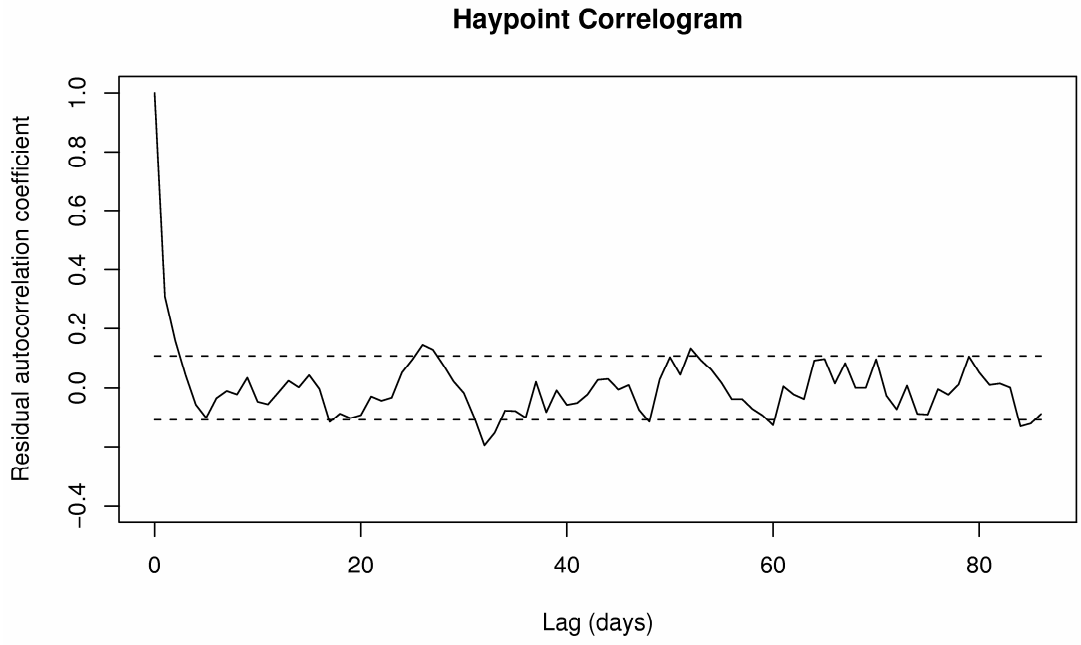

Esperance residual time series
Residuals
2
1
0
−1
−2
0
500
1000
1500
Time (days)

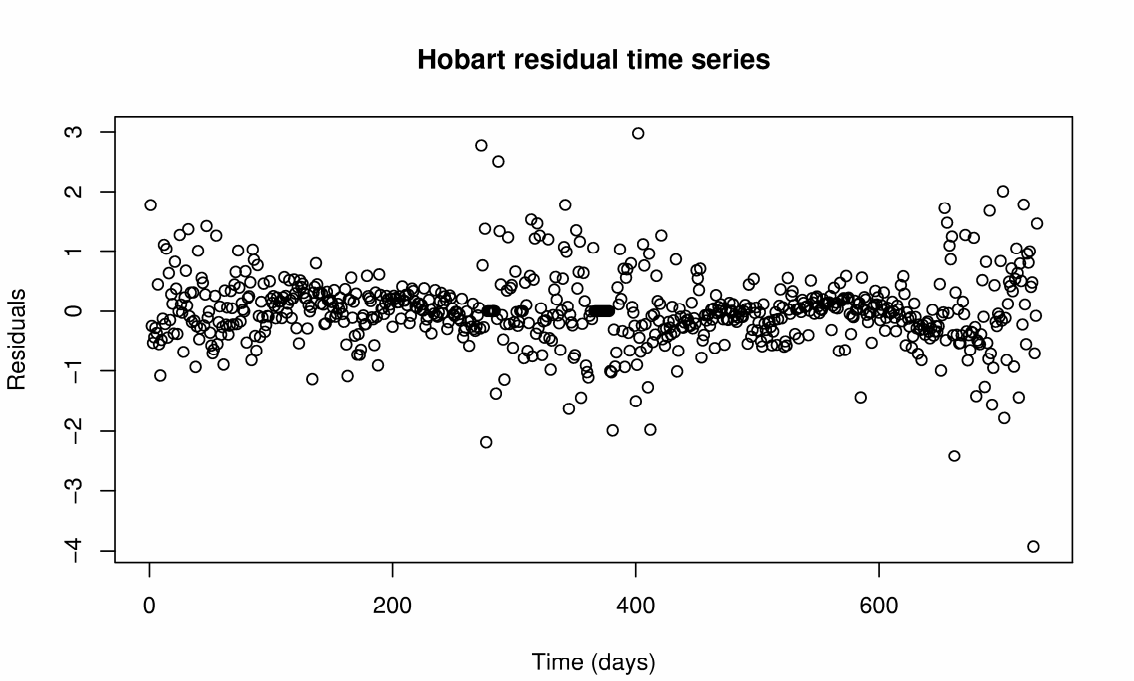

Esperance Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

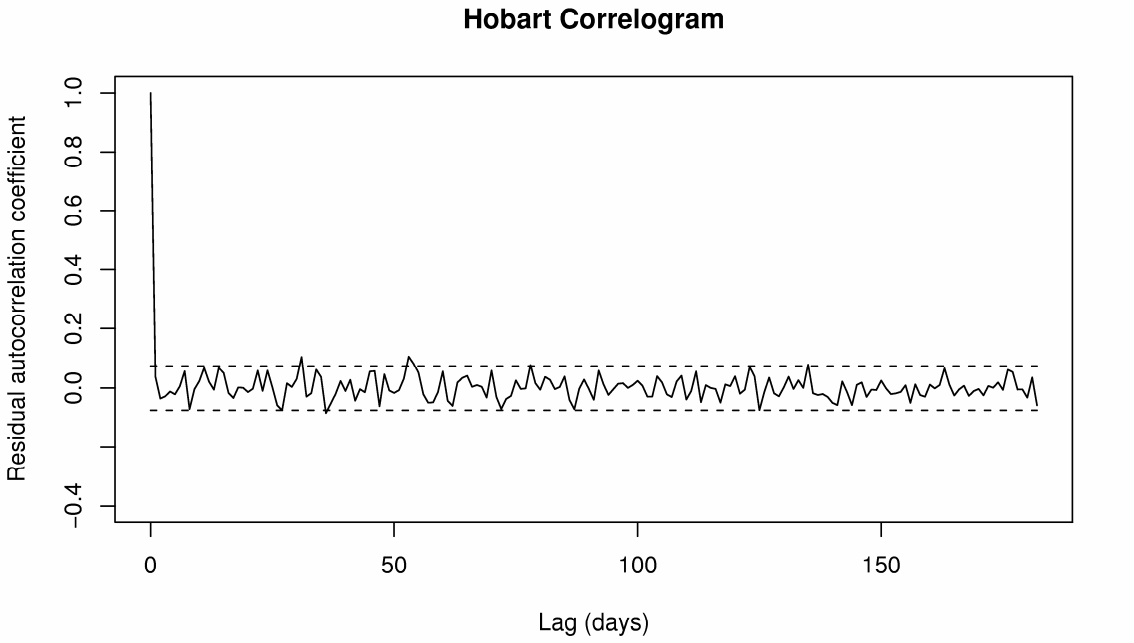

Groote_Eylandt residual time series
Residuals
2
1
0
−1
−2
−3
0
500
1000
1500
2000
Time (days)

