September 1996

CENTRE FOR RESEARCH ON INTRODUCED MARINE PESTS

TECHNICAL REPORT NUMBER 7

ENVIRONMENTAL TOLERANCES OF LARVAE OF THE EUROPEAN PARASITIC BARNACLE, *SACCULINA CARCINI* (RHIZOCEPHALA: SACCULINIDAE): IMPLICATIONS FOR USE AS A BIOLOGICAL CONTROL AGENT AGAINST THE INTRODUCED EUROPEAN CRAB, *CARCINUS MAENAS*

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Thresher, Ronald E., 1949 –

Environmental tolerances of larvae of the parasitic barnacle Sacculina carcini (Rhizocephala: Sacculinidae): implications for its use as a biological control agent for the introduced european shore crab, Carcinus maenas.

ISBN 0 643 05954 7.

1. Sacculina carcini – Effect of temperature on.
2. Sacculina carcini – Larvae.
3. Carcinus maenas – Biological control.
   I. Centre for Research on Introduced Marine Pests (Australia).
   II. CSIRO Division of Fisheries.
   III. Title. (Series: Technical report (Centre for Research on Introduced Marine Pests (Australia)): no. 7).

595.35

CONTENTS

ABSTRACT

Experimental studies were carried out to test the tolerance of larvae of the parasitic barnacle, *Sacculina carcini*, to elevated temperatures, reduced and elevated salinities and desiccation. Results indicate the larvae are extremely intolerant of temperatures greater than or equal to 40°C. and to elevated salinities. The larvae were quite tolerant of reduced salinities, and short term desiccation. The data, plus information from the literature, suggests that in Australian waters larvae of *S. carcini* would eventually be distributed in most estuarine and open coastal environments from southwestern Western Australia to northern NSW. This encompasses all of the known or expected geographic and habitat range of its host species, *Carcinus maenas*, in Australia. The extreme sensitivity of the larvae to elevated temperatures suggests heat treatment of wastewater is a practical means of preventing accidental escape of the larvae, while their tolerance to desiccation dictates protocols be put in place to ensure larvae are not accidentally released on damp clothing. However, even in a catastrophe (e.g. earthquake, fire), permanent introduction of the parasite following a release of larvae can be prevented by destroying or removing all remaining stocks, as the life history of the parasite requires two sets of larval releases, spaced one year apart, for the parasite to reach sexual maturity, breed and survive.

1. INTRODUCTION

The cirripede order Rhizocephala comprises some 260 species of highly specialized internal parasites that attack, primarily, marine Crustacea (Hoeg and Lutzen, 1995). The life cycle of the parasites consists of a free-swimming, lethicotrophic larval stage, during which the abbreviated planktonic development is similar to that of non-parasitic barnacles, followed by a much longer maturing and adult stage, in which the parasite is endoparasitic in a crustacean host (Fig.1). During the endoparasitic stage, the parasite grows from a few cells to a large interna that ramifies throughout the host. The effects of the parasite on the host are complex, but chief among them are castration of both males and females and feminization of the males. Following a period that varies among species from a few months to a few years, the parasite develops a reproductive externa, which replaces the normal egg sac of the host and which is fertilized by free-swimming, larval male parasites. The males attach as dwarves to the much larger females, after which the female produces one to many broods of larvae.

Many details about the life history of rhizocephalan barnacles remain poorly known. However, their parasitic activity and its apparent effect of irreversibly castrating the host species suggests they may be useful as biological control agents. Of the rhizocephalan host species, one is of particular interest in this context. The European green crab, *Carcinus maenas*, is an ecologically destructive and hardy species that has been widely introduced globally, and that is suspected of causing considerable ecological and economic damage outside of its native range (Cohen *et al.*, 1995; Grosholz and Ruiz, 1995). The species was first detected in Australia in the late 1800's (Fulton and Grant, 1900) and is now widely distributed and abundant in sheltered areas along the temperate Australian cost (Thresher *et al.*, in prep.). Its impacts on the Australian marine flora and fauna have not yet been investigated, but based on overseas experience they are likely to be substantial. To date, no effective means have been developed to minimize these impacts on anything but a very small scale. In European waters, *C. maenas* is the major host species of the rhizocephalan, *Sacculina carcini*. Studies are currently underway by the CSIRO Centre for Research on Introduced marine Pests to evaluate the potential of this parasite for controlling populations of *C. maenas* now established in Australia.

The present study on short-term environmental tolerances of *S. carcini* was undertaken in support of this evaluation, for two specific purposes. First, the effectiveness of *S. carcini* as a control agent against the green crab depends in part on the extent of habitat overlap between the parasite and its host. *C. maenas* is tolerant of a wide range of temperature and salinities, and may be able to maintain extensive refuge populations in some habitats if the environmental tolerances of the parasite are not as wide as the host species. Hence the first purpose of the current study was to assess for the first time the

FIGURE 1

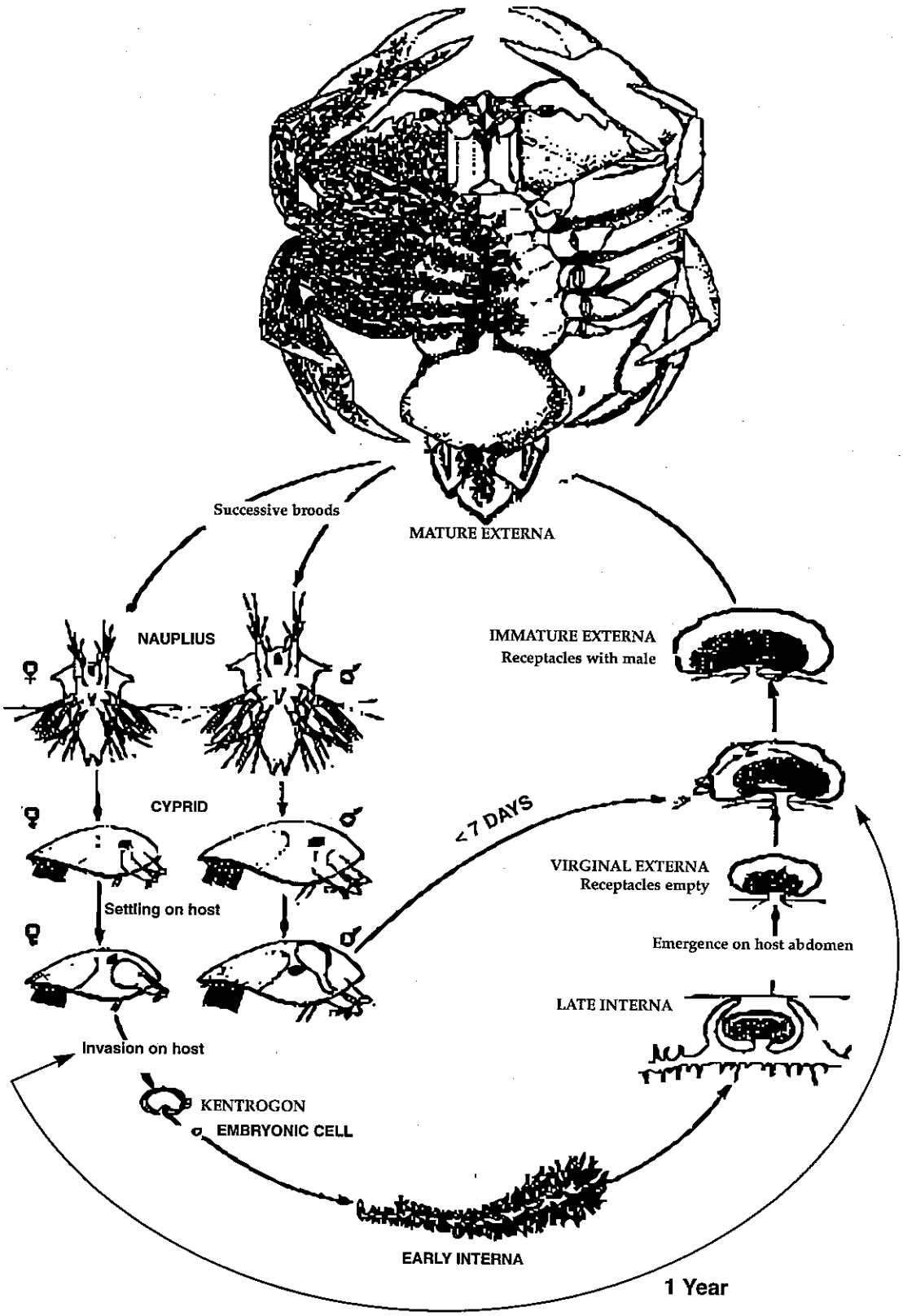


Fig. 1. Life cycle of the rhizocephalan barnacles, *Sacullina carcini*. Based on Hoeg and Lutzen (1995).

short-term tolerances of the parasite's larvae, for comparison with the environmental tolerances of *C. maenas*. On that basis, the habitat and ultimate geographic distribution of *S. carcini*, if established as a reproducing population in Australia, can be predicted.

The second purpose is to determine the conditions required for effective containment of *S. carcini* in experimental aquaria, prior to its release into the Australian environment, if ever. An acceptable biologically secure facility is an essential pre-condition for establishing laboratory populations of *S. carcini* in Australia for experimental purposes. Biological security requires 1) a means of treating waste water as to ensure any *S. carcini* present are killed during the process and 2) adequate protocols for handling the larvae as to ensure they are not inadvertently allowed into the environment. The present study examines the temperatures and salinities required to kill larvae of *S. carcini*, and examines their ability to survive exposure to air, as would be the case of, for example, larvae being present on clothing following a spill.

2. GENERAL MATERIALS AND METHODS

All trials were carried out at the Kristineberg Marine Research Station, Fiskebackskil, Sweden, during August, 1996. Larvae were obtained from *S. carcini*-bearing *C. maenas*, held in small static water aquaria. Prior to their use in the experiments, the larvae were reared in an open, flow-through aquarium, using 'deep water'. Temperature and salinity of this water are monitored continuously. For the duration of the project, deep-water temperature and salinity varied little from means of 13.5°C. and 32 ppt, respectively.

Experimental trials involved stage 3 naupliar larvae. Larval development of *S. carcini* is described and illustrated by Collis and Walker (1994). General larval characteristics of rhizocephalans, including what is known about their behaviour and ecology, are discussed by Hoeg (1995) and Hoeg and Lutzen (1995). All temperature trials and the desiccation trials were done using larvae drawn from the same brood; larvae from a second brood were used for the salinity tolerance test. In both cases, the positively photo-tactic larvae were collected from the rearing aquaria by attracting them towards a light, and then pippetting them into a small beaker. This procedure not only concentrated the larvae, but also ensured only healthy ones were used.

The criterion for 'death' was complete lack of movement for more than one minute. When healthy, the nauplii are extremely mobile, swimming rapidly about the aquarium or attaching briefly to the bottom while sporadically kicking their appendages vigorously. They are also largely transparent, except for a single, red-pigmented eye. On apparent death, the larvae either lay

unmoving on the bottom or drifted un-oriented in the water. Following a few hours death, the body also develops a white coloration.

The impact of each treatment was assessed by drawing a sample of water containing the larvae, after stirring the test container gently, and pippetting the sample into a petri dish. The sample was then examined using a Wild M8 dissecting microscope. Larvae thought dead were examined in detail using the 50X setting of the zoom lens. The effect of each treatment was quantified as the relative number of live and dead larvae in each sample. All larvae in the sample were examined. The number of larvae in each sample varied depending on chance and the number of larvae in the starting conditions. I attempted to have at least 10 larvae in each sample, although in a few instances this was not possible. The mean number of larvae examined per sample was about 15.

3. EFFECTS OF SHORT-TERM TEMPERATURE STRESS

3.1 EXPERIMENT #1. EFFECTS OF RAPID TEMPERATURE INCREASE

METHODS

Larvae, held in 50 ml plastic beakers, were divided into two sets and allowed to acclimate to either 13.5°C (the normal condition) or room temperature (19.2°C) for 12 hours. Salinity was the laboratory-normal 32 ppt. For the temperature trials, the larvae were transferred to a 100 ml Pyrex beaker, which was placed on an Ikamag Ret-G heating plate/stirrer. Water temperature was raised continuously at about 1° C per minute, by adjusting as needed the temperature setting of the heating plate. During heating, the water was stirred using a magnetic stirrer rotating at 50 rpm. Water temperature in the beaker was monitored using a mercury thermometer.

Samples containing larvae were drawn at 20, 30, 40, 50, 60 and 70°C. The first sample at each temperature step was drawn 'immediately', in effect within 5 sec. of the target temperature being reached. Subsequent samples at each nominal temperature were drawn 30, 60, 120 and 300 sec. Because water temperature was rising steadily, the sample drawn five minutes after reaching the target temperature was usually 4-5°C warmer than the samples drawn at the beginning of each step.

Samples were examined immediately after they were drawn, and then discarded.

RESULTS

Larval survival was markedly affected by water temperature. At temperatures of 20 and 30°C., the proportion of live larvae in a sample was generally 50% or

more, whereas at all temperatures ≥ 40°C., all larvae in all samples were dead (Figs. 2 and 3). Acclimating the larvae to 19.2°C prior to the trial had no effect survival at temperatures ≥ 40°C. It did effect on survival at 30°C., however. Larvae acclimated at 13.5°C had a global mean survival (all samples pooled) of 42.3%, ranging from 20 to 75%, as compared with 88.8% (range 75 to 96.4%) for larvae acclimated at 19.2°C. The survival rate of larvae acclimated to the lower temperature was consistently less than the proportion surviving at 20°C at the same exposure time (Fig. 2), whereas survival of the warm water acclimated larvae was similar at both 20 and 30° (Fig. 3). At the lower acclimation temperature, there is a weak (non-significant) indication of lower survival at the longest exposure time (300 sec.). It should be noted, however, that because temperatures were rising steadily, this was also the highest temperature sampled at that step (about 35°C).

Although survival at 30° was relatively high, at that temperature the larvae did not appear to be in good condition. At 20°C, the larvae were actively swimming rapidly about the sample volume, but at 30°C virtually all were lying on the bottom, kicking their appendages occasionally. J. Hoeg (pers. comm.) observed the larvae at 30°C, and commented that, though alive they were clearly 'not healthy'.

3.2 EXPERIMENT #2. EFFECTS OF RATE OF TEMPERATURE INCREASE ON SURVIVAL

METHODS

The experimental apparatus was identical to that used above. All larvae were acclimated to room temperature prior to the experiment. The temperature of the heating plate was adjusted to produce rates of temperature increase in the larvae-containing beaker that varied from target rates of 0.33 to 7°C per minute. The actual mean rates achieved were 0.29, 2.31 and 6.94° per minute. The results of these tests were combined with the room-temperature acclimated series in experiment # 1, which had a mean rate of temperature increase to 40°C. of 1.96°C per minute.

Samples were taken 5 and 30 sec. after reaching temperatures on 30 and 40°C. As above, the samples were examined immediately after they were taken.

RESULTS

Rates of temperature increase were essentially linear for all but the slowest rate tested, which increased more rapidly to about 25°C that preferred (Fig. 4a).

The proportion of larvae surviving at 40°C was unaffected by any of the rates of temperature rise tested; in all cases and at both times sampled, all larvae at 40°C were dead (Fig. 4b). Survival of larvae at 30°C was similar in magnitude to that