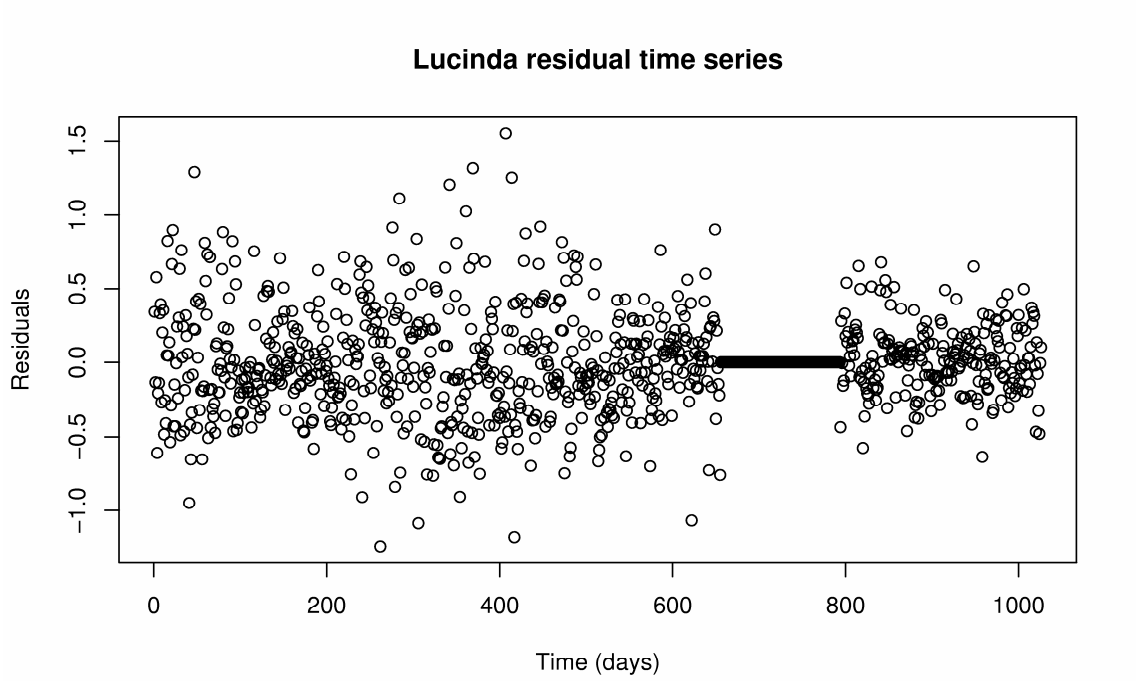

Groote_Eylandt Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

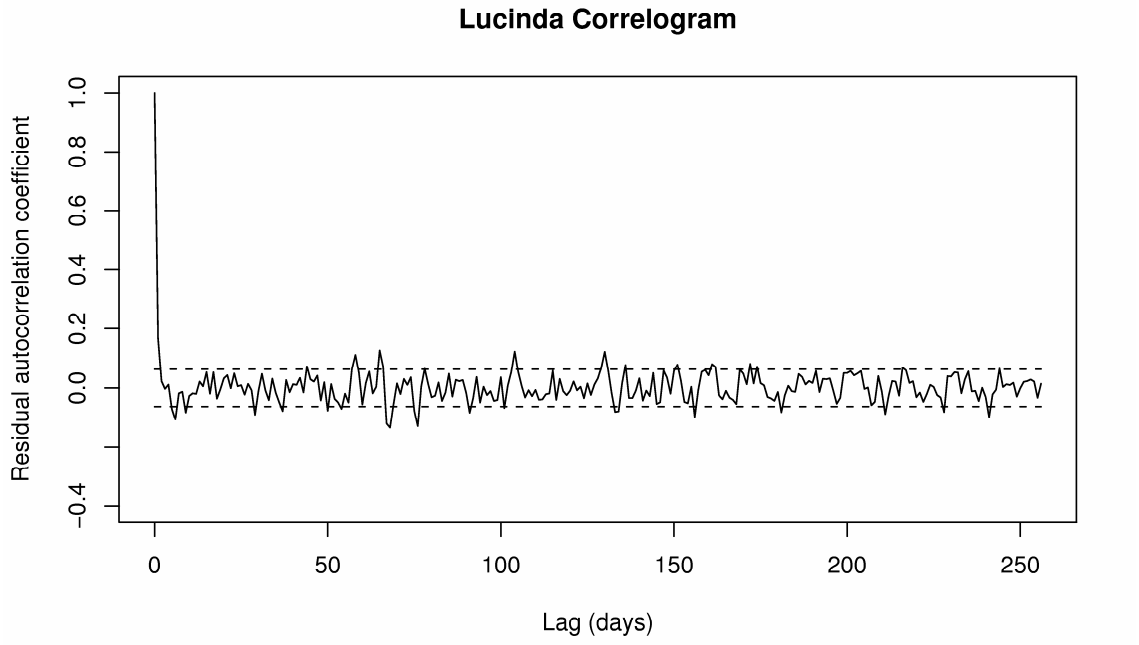

Haypoint residual time series
Residuals
−0.5
0.0
0.5
1.0
1.5
0
50
100
150
200
250
300
350
Time (days)

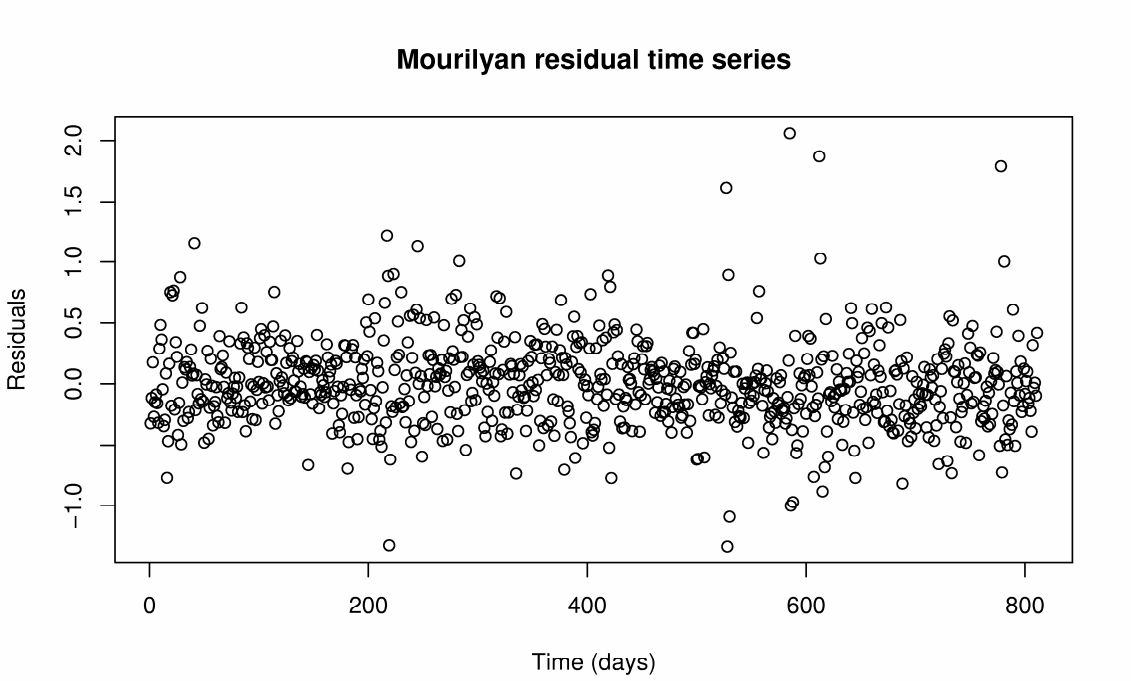

Haypoint Correlogram
Residual autocorrelation coefficient
−0.4
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
Lag (days)

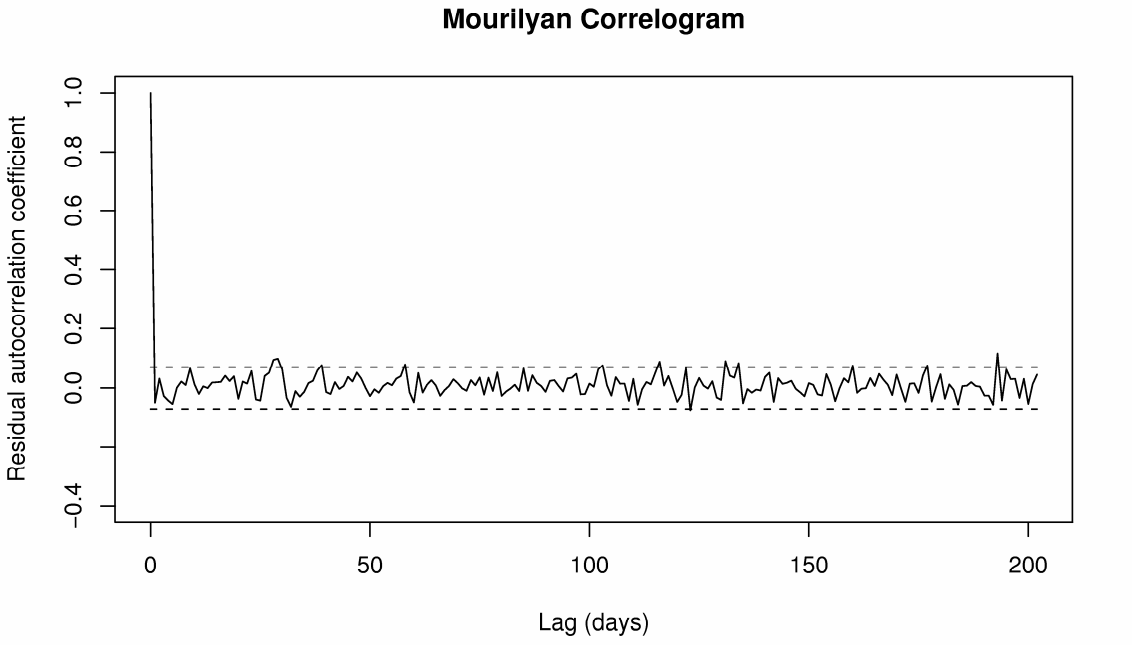

Hobart residual time series
Residuals
3
2
1
0
−1
−2
−3
−4
0
200
400
600
Time (days)

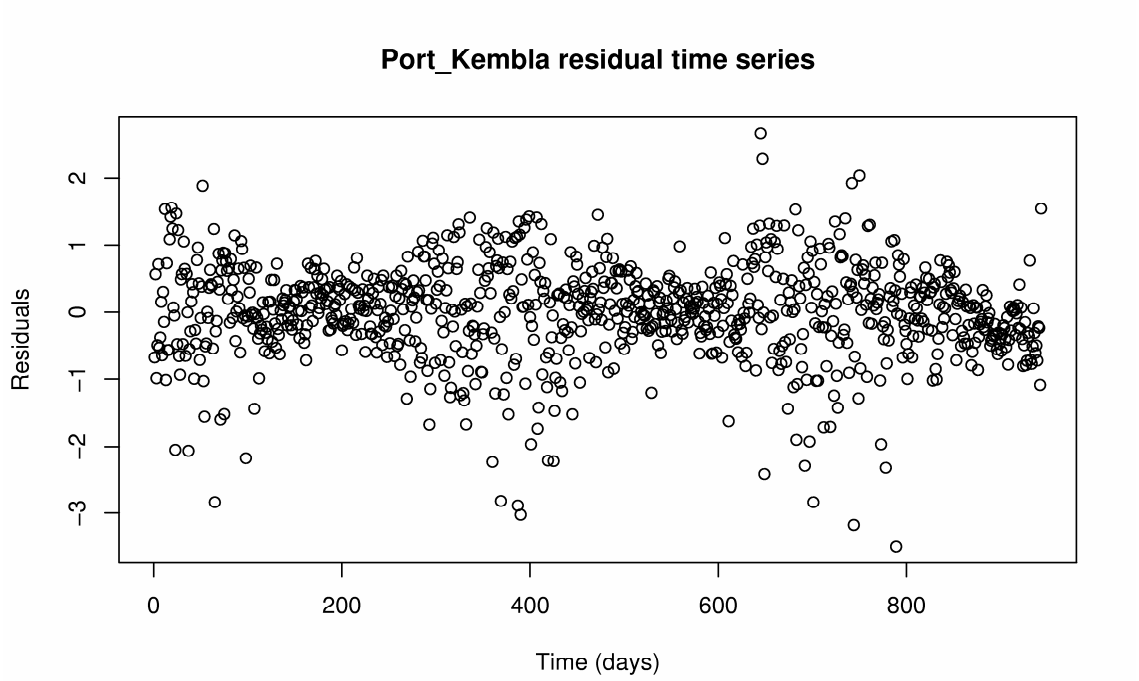

Hobart Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
Lag (days)