FIGURE 2

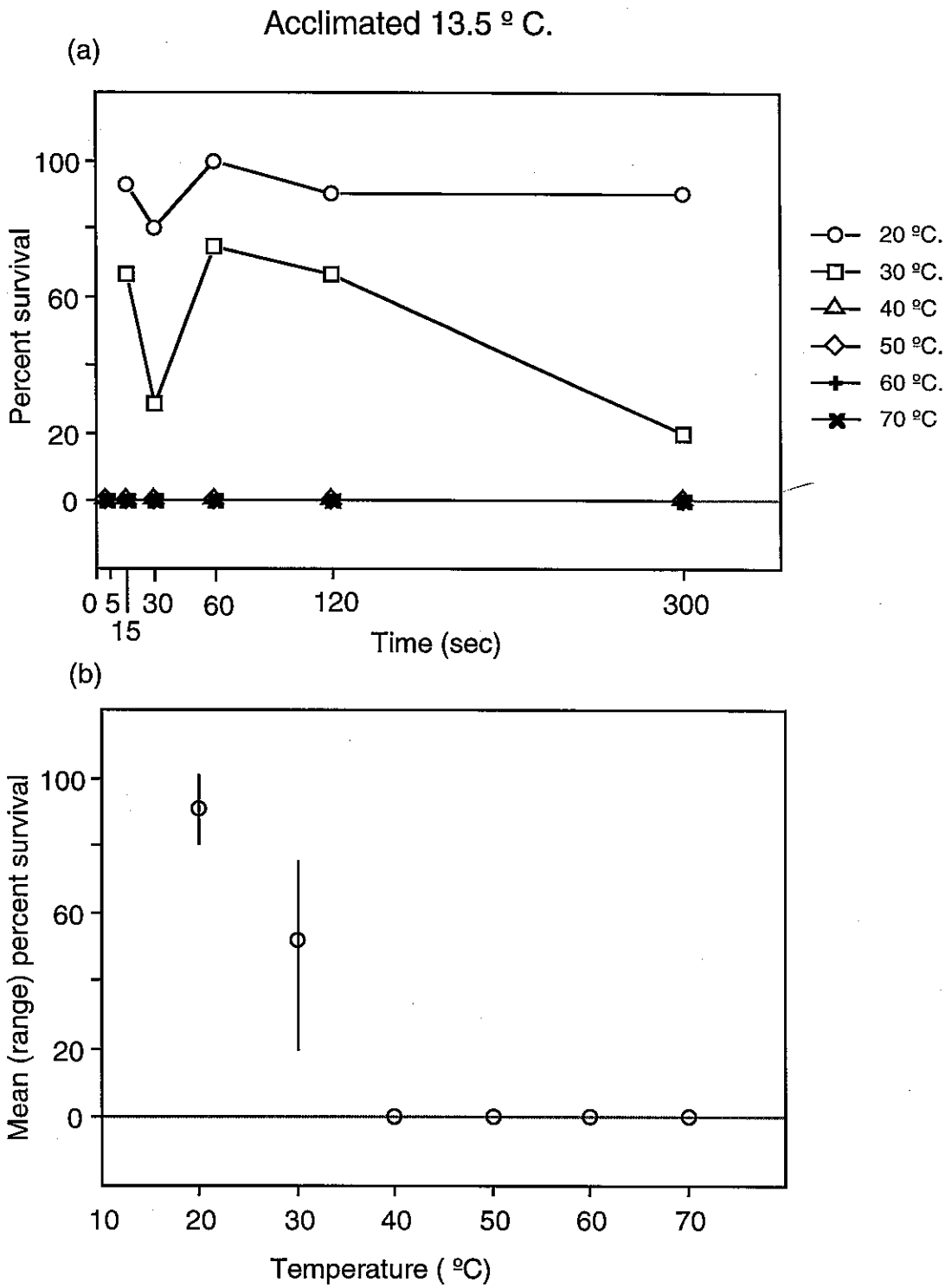


Figure 2. (a) Percent survival of naupliar larvae at fixed time intervals after reaching target temperatures ranging from 20 to 70 ºC. (b) The mean and range of percent survival of larvae at target temperatures, summed over exposure times. Larvae acclimated at 13.5º C. prior to trials.

FIGURE 3

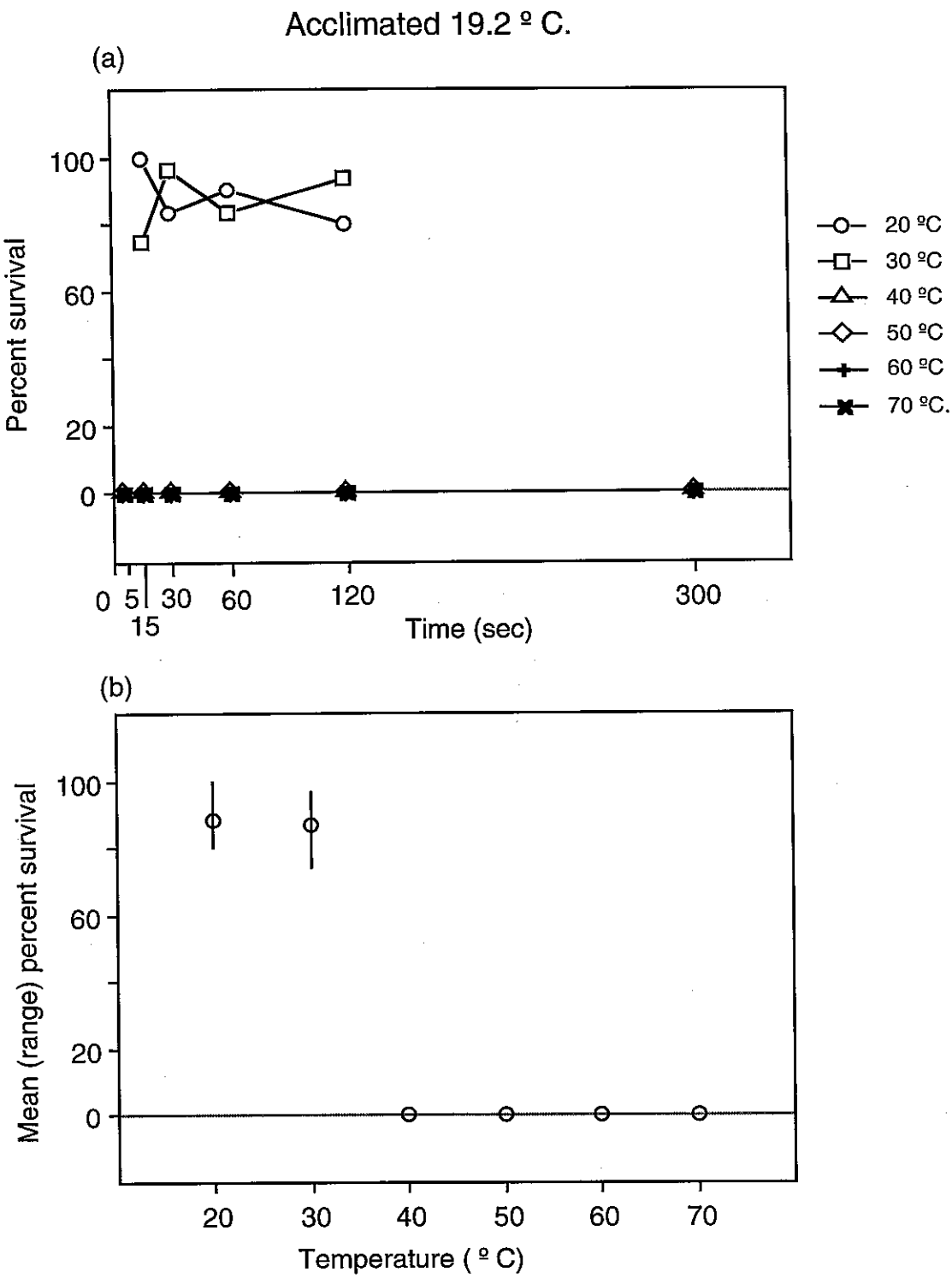


Figure 3. (a) Percent survival of naupliar larvae at fixed time intervals after reaching target temperatures ranging from 20 to 70 º C. (b) The mean and range of percent survival of larvae at target temperatures, summed over exposure times. Larvae acclimated at 19.2 º C. prior to trials.

FIGURE 4

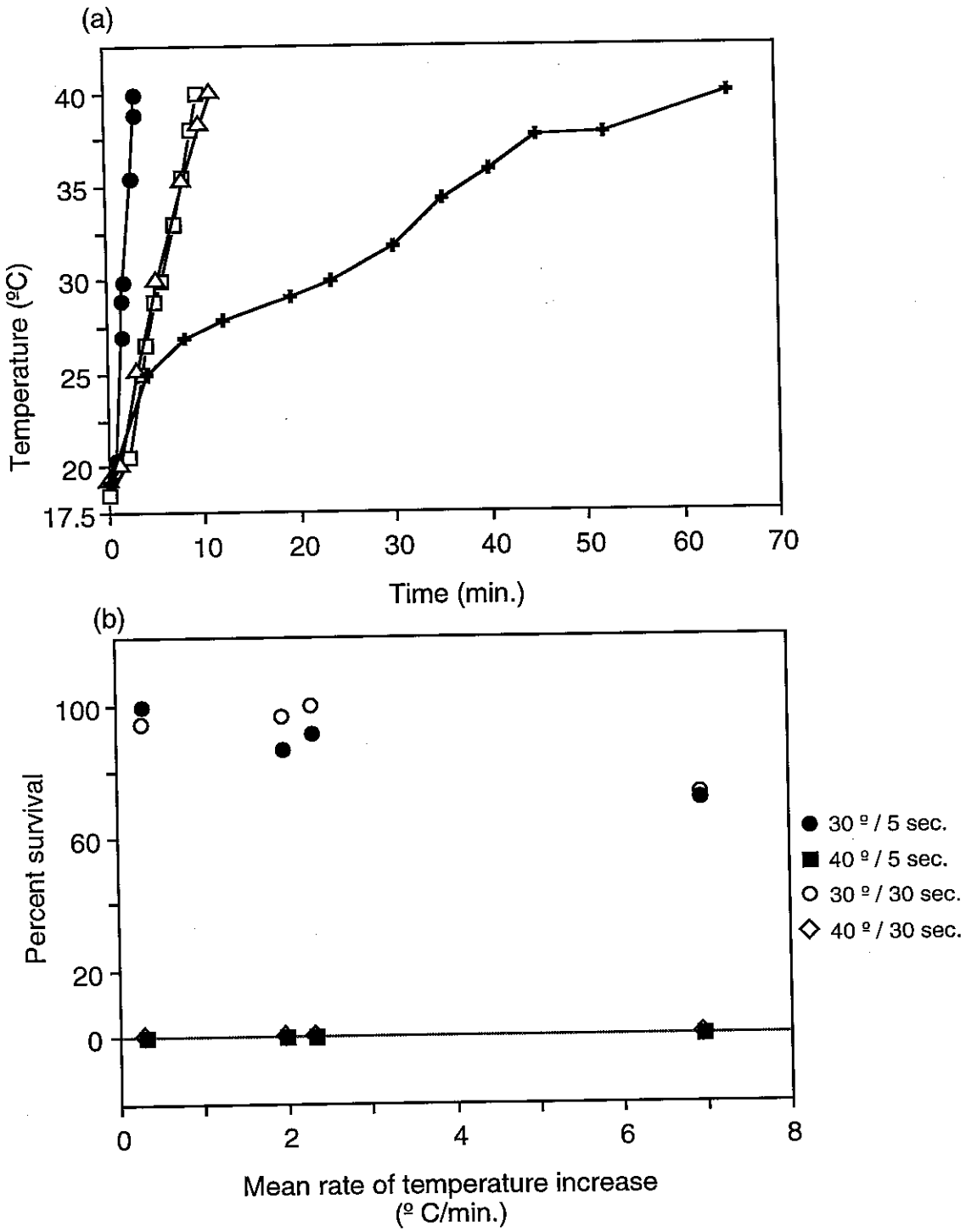


Figure 4. (a) Time-temperature relationships for experiments on effects of rate of temperature increase on survival. (b) Percent survival of naupliar larvae at 5 and 30 sec. after reaching temperatures of 30 and 40º C., as a function of the rate of temperature increase.

in Experiment #1, averaging 89.0% (range 71.4–100%). The proportion of larvae surviving to 30°C correlated negatively ($p < 0.01$) with the rate of temperature rise, when all data are pooled (Fig. 5). Finally, although the data are too few for a statistically powerful test, there does not appear to be a significant difference between survival at 5 and 30 sec. at 30°C, an observation consistent with the results of Experiment #1.

3.3 EXPERIMENT #3. TEMPERATURE EFFECTS ON LONGER TERM SURVIVAL

METHODS

To assess rates of survival at elevated temperatures over periods longer than 5 minutes, the procedure used above was modified as to hold temperatures at three target points, each in a different trial. The target temperatures were 20, 25 and 30°C. A brood of larvae was acclimated to deep-water temperatures (13.5°C), then divided into three parts for the trials. Samples were taken beginning at the time the trial reached its target temperature, and then at 10, 20, 30, 40, 50 and 60 minutes.

RESULTS

Temperature trajectories for the three trials are given in Fig. 6a. In all three trials, the temperatures achieved remained within ± 1 C. degree of the target once the sampling was started.

Mean rates of survival were highest at 20°C, and lowest at 30°C (Fig. 6b). The survival rate at 20°C did not decline with increased time at that temperature, to the 60 min. tested (regression between elapsed time and survival rate $R^2 = 0.04$, NS), whereas there was a significant decline in survival at 30°C ($R^2 = 0.58$, $p < 0.05$). At the intermediate temperature, 25°C, survival was lowest at the two longest elapsed times sampled (50 and 60 minutes), although the regression between elapsed time and survival rate is not significant ($R^2 = 0.41$, $p < 0.2$).

As in the previous experiments, there was a marked difference in the behaviour of the larvae in the three trials, with larvae at 25 and 30°C lying predominantly (>90%) on the bottom and kicking sporadically, whereas more than 90% of the larvae in the 20°C trials were actively swimming. At the end of the 60 minute trials, larvae at 25 and 30°C were still on the bottom.

4. EFFECTS OF SHORT-TERM SALINITY STRESS

METHODS

To assess the impacts of an abrupt change in salinity on larval survival, stock solutions of different salinity were made by, on the one hand, diluting seawater

FIGURE 5

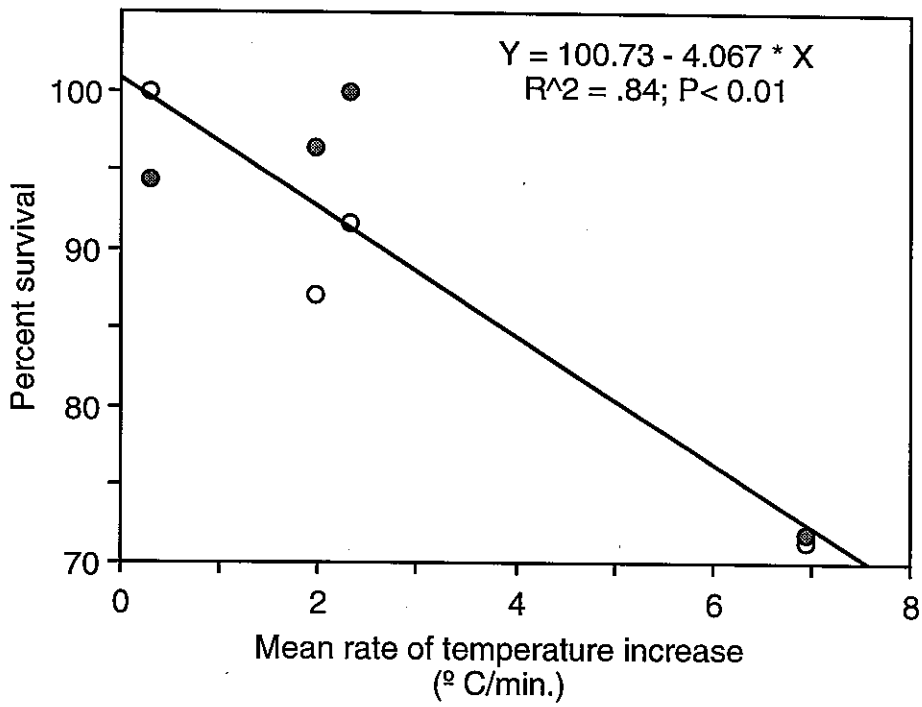


Figure 5. Percent survival of naupliar larvae 5 min (open circles) and 30 min (filled circles) after reaching 30º C., as a function of the rate of temperature increase.