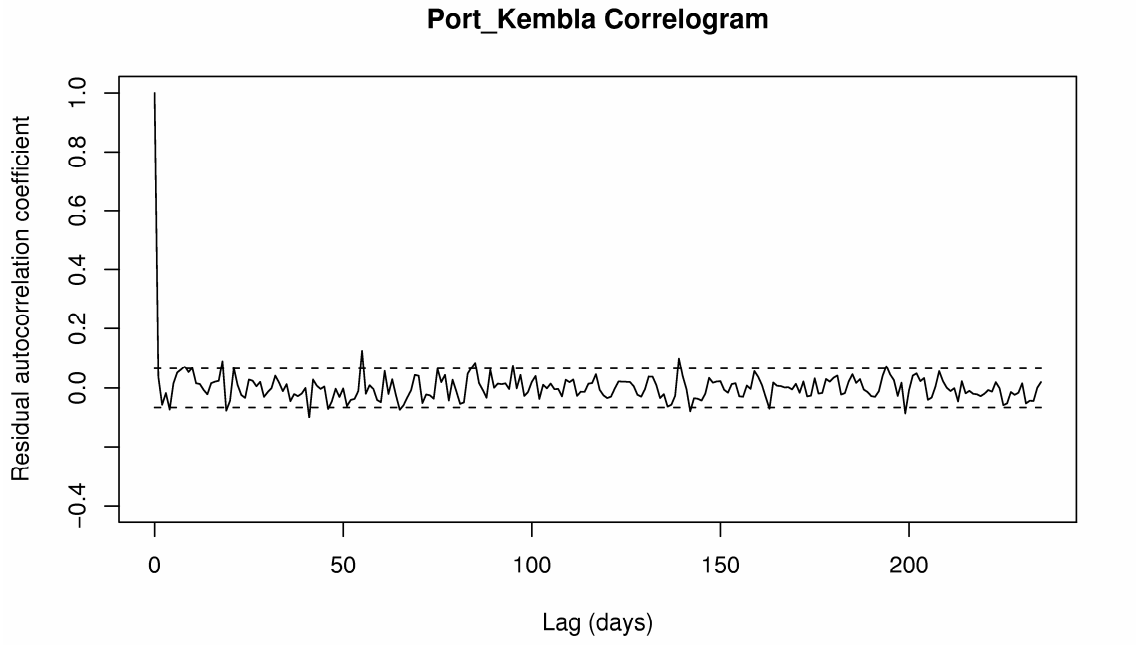

Lucinda residual time series
Residuals
1.5
1.0
0.5
0.0
−0.5
−1.0
0
200
400
600
800
1000
Time (days)

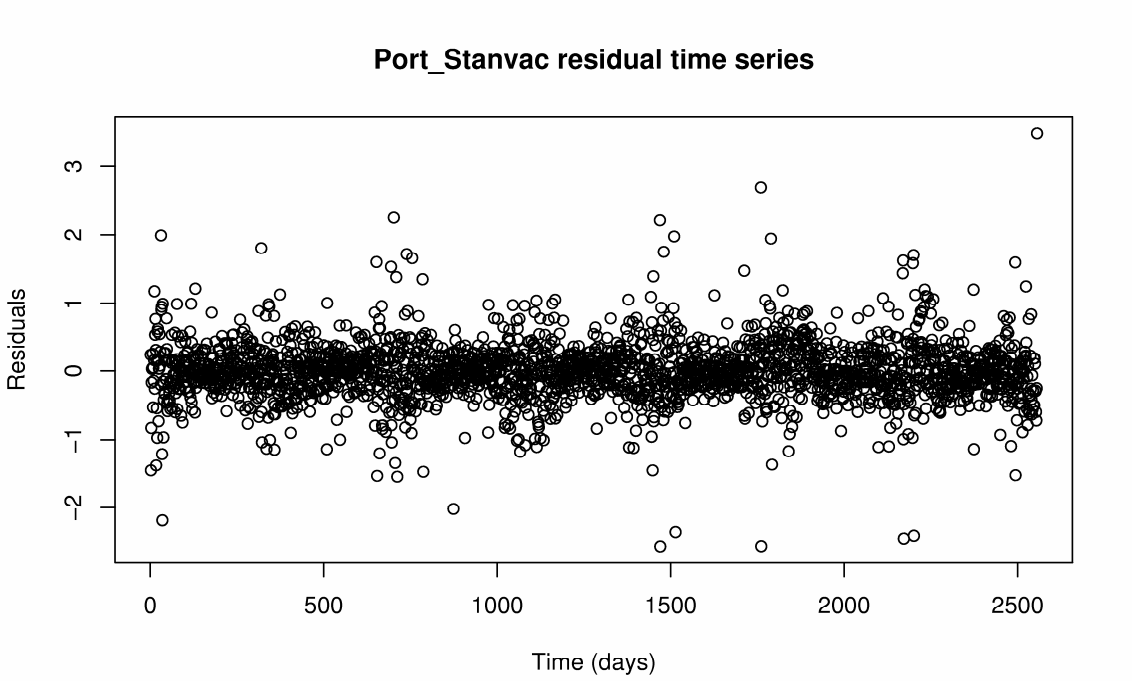

Lucinda Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

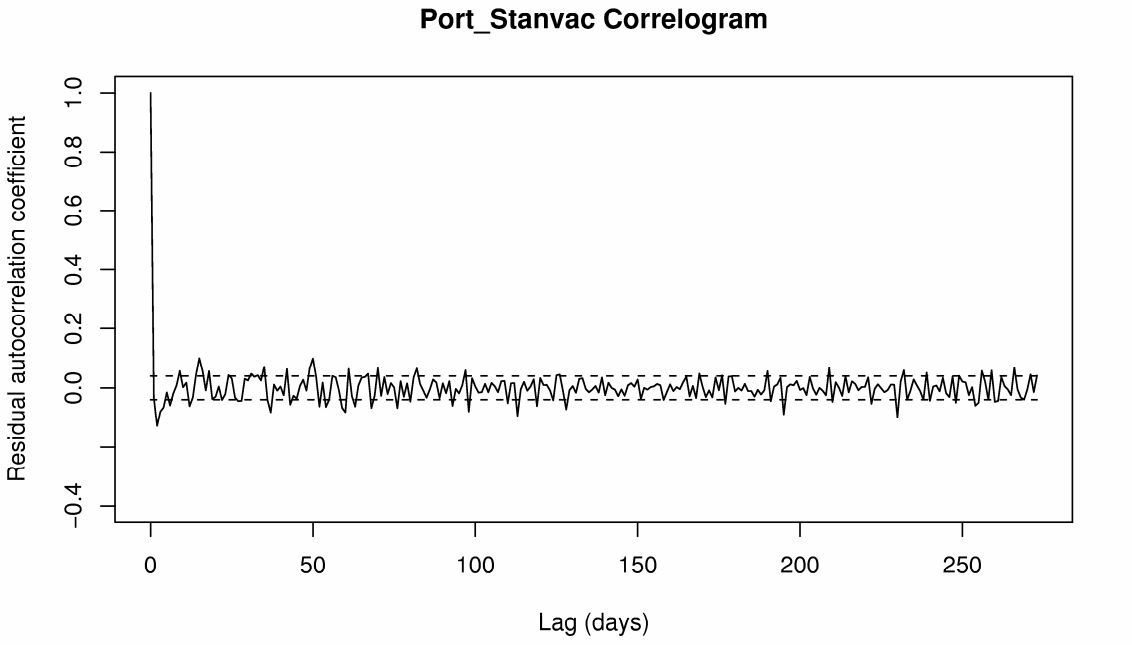

Mourilyan residual time series
Residuals
2.0
1.5
1.0
0.5
0.0
−1.0
0
200
400
600
800
Time (days)

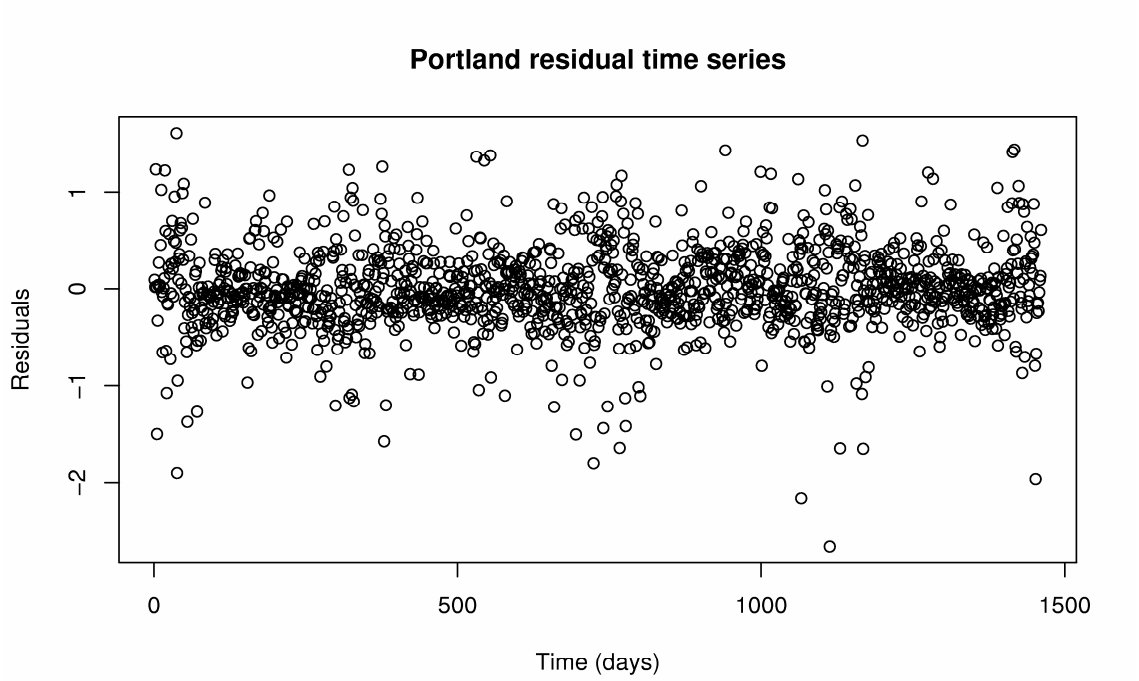

Mourilyan Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
Lag (days)

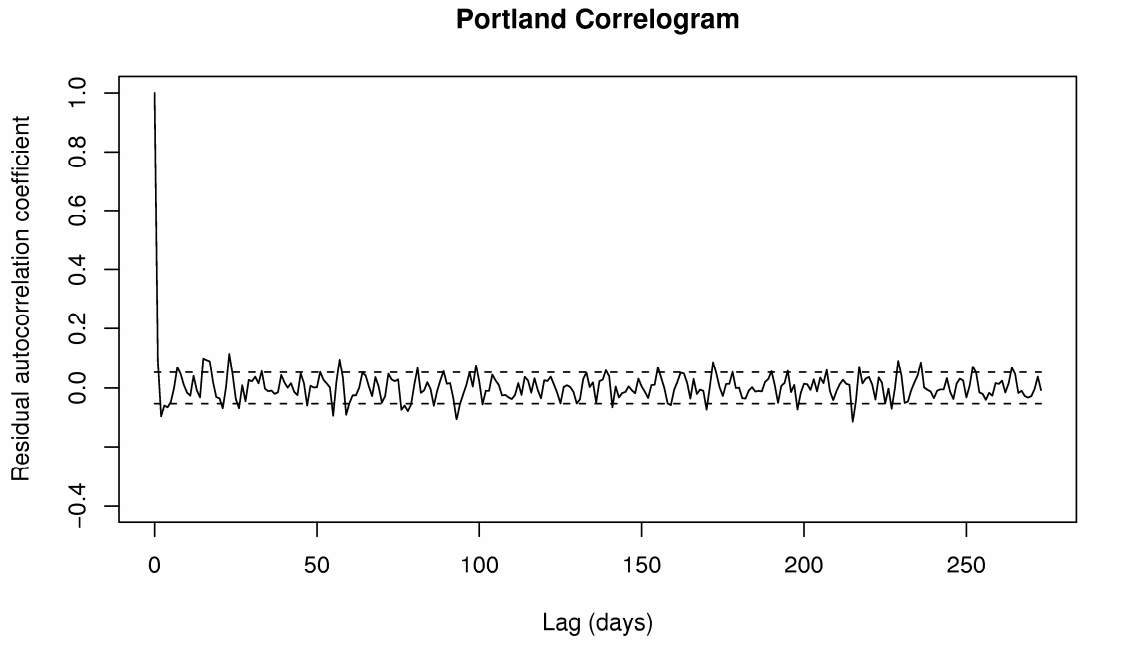

Port_Kembla residual time series
Residuals
−3
−2
−1
0
1
2
0
200
400
600
800
Time (days)