FIGURE 6

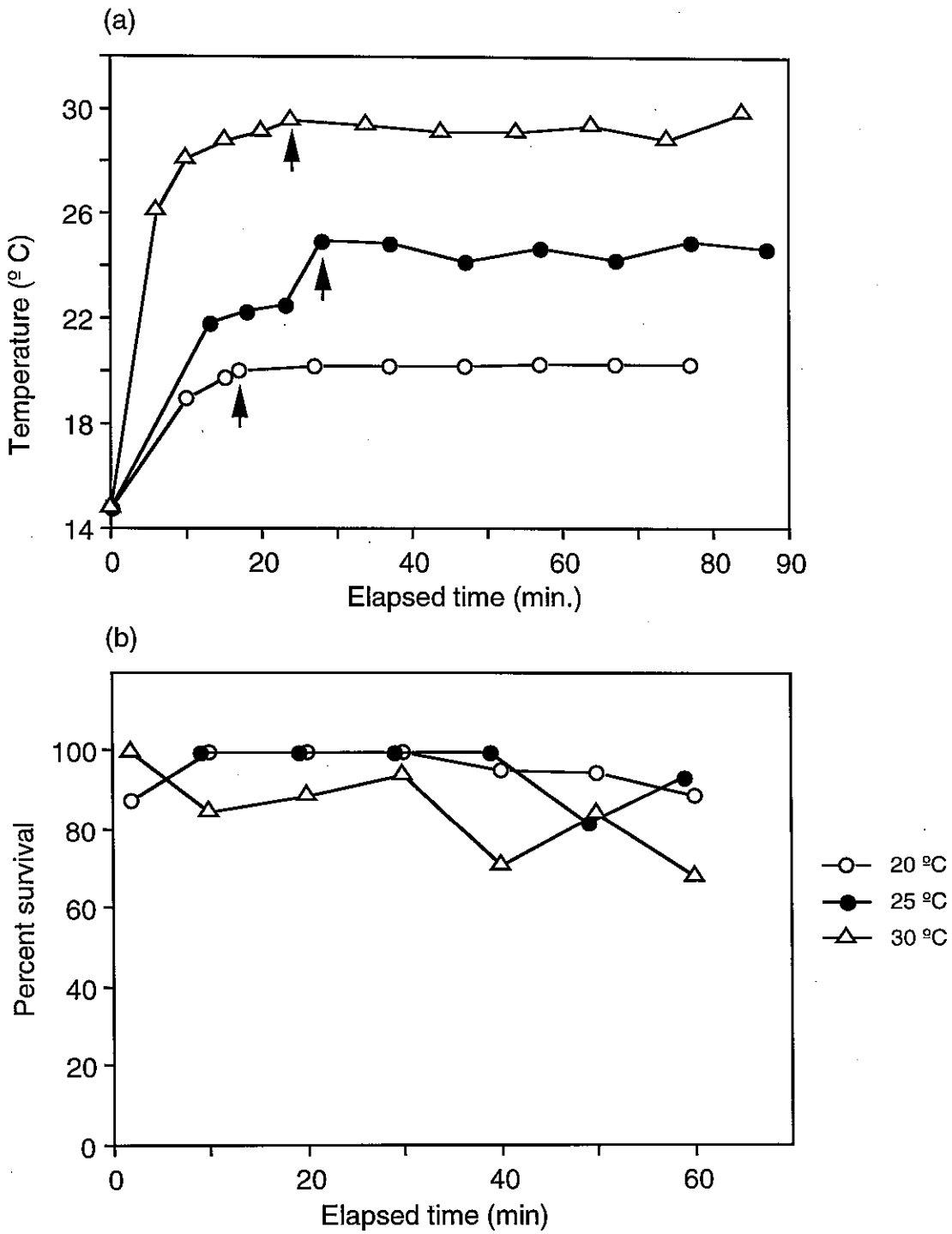


Figure 6. (a) Time-temperature relationships for experiments testing effects of longer term exposure on larval survival. Arrows indicate start of measured exposure time at target temperatures. (b) Percent survival of naupliar larvae as a function of exposure time at target temperatures of 20, 25 and 30º C.

with distilled water and, on the other, adding artificial sea salt. Salinities were calculated from volumetric relationships and confirmed approximately (± about 1 ppt) using an AO Scientific Instruments refractometer. The artificial sea salt used was Ektozon (produced in Germany).

The 50 ml stock solutions made were 0.01, 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 2 times normal 'deep water' salinity (32 ppt). Salinities as measured by the refractometer were correspondingly: no measurement (too dilute), about 2, about 7, 15, 23-24, 32, 40, 47-48 and 63 ppt. As these are close to expectations, given the imprecision of the refractometer, it was assumed that volumetric estimates of salinity (e.g. diluting deep water 50% with distilled water generates 16 ppt water) were correct. All solutions were at room temperature, to which the larvae had also been acclimated overnight.

The effects of salinity stress were measured by pippetting larvae from a common source into each stock solution, while the latter was stirred gently. Samples were then drawn from the stock solutions at 1 min, 30 min, 60 min and 24 hours after the nauplii were added. Each sample was examined immediately using the dissecting microscope, and scored as above.

RESULTS

Short-term (≤ 60 min) survival of larvae was high over a wide range of salinities, from 16 to 47 ppt (0.5 to 1.5 X normal sea water)(Fig. 7). At salinities less than 16 ppt, there was evidence of extensive rupturing of the exoskeleton, and all larvae sampled were clearly dead. There was also no survival, at any elapsed time sampled, of larvae exposed abruptly to 2X normal seawater. One larvae very feebly moved a leg, when examined, 30 min into the trial, but otherwise looked and acted dead.

Larvae in normal and 1.25 normal seawater swam consistently during the first 60 minutes of the trials. At higher and lower salinities, the larvae were largely immobile, lying on the bottom and quivering their appendages sporadically.

Twenty-four hours after the induced salinity stress, the range of salinities with live larvae had narrowed substantially. No live larvae were found in any sample with salinities higher than 32 ppt (Fig. 7). In contrast, survival at salinities down to 16 ppt were comparable to those in normal seawater. However, in contrast to normal sea water, larvae in the low salinities solutions were still largely immobile, except for occasional leg movements, and many were fouled with adhered detritus.

FIGURE 7

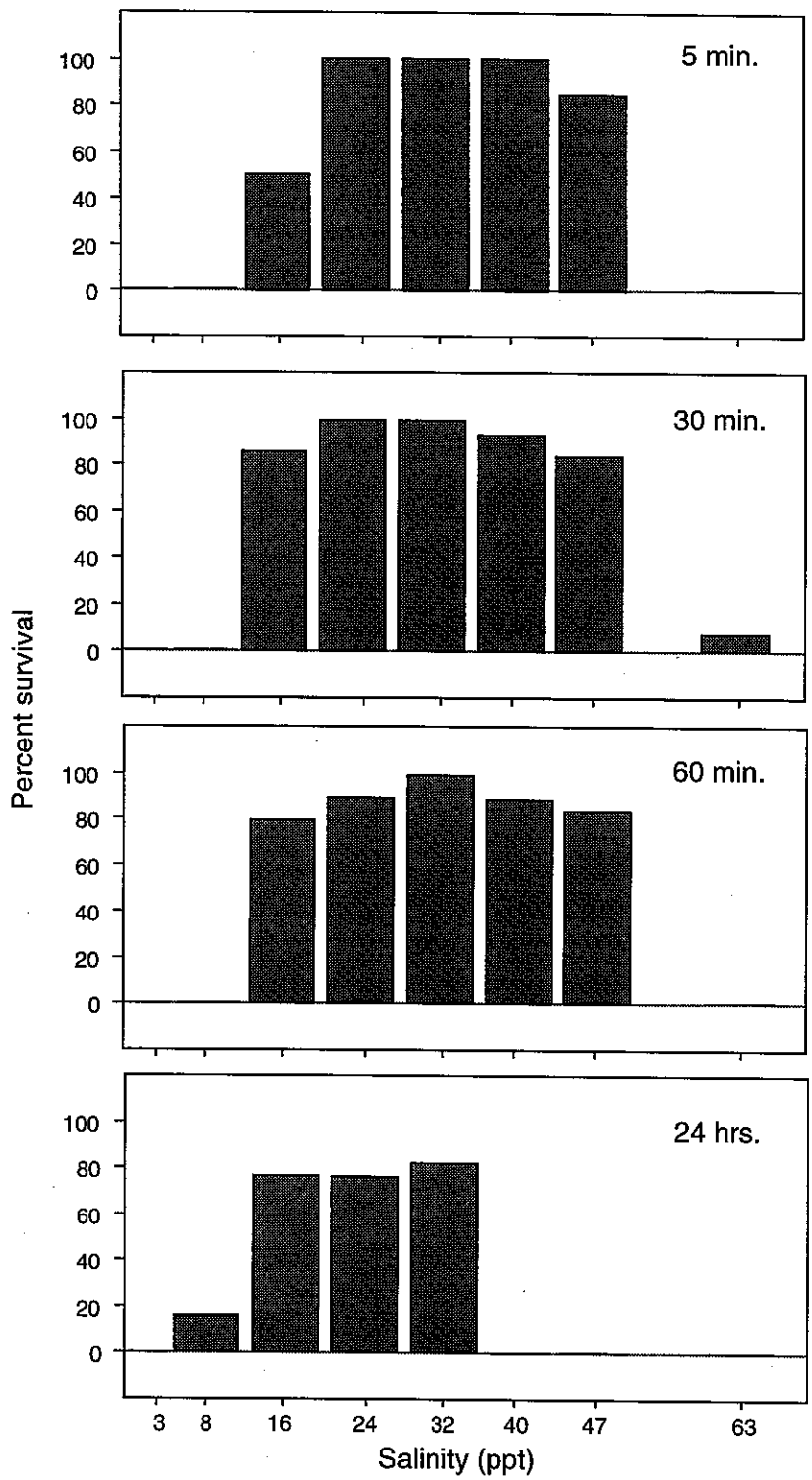


Figure 7. Effects of acute salinity stress at salinities ranging from 3 to 63 ppt, on survival of naupliar larvae 5, 30 and 60 min and 24 hours after onset of exposure.