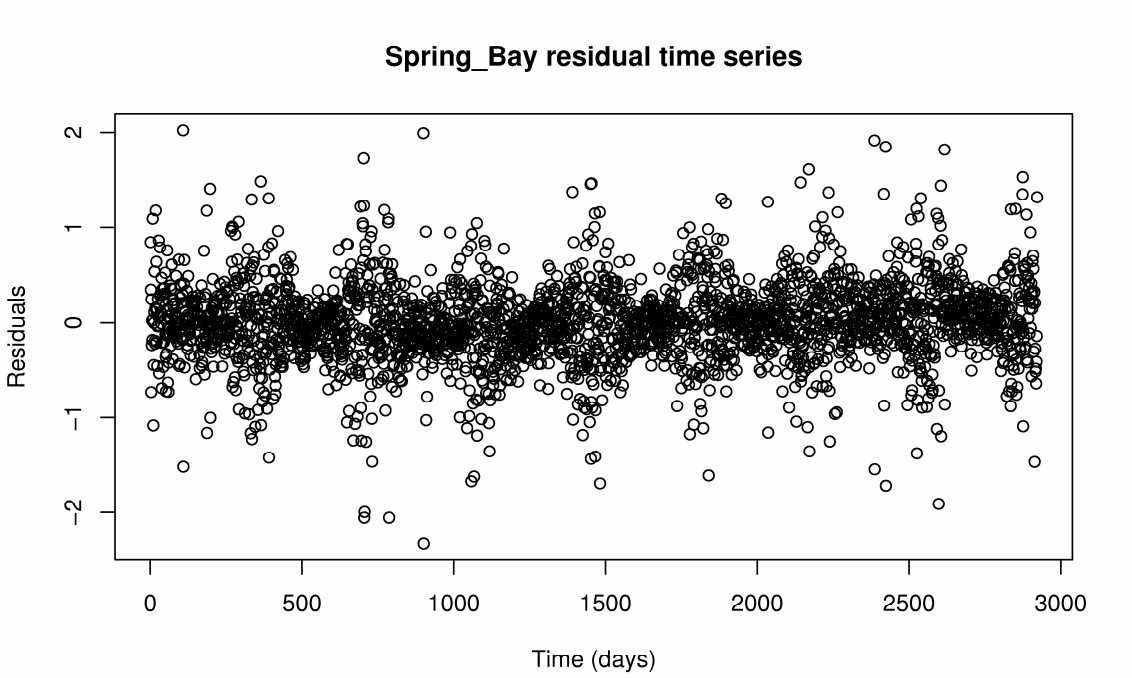

Port_Kembla Correlogram
Residual autocorrelation coefficient
−0.4
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
Lag (days)

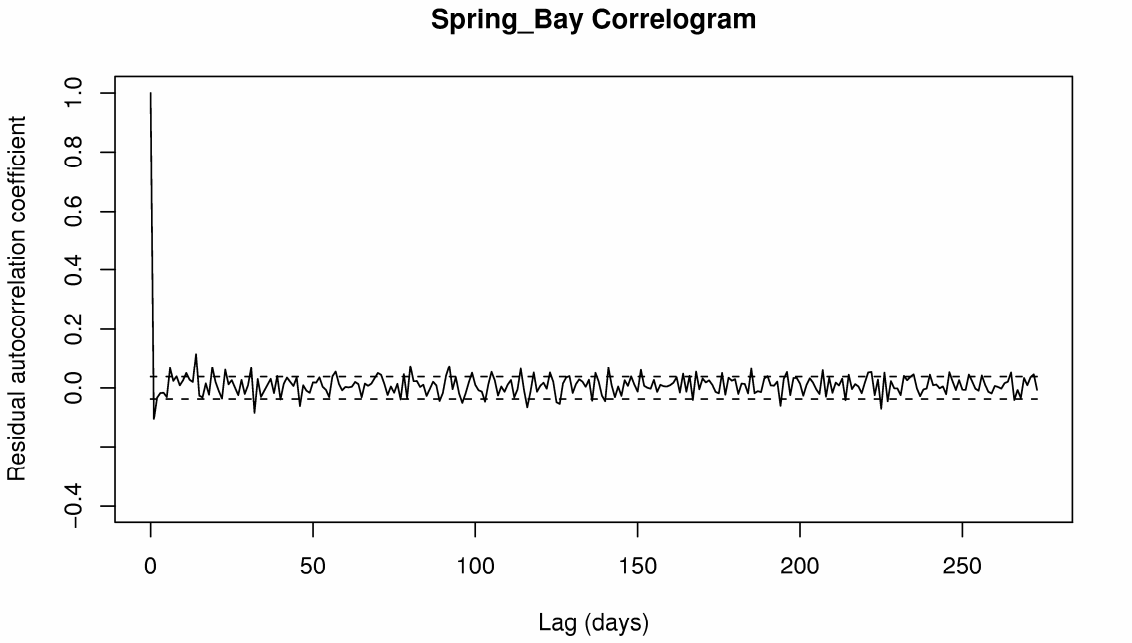

Port_Stanvac residual time series
Residuals
−2
−1
0
1
2
3
0
500
1000
1500
2000
2500
Time (days)

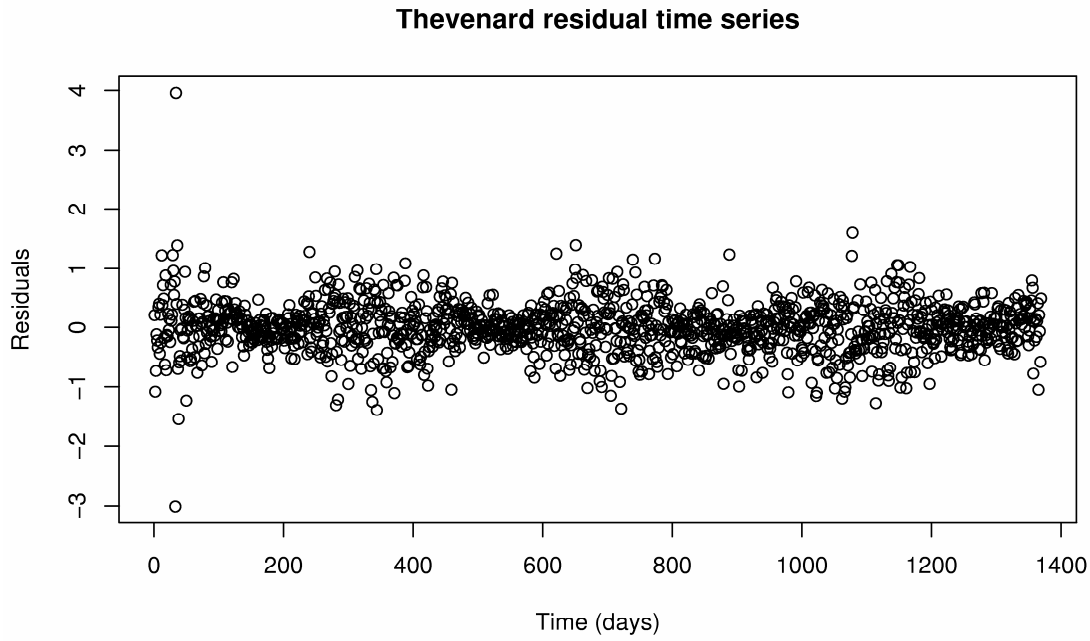

Port_Stanvac Correlogram
Residual autocorrelation coefficient
−0.4
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
Lag (days)

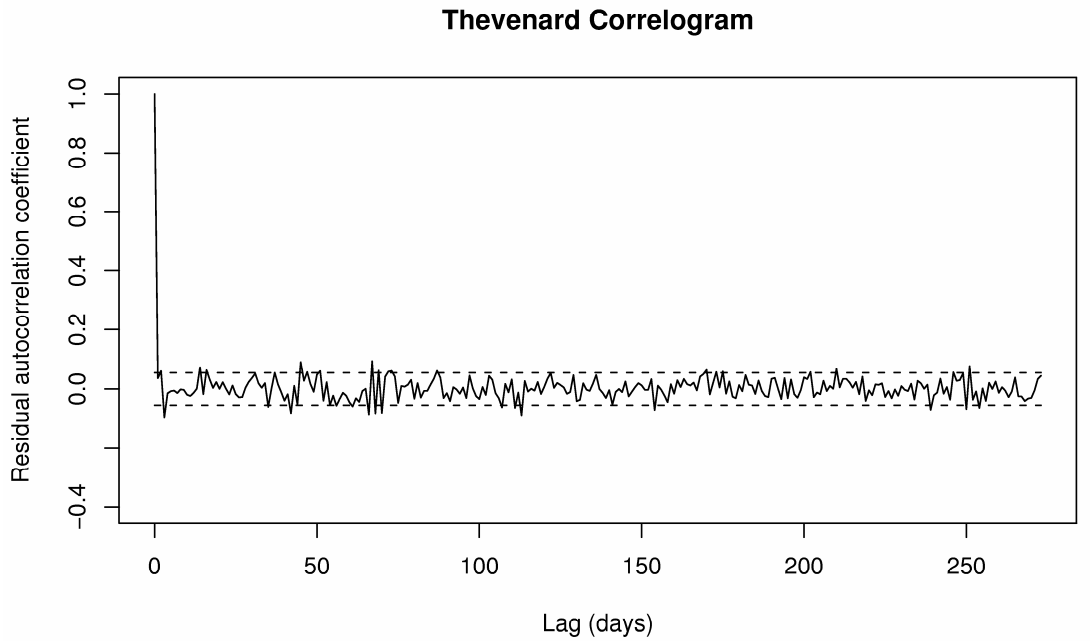

Portland residual time series
Residuals
1
0
−1
−2
0
500
1000
1500
Time (days)

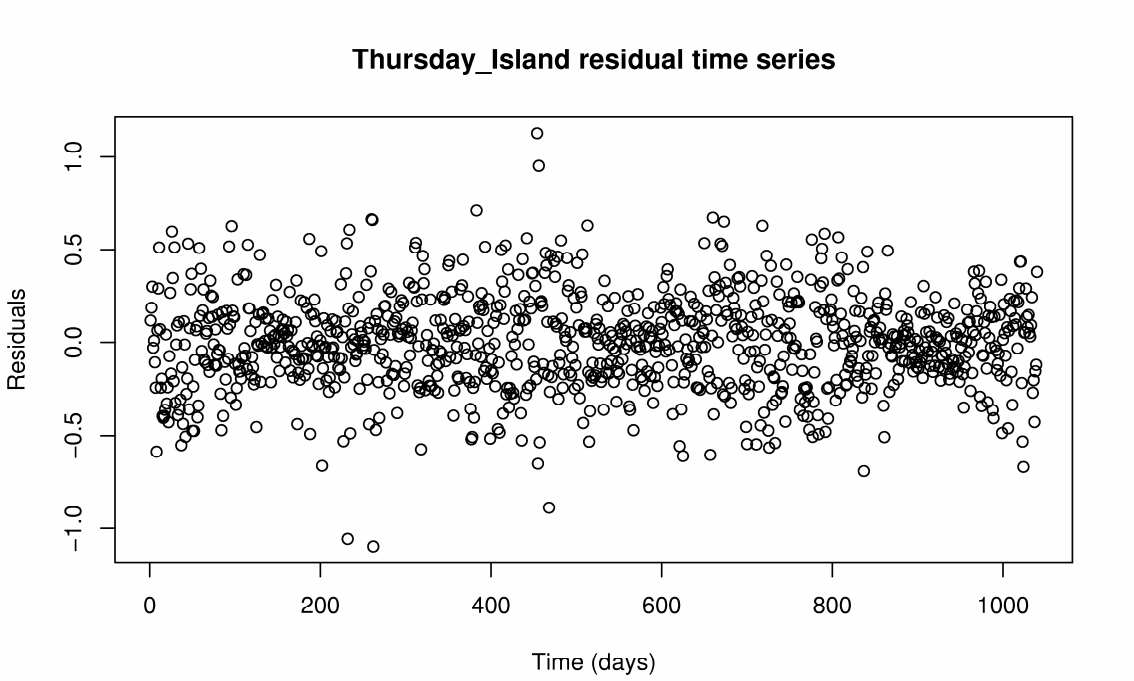

Portland Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

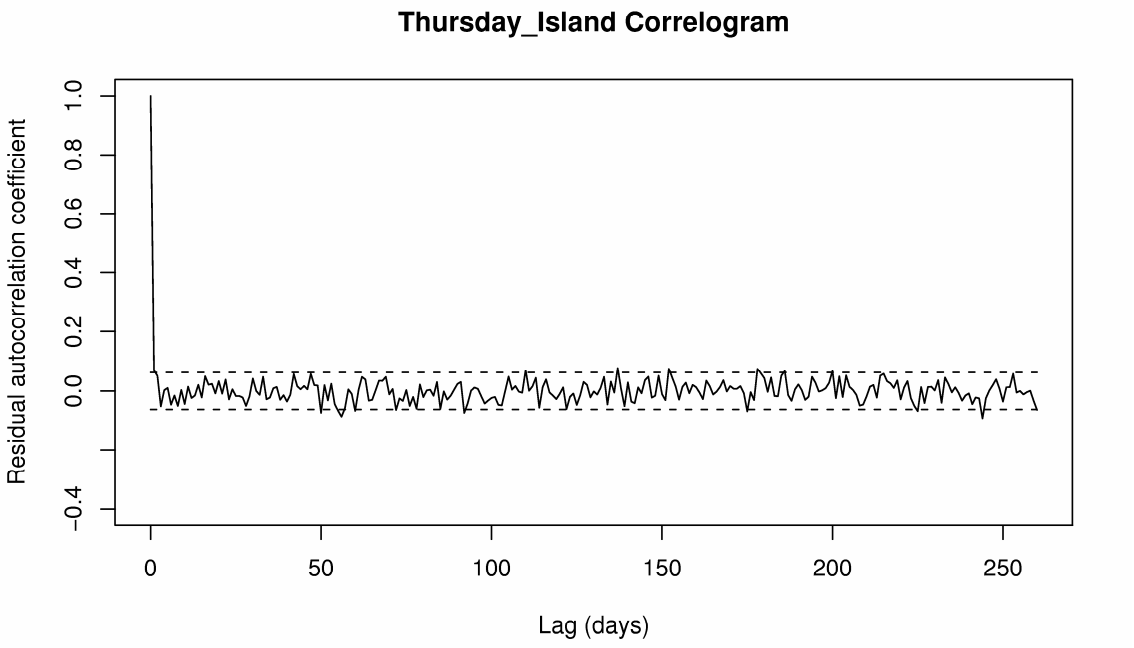

Spring_Bay residual time series
Residuals
2
1
0
−1
−2
0
500
1000
1500
2000
2500
3000
Time (days)

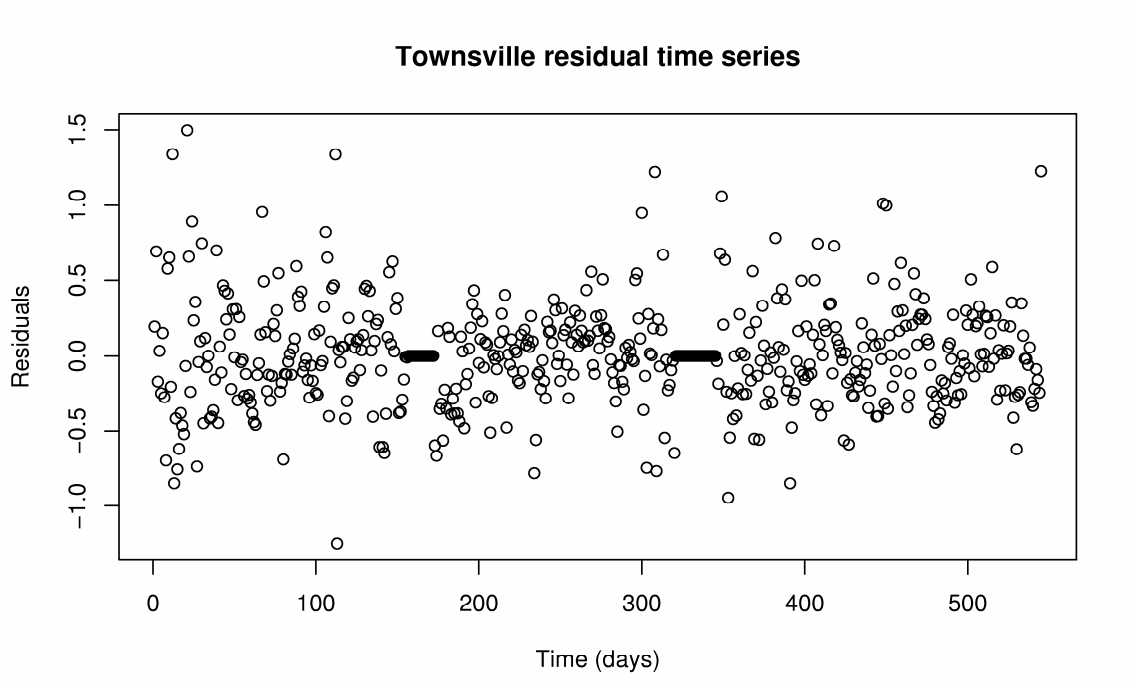

Spring_Bay Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)

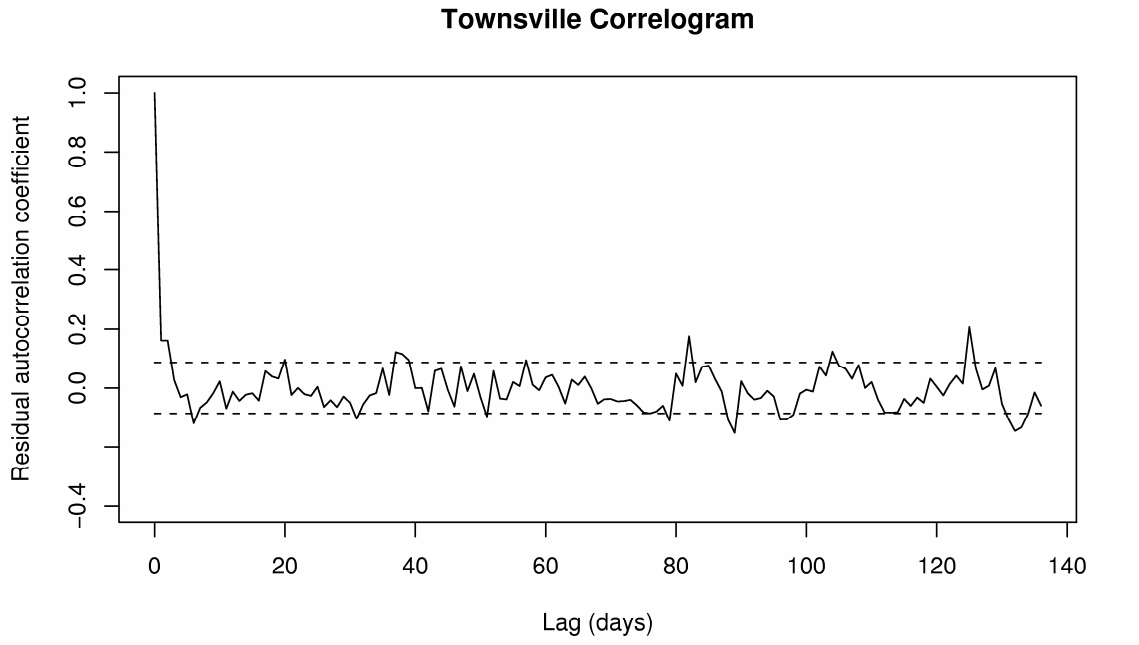

Thevenard residual time series
Residuals
−3
−2
−1
0
1
2
3
4
0
200
400
600
800
1000
1200
1400
Time (days)


Thevenard Correlogram
Residual autocorrelation coefficient
−0.4
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
Lag (days)


Thursday_Island residual time series
Residuals
1.0
0.5
0.0
−0.5
−1.0
0
200
400
600
800
1000
Time (days)


Thursday_Island Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
50
100
150
200
250
Lag (days)