5. EFFECTS OF SHORT-TERM DESICCATION

METHODS

The ability of the nauplii to survive short-term exposure to air was tested by filtering samples containing larvae through a small square of 90 µm plankton netting, after which the netting and larvae were allowed to lie above a damp cloth for varying periods of times. The longest time tested was 20 minutes. Following the exposure, the net was inverted into a petri dish and washed with clean sea water. A sample of the water was drawn using a pipette, and examined for live and dead nauplii.

RESULTS

Larval survival following exposure to air on a damp net varied from 87.5 to 100%, and was uncorrelated with the duration of exposure (Fig. 8). After 20 min. exposure to air in a damp environment, all of the sixteen larvae examined in the sample were kicking vigorously, although none were actively swimming. Most had a variety of detritus adhering to their ecto-skeletons, but none appeared incapacitated by it.

6. DISCUSSION

6.1 COMPARISONS WITH PREVIOUS STUDIES

The tests conducted focus primarily on effects of short-term, rapid changes in environmental conditions on survival of *S. carcini* nauplii, and hence on the abilities of these nauplii to cope with extreme physiological stress. Extrapolating the results into an ecological context, where environmental changes are more often longer-term and gradual (spaced over days and weeks), should be made with caution. Nonetheless, the data are likely to indicate the broad capabilities of the larvae to tolerate changes in salinity and temperature. Slowing the rate of change markedly is likely to only broaden slightly the tolerance ranges, rather than alter them radically.

A strong piece of evidence supporting this conclusion is the general similarity between the results of this study and other work on *S. carcini* temperature and salinity tolerances. The European distribution of *S. carcini* ranges from cold temperate conditions, in Scandinavia, through to sub-tropical areas off the Atlantic coast of Spain and in the Mediterranean (Hoeg and Lutzen, 1985). Information on spawning seasons of the species in sub-tropical regions is sparse, but at least some spawning occurs in the summer (J. Hoeg, pers. comm.), implying tolerance as larvae of water temperatures at least into the mid-20s. At the other extreme, spawning in Scandinavian regions occurs predominantly in the summer, at water temperatures in the teens. However, off England, at least some spawning occurs year-round, at water temperatures as

FIGURE 8

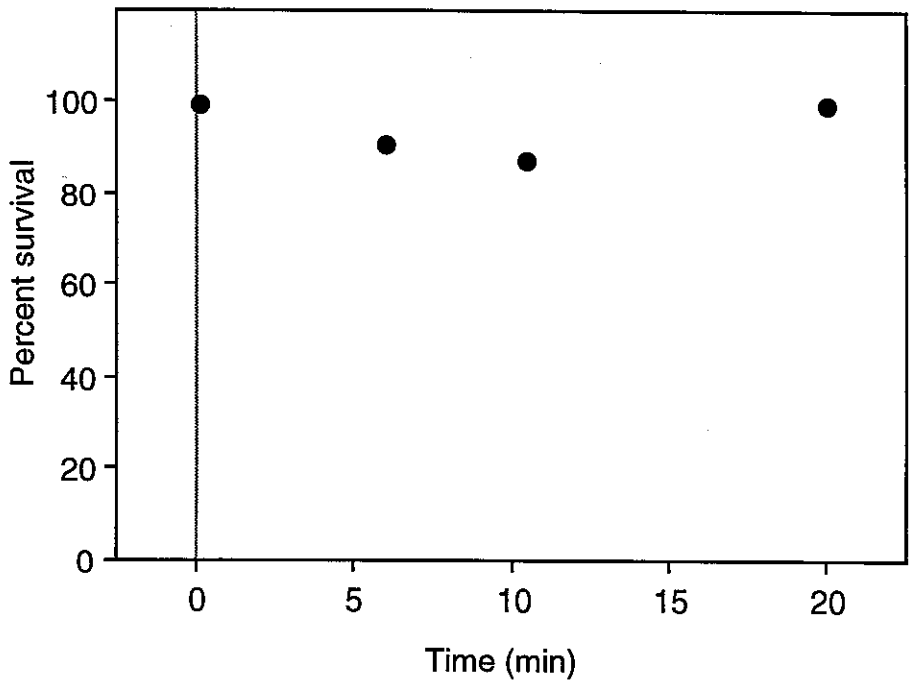


Figure 8. Effect of exposure time on percent survival of naupliar larvae in damp filter paper.

low as 3-6°C, though there is some doubt that the larvae can complete their development at the lowest temperatures (Walker, 1988). Salinity tolerances are *S. carcini* also appear to be broad, as evidenced by the distribution of infected crabs and direct developmental studies. *Carcinus maenas*, the primary host for *S. carcini*, commonly inhabits estuarine areas (Crothers, 1967), where despite the low salinity infection rates by the rhizocephalan can be high. Tolerance of low salinities is also supported by laboratory study of embryonic and naupliar development. Ramault (1935) reports *S. carcini* embryos develop normally at salinities ranging from 17.5 to 40.1 ppt, and complete development of nauplii to the cyprid stage between 24.5 and 35 ppt.

The upper thermal tolerance of naupliar *S. carcini* in the present study was in the range between 20 to 40°C. Finer definition of the upper lethal limit was not attempted, but the data, and particularly behavioural observations, suggest the limit is lower in this range, rather than higher. First, even at temperatures as low as 25°C, there was a very marked reduction in the activity levels of the larvae. Whereas larvae at 20°C were typically swimming rapidly and vigorously about the sample container, those at 25 and 30°C much more often where lying on the bottom of the container, kicking their appendages sporadically. Even after an hour at these temperatures, there was no indication of the larvae returning to their normal activity levels. Second, prolonging exposure to 30°C significantly increased mortality. Whether this rate of increase would have stabilized over time is not known, but the implication is that at 30° the animals were under considerable thermal stress, even if the majority were still alive. Increased mortality was also evident for nauplii exposed to 25°C for 50 minutes or more, implying stress at that temperature as well. Tolerance to temperatures in the range of 20 to 25°C is consistent with field observations, reported above.

The salinity data produced in the present study are also broadly consistent with previous work. Not surprisingly, nauplii are unable to survive at very low ($\leq$ 8 ppt) or very high (63 ppt) salinities, even when exposed for very brief periods (one minute or less). At low salinities, the nauplii's exoskeletons were conspicuously rupturing due to the osmotic stress, and it is likely that the opposite effect (compression of vital organs) was occurring at the highest salinity tested.

However, otherwise larvae appeared to be remarkably tolerant of a broad range of salinities, at least for brief periods. Survival for up to an hour was very high at salinities ranging from 16 to 48 ppt, though apparently normal swimming behaviour was evident over a narrower salinity range — 32 to 40 ppt. The pattern of survival changed markedly when examined over a 24 hour period, however. Despite appearing to be in relatively good health at slightly elevated salinities early in the experiment, all larvae in tests where salinity exceeded the local norm (32 ppt) died within an elapsed time of 24 hours. The cause of death at slightly elevated salinities, other than an inferred osmotic stress, is

unclear. The consequence, however, is that naupliar *S. carcini* appear more tolerant of lowered, rather than elevated salinities. This is consistent with Ramault's (1935) observations.

6.2 IMPLICATIONS FOR HABITAT OCCUPANCY AND GEOGRAPHIC RANGE IN AUSTRALIA

To the extent that the data obtained bear on ecological processes, they indicate larval *S. carcini* are capable to inhabiting regions with peak temperatures up to about 25°C, and possibly slightly higher, and salinities from normal down to about half sea water. The literature suggests that the lowest temperatures conducive to larval development are in the range of 4 to 6°C. The data and literature also suggest very limited abilities of the larvae to tolerate elevated salinities.

These results imply a large potential geographic and habitat range in Australian waters. The area where summer surface isotherms are less than 25°C stretches from southwest Western Australia to northern NSW; nowhere in that area, including southern Tasmania, do temperatures routinely decline to less than 4-6°C for extended periods. Temperatures in excess of 25°C would occur only in the hyper-saline South Australian gulfs, where elevated salinities would also prevent successful local reproduction by the parasite. In contrast, the abilities of the larvae to survive in relatively low salinities indicates the species could successfully colonize most of southern Australia's estuaries.