Townsville residual time series
Residuals
1.5
1.0
0.5
0.0
−0.5
−1.0
0
100
200
300
400
500
Time (days)


Townsville Correlogram
Residual autocorrelation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
−0.4
0
20
40
60
80
100
120
140
Lag (days)

APPENDIX B EFFECT OF CUT-OFF ON RISK ASSESSMENT RESULTS FOR 7 SPECIES

Donor port	Cut-off	Newcastle	Melbourne	Port_Kembla	Gladstone	Brisbane	Fremantle	Botany_Bay	Geelong	Dalrymple_Bay	Port_Hedland	Sydney	Weipa	Townsville	Adelaide	Devonport	Dampier	Geraldton	Burnie	Westernport	Portland	Launceston	Port_Stanvac	Hobart	Bunbury	Mackay	Whyalla	Wallaroo	Esperance	Albany	Cairns
Newcastle	0.05	0	7	0	4	7	7	0	7	4	2	0	2	7	7	0	0	6	0	7	7	0	7	0	7	3	7	7	7	7	4
Newcastle	0.8	0	7	0	0	0	0	0	7	0	0	0	0	0	5	0	0	0	0	7	7	0	7	0	4	0	4	4	4	6	0
Melbourne	0.05	12	0	12	12	12	12	12	0	12	12	12	12	12	12	12	12	12	12	12	12	12	12	8	12	12	12	12	12	12	12
Melbourne	0.8	12	0	12	8	10	12	12	0	8	8	12	5	9	12	12	7	8	12	12	12	12	12	8	12	8	12	12	12	12	7
Port_Kembla	0.05	0	7	0	4	7	7	0	7	4	2	0	2	7	7	0	0	6	0	7	7	0	7	0	7	3	7	7	7	7	4
Port_Kembla	0.8	0	7	0	0	0	0	0	7	0	0	0	0	0	5	0	0	0	0	7	7	0	7	0	4	0	4	4	4	6	0
Gladstone	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gladstone	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brisbane	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brisbane	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fremantle	0.05	11	0	12	12	12	0	11	0	12	12	11	12	12	0	7	12	12	8	8	8	8	8	8	11	12	11	11	12	11	12
Fremantle	0.8	8	0	8	8	10	0	8	0	8	8	8	5	9	0	0	7	8	8	8	8	8	8	8	5	8	8	8	6	4	7
Botany_Bay	0.05	0	7	0	4	7	7	0	7	4	2	0	2	7	7	0	0	6	0	7	7	0	7	0	7	3	7	7	7	7	4
Botany_Bay	0.8	0	7	0	0	0	0	0	7	0	0	0	0	0	5	0	0	0	0	7	7	0	7	0	4	0	4	4	4	6	0
Geelong	0.05	12	0	12	12	12	12	12	0	12	12	12	12	12	12	12	12	12	12	12	12	12	12	8	12	12	12	12	12	12	12
Geelong	0.8	12	0	12	8	10	12	12	0	8	8	12	5	9	12	12	7	8	12	12	12	12	12	8	12	8	12	12	12	12	7

Donor port	Cut-off	Newcastle	Melbourne	Port_Kembla	Gladstone	Brisbane	Fremantle	Botany_Bay	Geelong	Dalrymple_Bay	Port_Hedland	Sydney	Weipa	Townsville	Adelaide	Devonport	Dampier	Geraldton	Burnie	Westernport	Portland	Launceston	Port_Stanvac	Hobart	Bunbury	Mackay	Whyalla	Wallaroo	Esperance	Albany	Cairns
Dalrymple_Bay	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dalrymple_Bay	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Port_Hedland	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Port_Hedland	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sydney	0.05	0	7	0	4	7	7	0	7	4	2	0	2	7	7	0	0	6	0	7	7	0	7	0	7	3	7	7	7	7	4
Sydney	0.8	0	7	0	0	0	0	0	7	0	0	0	0	0	5	0	0	0	0	7	7	0	7	0	4	0	4	4	4	6	0
Weipa	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Weipa	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Townsville	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Townsville	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adelaide	0.05	12	0	12	12	12	12	12	0	12	12	12	12	12	0	7	12	12	8	8	12	8	12	8	12	12	12	12	12	12	12
Adelaide	0.8	8	0	8	8	10	3	8	0	8	8	8	5	9	0	0	7	8	8	8	12	8	8	8	5	8	8	8	9	6	7
Devonport	0.05	12	7	12	11	12	12	12	7	9	9	12	7	10	12	0	8	12	8	8	12	12	12	8	12	9	12	12	12	12	8
Devonport	0.8	12	7	12	7	8	12	12	7	5	4	12	0	6	12	0	0	8	8	8	12	12	12	8	12	5	12	12	12	12	1
Dampier*	0.05	12	0	12	12	12	12	12	0	12	12	12	12	12	12	12	0	12	12	12	12	12	12	8	12	12	12	12	12	12	12
Dampier*	0.8	5	0	7	8	10	0	5	0	8	8	5	5	9	0	0	0	7	0	0	0	0	5	0	5	8	5	5	6	4	7
Geraldton	0.05	12	7	12	12	12	12	12	7	12	12	12	12	12	12	12	12	0	12	12	12	12	12	8	12	12	12	12	12	12	12
Geraldton	0.8	12	0	12	8	10	12	12	0	8	8	12	5	9	12	12	7	0	12	12	12	12	12	8	12	8	12	12	12	12	7
Burnie	0.05	12	7	12	11	12	12	12	7	9	9	12	5	10	12	0	7	12	0	7	12	12	12	0	12	9	12	12	12	12	7
Burnie	0.8	12	7	12	4	4	12	12	7	2	0	12	0	2	12	0	0	8	0	7	12	12	12	0	12	1	12	12	12	12	0

Donor port	Cut-off	Newcastle	Melbourne	Port_Kembla	Gladstone	Brisbane	Fremantle	Botany_Bay	Geelong	Dalrymple_Bay	Port_Hedland	Sydney	Weipa	Townsville	Adelaide	Devonport	Dampier	Geraldton	Burnie	Westernport	Portland	Launceston	Port_Stanvac	Hobart	Bunbury	Mackay	Whyalla	Wallaroo	Esperance	Albany	Cairns
Westernport	0.05	12	0	12	12	12	12	12	0	12	12	12	12	12	12	7	12	12	5	0	12	12	12	4	12	12	12	12	12	12	12
Westernport	0.8	12	0	12	8	10	12	12	0	8	8	12	5	9	12	0	7	8	0	0	12	12	12	0	12	8	12	12	12	12	7
Portland	0.05	12	0	12	12	12	12	12	0	12	12	12	12	12	12	7	12	12	5	0	0	12	12	4	12	12	12	12	12	12	12
Portland	0.8	12	0	12	8	10	12	12	0	8	8	12	5	9	12	0	7	8	0	0	0	12	12	0	12	8	12	12	12	12	7
Launceston	0.05	12	7	12	12	12	12	12	7	12	12	12	12	12	7	7	12	12	5	7	12	0	12	4	12	12	12	12	12	12	12
Launceston	0.8	5	7	7	8	10	3	5	7	8	8	5	5	9	5	0	7	7	0	7	12	0	7	0	5	8	5	5	9	6	7
Port_Stanvac*	0.05	12	7	12	12	12	12	12	7	12	12	12	12	12	12	12	12	12	12	12	12	12	0	8	12	12	12	12	12	12	12
Port_Stanvac*	0.8	12	7	12	8	10	12	12	7	8	8	12	5	9	12	12	7	8	12	12	12	12	0	8	12	8	12	12	12	12	7
Hobart	0.05	12	7	12	11	12	12	12	7	9	9	12	5	10	12	12	7	12	12	12	12	12	12	0	12	9	12	12	12	12	7
Hobart	0.8	12	7	12	4	4	12	12	7	2	0	12	0	2	12	12	0	8	12	12	12	12	12	0	12	1	12	12	12	12	0
Bunbury	0.05	8	0	8	8	8	0	8	0	8	8	8	7	8	0	0	8	8	8	8	8	8	8	8	0	8	8	8	0	0	8
Bunbury	0.8	8	0	8	7	8	0	8	0	5	4	8	0	6	0	0	0	8	8	8	8	8	8	8	0	5	8	8	0	0	1
Mackay	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mackay	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Whyalla*	0.05	12	7	12	12	12	12	12	7	12	12	12	12	12	12	12	12	12	12	12	12	12	12	8	12	12	0	12	12	12	12
Whyalla*	0.8	12	7	12	8	10	12	12	7	8	8	12	5	9	12	12	7	8	12	12	12	12	12	8	12	8	0	12	12	12	7
Wallaroo*	0.05	12	7	12	12	12	12	12	7	12	12	12	12	12	12	12	12	12	12	12	12	12	12	8	12	12	12	0	12	12	12
Wallaroo*	0.8	12	7	12	8	10	12	12	7	8	8	12	5	9	12	12	7	8	12	12	12	12	12	8	12	8	12	0	12	12	7
Esperance	0.05	8	0	8	8	8	0	8	0	8	8	8	7	8	0	0	8	8	8	8	8	8	8	8	0	8	8	8	0	0	8
Esperance	0.8	8	0	8	7	8	0	8	0	5	4	8	0	6	0	0	0	8	8	8	8	8	8	8	0	5	8	8	0	0	1

Donor port	Cut-off	Newcastle	Melbourne	Port_Kembla	Gladstone	Brisbane	Fremantle	Botany_Bay	Geelong	Dalrymple_Bay	Port_Hedland	Sydney	Weipa	Townsville	Adelaide	Devonport	Dampier	Geraldton	Burnie	Westernport	Portland	Launceston	Port_Stanvac	Hobart	Bunbury	Mackay	Whyalla	Wallaroo	Esperance	Albany	Cairns
Albany	0.05	8	0	8	8	8	0	8	0	8	8	8	7	8	0	0	8	8	8	8	8	8	8	8	0	8	8	8	0	0	8
Albany	0.8	8	0	8	7	8	0	8	0	5	4	8	0	6	0	0	0	8	8	8	8	8	8	8	0	5	8	8	0	0	1
Cairns	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cairns	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX C *C. GIGAS* SAMPLING RESULTS