Salinity tolerances of the Australian population of *C. maenas* have not been determined. Preliminary survey data in Tasmania by the Centre for Research on Introduced Marine Pests suggests *C. maenas* locally is uncommon in areas of low salinity (Thresher *et al.*, in prep.), though reports for overseas populations indicate *C. maenas* tolerates conditions to nearly pure freshwater, at least temporarily. On that basis, I predict that there will be few, if any low salinity refugia for *C. maenas* in Australian waters, where it cannot be attacked by *S. carcini* cyprids.

6.3 IMPLICATIONS FOR REQUIREMENTS FOR BIOLOGICAL SECURITY IN EXPERIMENTAL FACILITIES

Adequate facilities for experimental studies of *S. carcini* in Australia need to include precautions that prevent the accidental release of the parasite. Biological security consists of two components: 'sterilization' of waste water and prevention of deliberate or accidental release by personnel in contact with the parasite. Other forms of accidental release, such as via air-borne vectors, are not relevant in this species, given the relatively large size (200–300 µm) of the larvae.

A variety of techniques can be used to 'sterilize' waste water, including filtration, heating and exposure to sterilizing radiation. The simplest systems

would involve coarse filtration to remove large particulates (which are then incinerated), followed by heating prior to venting to either the ocean or a domestic sewerage system.

The data on short-term temperature tolerances document that larval *S. carcini* would be very susceptible to heat treatment. No combination of heating rates was found that resulted in survival of any larvae at temperatures as low as 40°C. In all cases, even a 5 sec. exposure to this water temperature resulted in a complete kill of the larvae. The data further indicate that long exposure to lower temperatures (one hour at 30°C) also resulted in relatively low survival, though not a complete kill, implying long-term survival at temperatures in excess of 30°C may be unlikely.

In contrast, 'sterilization' by means of mixing waste water with freshwater, to kill via osmotic shock, appears to be less effective. After 24 hours, larvae were still alive (if perhaps apparently not healthy) at salinities as low as 8 ppt, i.e. 1/4 normal sea water. Dilution of waste sea water to salinities less than this would result in complete death, however.

Practical requirements for a system that ensures a complete kill of *S. carcini* in waste water, therefore, appear to be technologically simple. The temperature required to ensure a complete kill of larvae is comparable to that of a hot shower. Domestic water heaters routinely heat to temperatures of 70–80°C, i.e. very much higher than that required to kill *S. carcini* larvae. The virtually instantaneous death of the larvae at 40°C indicates that holding high temperatures for long periods in a waste water treatment system is not required. So long as temperatures exceed 40°C, any exposure time is apparently lethal to the larvae. To be on the safe side, however, adjusting flow-rates in a waste water heating system as to achieve temperatures of, perhaps, 50°C for at least 30 sec. would virtually guarantee a complete larval kill. The flow-rate that results in this temperature-time combination would have to be determined empirically. Published literature from the Rheem Company (Appendix 1), however, indicate that commercially available heaters can maintain temperatures of 60°C at flow-through rates of up to 3000 l per hour.

Additional biological security could be achieved by diluting the waste water with large volumes of hot freshwater (in a ratio of at least 5 parts of freshwater per 1 part seawater). Exposure to salinities this low for as little as 60 seconds resulted in complete larval death due to exoskeletal rupturing. The diluted wastewater could then be vented to sea, or linked into a domestic sewerage system, where presumably salinities would rapidly approach near pure freshwater.

Security from deliberate or accidental human-mediated release of the larvae is more difficult to ensure, and requires strict maintenance of suitable protocols for site security and handling. The desiccation trials, though not

comprehensive, indicate that larval *S. carcini* are very tolerant to exposure to air in damp conditions, surviving in high numbers following exposures as long as twenty minutes. Therefore, there is a very high probability that larvae spilled or splashed onto clothing, if it remained damp, would still be alive for quite some time afterwards. The very small (by human standards) size of the larvae would make it unlikely to them to be seen when on clothing; without magnification they are difficult to see in a petri dish.

The solution to this level of biological security is also simple in principle. Laboratory workers should be required to wear protective clothing while in the laboratory, which is then washed in hot freshwater before exiting the facility. As well, all exposed skin should also be washed in hot freshwater before exiting the laboratory. There are no practical impediments to maintaining this procedure, though processes would have to be put in place to ensure protocols were strictly adhered to.

The broader issue of deliberate human-mediated release of the larvae is more difficult to deal with. Larvae captured and held in a small, damp hand net are likely to remain alive for long periods, and could easily be transported to a release site. Clearly, an essential requirement is that access to any experimental aquaria be extremely limited.

Beyond this, however, the reproductive biology of *S. carcini* (and all rhizocephalans, for that matter) mitigates strongly against deliberate or accidental premature release in Australian waters. Only female larvae are capable of parasitizing crabs. Male larvae are attracted to mature females only, and attach in special receptive organs, before developing into sexually mature dwarf males on the relatively large female externa (Fig.1). In *S. carcini*, females require a year to reach maturity. The consequence of this life history cycle is that for *S. carcini* to be established as a reproductive population, the larvae must be released twice, and exactly a year apart. One release of a mixture of male and female larvae might result in females attacking host crabs, but all of the male larvae, searching for mature females that are not there, would die within a few days of release (Hoeg, 1995; Hoeg and Lutzen, 1995). While the females might survive this period, if no further release of larvae occur, they too will die a year later, when there are no males available to fertilize them. Moreover, males released at any time other than the roughly month-long period of female receptively would also die, and have no affect on viability of the introduced population.

The consequence of this life history is that even if larvae were stolen and deliberately released, were released accidentally during laboratory operations, or were released as a result of a major catastrophe (earthquake, fire), destruction or removal of all remaining stocks in Australia at any time up to 9 months later guarantees the species would not establish in Australian waters, or be present for more than a year (as un-fertilized females) after the initial release.

7. CONCLUSIONS

1. Temperature and salinity tolerances of larvae of the parasitic barnacle, *Sacculina carcini*, as determined from the literature and short-term experiments, suggest that if released into Australian waters the species would eventually inhabit most of southern Australian coastal waters. The northern limits of its distribution (as reproductive individuals) would probably be northern NSW and southwestern Western Australia. This geographic distribution includes all of that known, or expected for, its host species, *Carcinus maenas*.
2. The larvae are incapable of surviving for extended periods high salinities or very high temperatures. Therefore they would not exist as competent larvae in, for example, the hyper-saline South Australian gulfs. The would, however, tolerate low salinities in estuarine conditions. The range of tolerated salinities appears comparable to that to *C. maenas* in Australian waters, suggesting there would be little or no physical refugia for the crab from the parasite.
3. *S. carcini* larvae are extremely intolerant of temperatures greater than or equal to 40°C. Heating wastewater for an experimental facility to this relatively low temperature (commercial water heaters routinely achieve temperatures in excess of 70°C) is a practical and easily achieved means of preventing release of the larvae. Dilution by freshwater and running wastewater into a sewerage system would act as a redundant form of waste water security.
4. The larvae are very tolerant of desiccation, indicating a rigorously maintained protocol for limiting access to the laboratory and washing clothing and skin in hot freshwater before leaving the facility is required.
5. However, even in the case of a catastrophic escape of larvae (or their deliberate release due to human sabotage) the life cycle of *S. carcini* mitigates strongly against its permanent introduction. Establishment of a permanent, reproductive population of the parasite requires two releases of larvae, exactly one year apart. This could easily be prevented by destruction or removal of all remaining stocks after the first larval release.

8. ACKNOWLEDGEMENTS

I thank Jens Hoeg for his enthsiastic assistance and advice throughout the planning and execution of this experimental study and Ib Svane, Malin Werner and Henrik Glenner for their assistance at the Kristineberg Marine Research Station (KMRS). This work was carried out at the KMRS, whose logistical support for the study is appreciated.

REFERENCES

Cohen, A.N., J.T. Carlton and M.C. Fountain. 1995. Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. Mar. Biol. 122: 225–237.

Collis, S. and G. Walker. 1994. The morphology of the nauplius stages of *Sacculina carcini* (Crustacea: Cirripedia: Rhizocephala). Acta Zool (Stockholm) 75: 297–308.

Crothers, J.H. 1967. The biology of the shore crab *Carcinus maenas* (L.) I. The background — anatomy, growth and life history. Fld. stud. 2: 407–434.

Furlani, D.M. 1996. A Guide to the Introduced Marine Species. CRIMP Tech. Rpt. #5. CSIRO Division of Fisheries, Hobart, Australia.

Fulton, S.W. and F.E. Grant. 1900. Note on the occurrence of the European crab, *Carcinus maenas*, Leach, in Port Phillip. Victoriuan Natur. 27: 147–148.

Grosholz, E.D. and G.M. Ruiz. 1995. Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. Mar. Biol. 122: 239–247.

Hoeg, J.T. 1995. The biology and life cycle of the Rhizocephala (Cirripedia). J. Mar. Biol. Assoc. UK 75: 517–550.

Hoeg, J.T. and J. Lutzen. 1985. Crustacea Rhizocephala. Marine Invertebrates of Scandanavia. 5. Norwegian Univ. Press., Oslo.

Hoeg, J.T. and J. Lutzen. 1995. Life cycle and reproduction in the Cirripedia Rhizocephala. Ann. Rev. Oceanogr. Mar. Biol. 33: 427–485.

Ramault, M. 1935. Observations of the embryonic and larval development in *Sacculina carcini* in changed osmotic conditions of medium. Bull. Intern. l'Acad. Polonaise Sci. Lettr. B II: Zool. 2: 87–109.

Walker, G. 1988. Observations on the larval development of *Sacculina carcini* (Cruatacea: Cirripedia: Rhizocephala). J. Mar. Biol. Assoc. UK 68: 377–390.

APPENDIX 1: RHEEM LITERATURE

Performance Data

Models	Number units in Parallel	Heater Delivery Rating (Litres)	Initial Storage Capacity (Litres)	Heating Units (Watts)	Recovery Litres/h 50°C rise	Litres Hot water at 50°C Rise over Peak Period of 1 Hour	2 Hours	3 Hours	5 Hours	8 Hours
603/050	1	50	60	3x3600	185	235	420	605	975	1530
				3x4800	245	295	540	785	1275	2010
603/315	1	315	340	3x3600	185	500	685	870	1240	1795
				3x4800	245	560	805	1050	1540	2275
				3x6000	310	625	935	1245	1865	2795
	2	630	680	3x3600	370	1000	1370	1740	2480	3590
				3x4800	495	1125	1620	2115	3105	4590
				3x6000	620	1250	1870	2490	3730	5590
	3	945	1020	3x3600	560	1505	2065	2625	3745	5425
				3x4800	745	1690	2435	3180	4670	6905
				3x6000	930	1875	2805	3735	5595	8385
606/315	1	315	340	6x3600	370	685	1055	1425	2165	3275
				6x4800	495	810	1305	1800	2790	4275
				6x6000	620	935	1555	2175	3415	5275
	2	630	680	6x3600	745	1375	2120	2865	4355	6590
				6x4800	990	1620	2610	3600	5580	8550
				6x6000	1240	1870	3110	4350	6830	10550
	3	945	1020	6x3600	1115	2060	3175	4290	6520	9865
				6x4800	1490	2435	3925	5415	8395	12865
				6x6000	1860	2805	4665	6525	10245	15825
	4	1260	1360	6x3600	1490	2750	4240	5730	8710	13180
				6x4800	1985	3245	5230	7215	11185	17140
				6x6000	2480	3740	6220	8700	13660	21100
	6	1890	2040	6x3600	2235	4125	6360	8595	13065	19770
				6x4800	2980	4870	7850	10830	16790	25730
				6x6000	3725	5615	9340	13065	20515	31690
					Recovery Litres/h 65°C rise	Litres Hot water at 65°C Rise over Peak Period of 1 Hour	2 Hours	3 Hours	5 Hours	8 Hours
603/050	1	50	60	3x3600	140	190	330	470	750	1170
				3x4800	190	240	430	620	1000	1570
603/315	1	315	340	3x3600	140	455	595	735	1015	1435
				3x4800	190	505	695	885	1265	1835
				3x6000	240	555	795	1035	1515	2235
	2	630	680	3x3600	285	915	1200	1485	2055	2910
				3x4800	380	1010	1390	1770	2530	3670
				3x6000	475	1105	1580	2055	3005	4430
	3	945	1020	3x3600	430	1375	1805	2235	3095	4385
				3x4800	570	1515	2085	2655	3795	5505
				3x6000	715	1660	2375	3090	4520	6665
606/315	1	315	340	6x3600	285	600	885	1170	1740	2595
				6x4800	380	695	1075	1455	2215	3355
				6x6000	475	790	1265	1740	2690	4115
	2	630	680	6x3600	570	1200	1770	2340	3480	5190
				6x4800	765	1395	2160	2925	4455	6750
				6x6000	955	1585	2540	3495	5405	8270
	3	945	1020	6x3600	860	1805	2665	3525	5245	7825
				6x4800	1145	2090	2335	4380	6670	10105
				6x6000	1430	2375	3805	5235	8095	12385

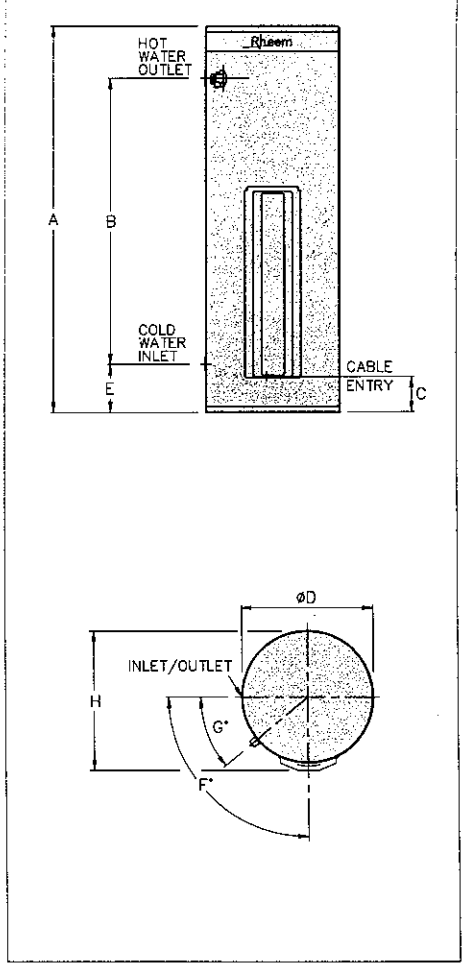
Technical Data

Model		603/050	603/315	606/315
Heating units		3	3	6
Storage Capacity	(litres)	60	340	340
Delivery Rating	(litres)	50	315	315
Relief Valve Setting	(kPa)	1000	1000	1000
Max. Supply Pressure				
with ECV Fitted	(kPa)	650	650	650
without ECV Fitted	(kPa)	800	800	800
Thermostat Setting - Factory	(°C)	75	75	75
Max. Thermostat Setting	(°C)	82	82	82

Heating Units(watts)	3x3600	3x4800	3x6000	6x3600	6x4800	6x6000
Recovery litres at 20°C Rise	465	620	775	930	1240	1550
Recovery litres at 30°C Rise	310	415	515	620	830	1035
Recovery litres at 40°C Rise	235	310	385	465	620	775
Recovery litres at 50°C Rise	185	245	310	370	495	620
Recovery litres at 60°C Rise	155	205	260	310	415	520
Recovery litres at 65°C Rise	140	190	240	285	380	475
Recovery litres at 70°C Rise	135	175	220	265	355	445

Nominal Dimensions (mm)

Model No.	603/050	603/315	606/315
A	664	1675	1675
B	405	1378	1378
C	100	135	135
D	425	607	607
E	96	96	96
F°	90°	90°	90°
G°	30°	32°	32°
H	464	646	646
Weight empty(kg)	34	100	102
Inlet/Outlet (kg)	RP 1¼/32	RP 1¼/32	RP 1¼/32
T & PR Valve Connection	RP ¾/20	RP ¾/20	RP ¾/20
Manifold - minimum centre to centre	625	810	810



Approximate Daily Energy Consumption - kWh

Daily Hot Water Usage at 50°C Rise	Heater Model 603/050	603/315	606/315
0	2.1	3.9	4.2
50	5.0	6.8	7.1
100	7.9	9.7	10.0
150	10.8	12.6	12.9
200	13.7	15.5	15.8
250	16.6	18.4	18.7
300	19.5	21.4	21.6
350	22.4	24.3	24.5
400	25.3	27.2	27.4
500	31.2	33.0	33.3
750	45.7	47.5	47.8
1000	60.2	62.0	62.3
1500	89.3	91.1	91.4