Date	Location	18S	*C. gigas*	Date	Location	18S	*C. gigas*
12/08/2003	RYC	1	0	4/11/2003	Domain	1	0
12/08/2003	RYC	1	0	4/11/2003	Domain	1	0
12/08/2003	RYC	1	0	4/11/2003	Domain	1	0
15/08/2003	RYC	1	0	12/11/2003	RYC	1	0
15/08/2003	RYC	1	0	12/11/2003	RYC	1	0
15/08/2003	RYC	1	0	12/11/2003	RYC	1	0
8/09/2003	RYC	1	0	19/11/2003	Domain	1	0
8/09/2003	RYC	1	0	19/11/2003	Domain	1	0
8/09/2003	RYC	1	0	19/11/2003	Domain	1	0
23/09/2003	RYC	1	0	21/11/2003	RYC	1	0
23/09/2003	RYC	1	0	21/11/2003	RYC	1	0
23/09/2003	RYC	1	0	21/11/2003	RYC	1	0
25/09/2003	RYC	1	0	24/11/2003	RYC	1	0
25/09/2003	RYC	1	0	24/11/2003	RYC	1	0
25/09/2003	RYC	1	0	24/11/2003	RYC	1	0
1/10/2003	RYC	1	0	8/12/2003	Domain	1	0
1/10/2003	RYC	1	0	8/12/2003	Domain	1	0
1/10/2003	RYC	1	0	8/12/2003	Domain	1	0
6/10/2003	RYC	1	0	2/02/2004	Domain	1	0
6/10/2003	RYC	1	0	2/02/2004	Domain	1	1
6/10/2003	RYC	1	0	2/02/2004	Domain	1	0
22/10/2003	RYC	1	0	3/02/2004	Domain	1	0
22/10/2003	RYC	1	0	3/02/2004	Domain	1	0
22/10/2003	RYC	1	0	3/02/2004	Domain	1	0
24/10/2003	RYC	1	0	10/02/2004	Domain	1	1
24/10/2003	RYC	1	0	10/02/2004	Domain	1	1
24/10/2003	RYC	1	0	10/02/2004	Domain	1	0
3/11/2003	Domain	1	0	25/02/2004	RYC	1	1
3/11/2003	Domain	1	0	25/02/2004	RYC	1	1
3/11/2003	Domain	1	0	25/02/2004	RYC	1	1

Date	Location	18S	*C. gigas*	Date	Location	18S	*C. gigas*
1/03/2004	RYC	1	0	10/05/2004	Domain	1	0
1/03/2004	RYC	1	1	10/05/2004	Domain	1	0
1/03/2004	RYC	1	1	10/05/2004	Domain	1	0
3/03/2004	Domain	1	1	20/05/2004	Domain	1	0
3/03/2004	Domain	1	1	20/05/2004	Domain	1	0
3/03/2004	Domain	1	1	20/05/2004	Domain	1	0
18/03/2004	Domain	1	0	11/06/2004	Domain	1	0
18/03/2004	Domain	1	0	11/06/2004	Domain	1	0
18/03/2004	Domain	1	0	11/06/2004	Domain	1	0
2/04/2004	RYC	1	0	14/07/2004	Domain	1	0
2/04/2004	RYC	1	0	14/07/2004	Domain	1	0
2/04/2004	RYC	1	0	14/07/2004	Domain	1	0
23/04/2004	Domain	1	0	19/07/2004	RYC	1	0
23/04/2004	Domain	1	0	19/07/2004	RYC	1	0
23/04/2004	Domain	1	0	19/07/2004	RYC	1	0
28/04/2004	RYC	1	0	23/09/2004	RYC	1	1
28/04/2004	RYC	1	0	23/09/2004	RYC	1	0
28/04/2004	RYC	1	0	23/09/2004	RYC	1	1
3/05/2004	Domain	1	0	4/10/2004	Domain	1	0
3/05/2004	Domain	1	0	4/10/2004	Domain	1	0
3/05/2004	Domain	1	0	4/10/2004	Domain	1	0

Date	Location	Weight (g)	ID	*C. gigas*	18s
07/12/05	Risdon	0.68	1	1	1
07/12/05	Risdon	0.75	2	1	1
07/12/05	Risdon	0.48	3	1	1
07/12/05	Geilston Bay	0.0625	4	0	1
07/12/05	Geilston Bay	0.2648	5	0	1
07/12/05	Geilston Bay	0.609	6	1	1
07/12/05	Macq. Wharf	0.6134	7	1	1
07/12/05	Macq. Wharf	1.4982	8	1	1
07/12/05	Macq. Wharf	1.163	9	0	1
07/12/05	Kangaroo Bluff	0.4204	10	1	1
07/12/05	Kangaroo Bluff	0.7664	11	1	1
07/12/05	Kangaroo Bluff	1.0971	12	1	1
07/12/05	Wrest Point	0.613	13	0	1
07/12/05	Wrest Point	0.4411	14	1	1
07/12/05	Wrest Point	0.2629	15	0	1
07/12/05	Tranmere	0.4239	16	0	1
07/12/05	Tranmere	1.0115	17	1	1
07/12/05	Tranmere	0.8008	18	1	1
07/12/05	Blackmans Bay	0.5464	19	0	1
07/12/05	Blackmans Bay	1.0971	20	1	1
07/12/05	Blackmans Bay	0.3921	21	1	1
07/12/05	East Dennes	0.0632	22	0	1
07/12/05	East Dennes	0.2131	23	0	1
07/12/05	East Dennes	0.1054	24	0	1
07/12/05	West Dennes	0.5756	25	1	1
07/12/05	West Dennes	0.2243	26	0	1
07/12/05	West Dennes	0.3857	27	1	1
07/12/05	Coningham	0.5655	28	0	1
07/12/05	Coningham	0.2433	29	0	1
07/12/05	Coningham	0.216	30	0	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
19/12/05	Risdon	1.8447	31	1	1
19/12/05	Risdon	1.6692	32	1	1
19/12/05	Risdon	1.8903	33	0	1
19/12/05	Geilston Bay	1.1826	34	0	1
19/12/05	Geilston Bay	1.3021	35	1	1
19/12/05	Geilston Bay	1.2219	36	0	1
19/12/05	Macq. Wharf	0.5959	37	0	1
19/12/05	Macq. Wharf	1.4968	38	1	1
19/12/05	Macq. Wharf	1.9966	39	1	1
19/12/05	Kangaroo Bluff	1.2454	40	1	1
19/12/05	Kangaroo Bluff	0.8072	41	1	1
19/12/05	Kangaroo Bluff	1.289	42	1	1
19/12/05	Wrest Point	1.0895	43	0	1
19/12/05	Wrest Point	2.7288	44	1	1
19/12/05	Wrest Point	1.3483	45	0	1
19/12/05	Tranmere	1.7821	46	1	1
19/12/05	Tranmere	1.5731	47	1	1
19/12/05	Tranmere	0.9392	48	1	1
19/12/05	Blackmans Bay	0.4112	49	0	1
19/12/05	Blackmans Bay	0.2236	50	0	1
19/12/05	Blackmans Bay	0.5246	51	1	1
19/12/05	East Dennes	0.1839	52	0	1
19/12/05	East Dennes	0.2094	53	0	0
19/12/05	East Dennes	0.337	54	0	1
19/12/05	West Dennes	0.3219	55	0	1
19/12/05	West Dennes	0.3265	56	0	1
19/12/05	West Dennes	0.2915	57	0	1
19/12/05	Coningham	0.4067	58	0	1
19/12/05	Coningham	0.6318	59	0	1
19/12/05	Coningham	0.4851	60	0	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
05/01/06	Risdon	0.8163	61	1	1
05/01/06	Risdon	0.936	62	1	1
05/01/06	Risdon	0.6946	63	1	1
05/01/06	Geilston Bay	0.493	64	1	1
05/01/06	Geilston Bay	0.2974	65	1	1
05/01/06	Geilston Bay	0.5098	66	1	1
05/01/06	Macq. Wharf	0.7285	67	1	1
05/01/06	Macq. Wharf	1.4973	68	1	1
05/01/06	Macq. Wharf	1.14	69	1	1
05/01/06	Kangaroo Bluff	1.586	70	1	1
05/01/06	Kangaroo Bluff	1.3319	71	1	1
05/01/06	Kangaroo Bluff	1.3981	72	1	1
05/01/06	Wrest Point	0.7844	73	1	1
05/01/06	Wrest Point	0.8939	74	1	1
05/01/06	Wrest Point	1.1328	75	1	1
05/01/06	Tranmere	0.3911	76	1	1
05/01/06	Tranmere	1.1243	77	1	1
05/01/06	Tranmere	0.6578	78	1	1
05/01/06	Blackmans Bay	1.687	79	1	1
05/01/06	Blackmans Bay	1.3723	80	1	1
05/01/06	Blackmans Bay	1.4139	81	1	1
05/01/06	East Dennes	0.8478	82	1	1
05/01/06	East Dennes	0.7732	83	1	1
05/01/06	East Dennes	0.5205	84	1	1
05/01/06	West Dennes	0.2758	85	1	1
05/01/06	West Dennes	0.4079	86	1	1
05/01/06	West Dennes	0.447	87	1	1
05/01/06	Coningham	1.1171	88	0	1
05/01/06	Coningham	0.9916	89	0	1
05/01/06	Coningham	1.0338	90	0	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
16/01/06	Risdon	3.2254	91	0	0
16/01/06	Risdon	2.5289	92	0	0
16/01/06	Risdon	2.5544	93	0	0
16/01/06	Geilston Bay	1.3008	94	1	1
16/01/06	Geilston Bay	1.3248	95	1	1
16/01/06	Geilston Bay	1.3884	96	1	1
16/01/06	Macq. Wharf	0.7502	97	1	1
16/01/06	Macq. Wharf	0.3436	98	1	1
16/01/06	Macq. Wharf	0.5288	99	1	1
16/01/06	Kangaroo Bluff	1.1727	100	1	1
16/01/06	Kangaroo Bluff	1.0648	101	1	1
16/01/06	Kangaroo Bluff	1.3036	102	1	1
16/01/06	Wrest Point	1.347	103	1	1
16/01/06	Wrest Point	1.5413	104	1	1
16/01/06	Wrest Point	1.31	105	1	1
16/01/06	Tranmere	1.7121	106	1	1
16/01/06	Tranmere	1.4874	107	1	1
16/01/06	Tranmere	1.6364	108	1	1
16/01/06	Blackmans Bay	1.9264	109	1	1
16/01/06	Blackmans Bay	1.7527	110	1	1
16/01/06	Blackmans Bay	1.2852	111	1	1
16/01/06	East Dennes	0.7879	112	1	1
16/01/06	East Dennes	1.1986	113	1	1
16/01/06	East Dennes	1.1614	114	1	1
16/01/06	West Dennes	0.7102	115	0	1
16/01/06	West Dennes	0.866	116	1	1
16/01/06	West Dennes	0.9008	117	1	1
16/01/06	Coningham	0.9587	118	1	1
16/01/06	Coningham	0.8654	119	1	1
16/01/06	Coningham	0.8414	120	1	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
30/01/06	Risdon	11.4089	121	0	0
30/01/06	Risdon	11.8778	122	0	0
30/01/06	Risdon	9.281	123	0	0
30/01/06	Geilston Bay	1.8237	124	1	1
30/01/06	Geilston Bay	1.3745	125	1	1
30/01/06	Geilston Bay	2.0803	126	1	1
30/01/06	Macq. Wharf	3.5916	127	1	1
30/01/06	Macq. Wharf	3.1357	128	1	1
30/01/06	Macq. Wharf	3.0892	129	1	1
30/01/06	Kangaroo Bluff	7.7439	130	1	1
30/01/06	Kangaroo Bluff	7.1948	131	1	1
30/01/06	Kangaroo Bluff	3.844	132	1	1
30/01/06	Wrest Point	7.3116	133	1	1
30/01/06	Wrest Point	6.2453	134	1	1
30/01/06	Wrest Point	6.6501	135	1	1
30/01/06	Tranmere	5.4395	136	0	1
30/01/06	Tranmere	8.5233	137	0	1
30/01/06	Tranmere	5.4772	138	1	1
30/01/06	Blackmans Bay	4.3131	139	1	1
30/01/06	Blackmans Bay	2.4279	140	1	1
30/01/06	Blackmans Bay	3.0888	141	1	1
30/01/06	East Dennes	1.8407	142	1	1
30/01/06	East Dennes	1.9478	143	1	1
30/01/06	East Dennes	1.471	144	1	1
30/01/06	West Dennes	0.9093	145	1	1
30/01/06	West Dennes	1.5884	146	1	1
30/01/06	West Dennes	1.2969	147	1	1
30/01/06	Coningham	2.6409	148	1	1
30/01/06	Coningham	2.1739	149	1	1
30/01/06	Coningham	2.5762	150	1	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
15/02/06	Risdon	5.2491	151	0	1
15/02/06	Risdon	8.2878	152	0	0
15/02/06	Risdon	8.4136	153	0	0
15/02/06	Geilston Bay	7.1174	154	1	1
15/02/06	Geilston Bay	6.6037	155	1	1
15/02/06	Geilston Bay	5.3507	156	1	1
15/02/06	Macq. Wharf	3.8197	157	1	1
15/02/06	Macq. Wharf	4.8283	158	0	1
15/02/06	Macq. Wharf	4.8747	159	1	1
15/02/06	Kangaroo Bluff	8.3493	160	1	1
15/02/06	Kangaroo Bluff	7.1882	161	0	1
15/02/06	Kangaroo Bluff	9.5007	162	0	1
15/02/06	Wrest Point	7.0197	163	0	1
15/02/06	Wrest Point	4.947	164	0	1
15/02/06	Wrest Point	6.2481	165	1	1
15/02/06	Tranmere	11.5023	166	1	1
15/02/06	Tranmere	5.6155	167	1	1
15/02/06	Tranmere	7.4905	168	0	1
15/02/06	Blackmans Bay	3.5118	169	1	1
15/02/06	Blackmans Bay	3.5471	170	1	1
15/02/06	Blackmans Bay	4.1975	171	1	1
15/02/06	East Dennes	6.5858	172	0	1
15/02/06	East Dennes	4.2766	173	1	1
15/02/06	East Dennes	4.0693	174	1	1
15/02/06	West Dennes	4.8313	175	1	1
15/02/06	West Dennes	3.9683	176	1	1
15/02/06	West Dennes	4.7587	177	1	1
15/02/06	Coningham	4.1897	178	1	1
15/02/06	Coningham	4.864	179	1	1
15/02/06	Coningham	3.5013	180	1	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
27/02/06	Risdon	13.4387	181	0	1
27/02/06	Risdon	13.3089	182	0	0
27/02/06	Risdon	11.5875	183	0	0
27/02/06	Geilston Bay	8.9799	184	1	1
27/02/06	Geilston Bay	7.9426	185	1	1
27/02/06	Geilston Bay	8.0552	186	1	1
27/02/06	Macq. Wharf	7.6705	187	1	1
27/02/06	Macq. Wharf	6.7432	188	1	1
27/02/06	Macq. Wharf	7.2643	189	1	1
27/02/06	Kangaroo Bluff	11.6652	190	1	1
27/02/06	Kangaroo Bluff	11.3833	191	0	1
27/02/06	Kangaroo Bluff	10.368	192	0	1
27/02/06	Wrest Point	8.2436	193	1	1
27/02/06	Wrest Point	12.2494	194	1	1
27/02/06	Wrest Point	12.7671	195	0	1
27/02/06	Tranmere	14.2359	196	0	1
27/02/06	Tranmere	18.3017	197	0	1
27/02/06	Tranmere	16.6954	198	1	1
27/02/06	Blackmans Bay	4.2648	199	1	1
27/02/06	Blackmans Bay	5.1468	200	1	1
27/02/06	Blackmans Bay	4.7361	201	1	1
27/02/06	East Dennes	6.8167	202	1	1
27/02/06	East Dennes	4.9844	203	1	1
27/02/06	East Dennes	5.1016	204	0	1
27/02/06	West Dennes	3.6222	205	0	1
27/02/06	West Dennes	2.901	206	1	1
27/02/06	West Dennes	4.1362	207	1	1
27/02/06	Coningham	4.7643	208	1	1
27/02/06	Coningham	4.3379	209	0	1
27/02/06	Coningham	4.5623	210	1	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
15/03/06	Risdon	1.0283	211	0	1
15/03/06	Risdon	1.4047	212	1	1
15/03/06	Risdon	0.8306	213	1	1
15/03/06	Geilston Bay	1.3274	214	0	1
15/03/06	Geilston Bay	1.2964	215	0	1
15/03/06	Geilston Bay	1.2301	216	1	1
15/03/06	Macq. Wharf	1.1323	217	0	1
15/03/06	Macq. Wharf	0.9745	218	0	1
15/03/06	Macq. Wharf	1.1829	219	0	1
15/03/06	Kangaroo Bluff	1.6346	220	0	1
15/03/06	Kangaroo Bluff	1.6116	221	1	1
15/03/06	Kangaroo Bluff	1.7557	222	0	1
15/03/06	Wrest Point	1.652	223	0	1
15/03/06	Wrest Point	1.7405	224	0	1
15/03/06	Wrest Point	1.4495	225	0	1
15/03/06	Tranmere	1.4028	226	0	1
15/03/06	Tranmere	2.0368	227	0	1
15/03/06	Tranmere	1.9965	228	1	1
15/03/06	Blackmans Bay	1.8822	229	1	1
15/03/06	Blackmans Bay	1.7484	230	1	1
15/03/06	Blackmans Bay	1.2318	231	0	1
15/03/06	East Dennes	1.0935	232	0	1
15/03/06	East Dennes	1.0913	233	1	1
15/03/06	East Dennes	0.8935	234	0	1
15/03/06	West Dennes	2.5726	235	0	1
15/03/06	West Dennes	2.4302	236	0	1
15/03/06	West Dennes	2.454	237	1	1
15/03/06	Coningham	3.2993	238	1	1
15/03/06	Coningham	1.8776	239	1	1
15/03/06	Coningham	2.0843	240	1	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
28/03/06	Risdon	1.9327	241	0	1
28/03/06	Risdon	2.3771	242	0	1
28/03/06	Risdon	2.1907	243	0	1
28/03/06	Geilston Bay	1.5888	244	0	1
28/03/06	Geilston Bay	1.9316	245	0	1
28/03/06	Geilston Bay	2.1879	246	0	1
28/03/06	Macq. Wharf	2.4515	247	0	1
28/03/06	Macq. Wharf	2.0267	248	1	1
28/03/06	Macq. Wharf	3.0634	249	0	1
28/03/06	Kangaroo Bluff	5.363	250	0	1
28/03/06	Kangaroo Bluff	5.4872	251	0	1
28/03/06	Kangaroo Bluff	6.8753	252	0	1
28/03/06	Wrest Point	3.7415	253	0	1
28/03/06	Wrest Point	4.2915	254	0	0
28/03/06	Wrest Point	3.9493	255	0	1
28/03/06	Tranmere	8.8556	256	0	1
28/03/06	Tranmere	7.6765	257	0	1
28/03/06	Tranmere	11.448	258	0	1
28/03/06	Blackmans Bay	4.3585	259	0	1
28/03/06	Blackmans Bay	3.5405	260	0	1
28/03/06	Blackmans Bay	3.6049	261	0	1
28/03/06	East Dennes	2.2521	262	0	1
28/03/06	East Dennes	3.5765	263	0	1
28/03/06	East Dennes	2.1568	264	0	1
28/03/06	West Dennes	2.2727	265	0	1
28/03/06	West Dennes	2.0238	266	0	1
28/03/06	West Dennes	2.7657	267	0	1
28/03/06	Coningham	1.0628	268	1	1
28/03/06	Coningham	0.9854	269	1	1
28/03/06	Coningham	1.5029	270	1	1

Date	Location	Weight (g)	ID	*C. gigas*	18s
10/04/06	Risdon	1.6763	271	0	1
10/04/06	Risdon	1.193	272	0	1
10/04/06	Risdon	1.4485	273	0	1
10/04/06	Geilston Bay	1.8474	274	0	1
10/04/06	Geilston Bay	1.488	275	1	1
10/04/06	Geilston Bay	0.9165	276	0	1
10/04/06	Macq. Wharf	2.6464	277	1	1
10/04/06	Macq. Wharf	3.0733	278	0	1
10/04/06	Macq. Wharf	3.3384	279	1	1
10/04/06	Kangaroo Bluff	6.2728	280	0	1
10/04/06	Kangaroo Bluff	5.5838	281	0	1
10/04/06	Kangaroo Bluff	4.191	282	0	1
10/04/06	Wrest Point	4.0014	283	0	1
10/04/06	Wrest Point	4.87	284	0	1
10/04/06	Wrest Point	4.374	285	0	1
10/04/06	Tranmere	4.2521	286	0	1
10/04/06	Tranmere	4.6385	287	0	1
10/04/06	Tranmere	3.859	288	0	1
10/04/06	Blackmans Bay	1.8065	289	0	1
10/04/06	Blackmans Bay	1.6077	290	0	1
10/04/06	Blackmans Bay	2.0456	291	0	1
10/04/06	East Dennes	0.6432	292	0	0
10/04/06	East Dennes	0.4625	293	0	0
10/04/06	East Dennes	0.4003	294	0	0
10/04/06	West Dennes	0.186	295	1	1
10/04/06	West Dennes	0.194	296	0	1
10/04/06	West Dennes	0.2326	297	0	1
10/04/06	Coningham	0.2398	298	1	1
10/04/06	Coningham	0.165	299	1	1
10/04/06	Coningham	0.1384	300	1	1