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Ecophysiological tolerances of the Pacific oyster, *Crassostrea gigas*, with regard to the potential spread of populations in South Australian waters

Prepared for

PIRSA Marine Biosecurity

Kathryn Wiltshire

July 2007

SARDI Aquatic Sciences Publication Number F2007/000499-1
SARDI Research Report Series Number 222

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This Publication may be cited as:
Wiltshire, K.H. (2007) Ecophysiological tolerances of the Pacific oyster, *Crassostrea gigas*, with regard to the potential spread of populations in South Australian waters. Prepared for PIRSA Marine Biosecurity. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, 29pp. SARDI Aquatic Sciences Publication Number F2007/000499-1. SARDI Research Report Series Number 222.

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Printed in Adelaide July 2007

SARDI Aquatic Sciences Publication Number F2007/000499-1
SARDI Research Report Series Number 222
ISBN Number 9780730853718

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Signed:
Date: July 2007
Distribution: PIRSA Marine Biosecurity
SARDI Aquatic Sciences Library
Circulation: Public domain

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ACKNOWLEDGEMENTS

I would like to thank the following people for their assistance in the preparation of this report: Suzanne Bennett for tracking down a number of the historical papers and technical reports, and Michael Sierp, Simon Bryars, Meegan Vandepeer and Robert Wiltshire for their constructive comments. This report was funded by PIRSA Marine Biosecurity.

EXECUTIVE SUMMARY

Pacific oysters (*Crassostrea gigas*) were introduced to South Australia in the 1960s for the purpose of establishing a fishery, and proved to be a highly successful aquaculture species. Farms are currently located on the West coast, Spencer Gulf, Gulf St Vincent and Kangaroo Island. Despite the size and age of the industry, and despite the fact that non-native oyster populations have established elsewhere, the occurrence of wild Pacific oysters in South Australia is rare.

It was previously believed that the relatively high salinity of South Australian waters would prevent survival of Pacific oyster larvae but given that oyster spat do occur, albeit in limited numbers, this is clearly not the case. Environmental factors may be influencing the reproductive success of the Pacific oyster in South Australia but which factors are limiting or otherwise important is unclear. In order to assess and manage the risk of wild populations establishing or expanding, a clear understanding of the ecophysiological tolerances of Pacific oysters is required.

This report aims to summarise the current state of knowledge regarding the ecophysiological tolerances of adult oysters, spat, and larvae. From this information, gaps in our current knowledge can be identified and directions for further research suggested.

A range of work has been carried out on the ecophysiology of Pacific oysters, but this has primarily focussed on optimal parameters for growth, with less attention being placed on determining the overall limits for survival and reproduction. These overall limits are the most relevant aspect for ascertaining the potential of the Pacific oyster to establish populations in the regions to which they have been introduced.

Adult oysters have a very wide range of tolerance and are able to tolerate periods of below freezing air temperature when emersed, as well as water temperatures <0ºC and short exposures to >40ºC. Juveniles tolerate a slightly lesser temperature range, and the extent of their salinity tolerance has not been tested, although it is expected to be greater than those of the larvae (discussed below). The lower salinity limit for survival is not clear but prolonged salinity below ~15‰ negatively affects growth. Gametogenesis requires a period of temperatures above 10ºC, and progresses faster at higher temperature. It may be impaired by salinity <15‰, but is not inhibited by high salinity or temperature, at least up to 41‰/27ºC. Spawning occurs when water temperatures exceed 18ºC, and in warm climates there may be several spawning events per season.

Conditions for larval development are more restricted, with the optimum temperature range between 20 and 30ºC and salinity between 25 and 30‰. Higher temperature may cause mortality, while lower temperatures and salinities outside the optimal range cause reduced growth but have little to no effect on short-term larval survival. Longer-term impacts have not been investigated, but biochemical models suggest that sub-optimal temperature/salinity combinations should lead to decreased settlement success. Additionally, reduced growth rates will lead to a prolonged larval period with a greater risk of predation or dispersal away from suitable settlement sites. However, neither of these theories has been experimentally tested.

Feed supply is an important factor for larval growth and survival. Feed supply may also have an interactive effect with temperature and salinity. The existence of a critical period for oyster larvae within which they must commence feeding, or perish, has been suggested but is unproven. There is evidence that oyster larvae can utilise dissolved organic matter as an energy source when algal feed is lacking. However, growth rates and settlement success are strongly correlated with quantity and quality of feed provided. Oligotrophic South Australian waters may provide a low feed supply and hence limit growth rates. A low feed supply has been suggested to limit the range of temperature and salinity combinations over which successful larval development will occur, but this has not been experimentally tested. Additionally, very few studies have examined the long-term impact of a reduced feed supply on development, particularly at temperature or salinity conditions outside the optimal range.

Our knowledge of the ecophysiological tolerances of Pacific oysters is far from complete. In particular, our knowledge of longer-term impacts of high salinity and reduced feed on larval growth and survival is lacking. It is unclear whether acclimation of adults can impact larval performance or what the optimum salinity is for fertilization success. Further research in these areas is required to supplement our existing knowledge of this species.

1. INTRODUCTION

The Pacific oyster (*Crassostrea gigas*) has been introduced to many regions of the world from Japan and is now one of the major shellfish species aquacultured and harvested globally (Olsen 1994, Shatkin et al. 1997, Ruesink et al. 2005). It comprises an estimated 80% of total world oyster production, with this percentage expected to increase (Chew 1990, Ayres 1991). The success of *C. gigas* as an aquaculture species is due to its wide ecophysiological tolerances and adaptability, combined with other features that make it desirable for aquaculture, such as a fast growth rate and disease resistance (Coleman 1986, Shpigel 1989, Chew 1990, Dinamani 1991, Shatkin et al. 1997). While the establishment of successful aquaculture operations over a wide range of environments is evidence of the adaptability and tolerances of the Pacific oyster, surprisingly few controlled studies have been carried out to confirm or test their ecophysiological tolerances.

Suitable conditions for successful reproduction of the Pacific oyster are believed to be more restricted than those required for adult growth (Coleman 1986, Vandepeer 1995) and have received more attention. These have mainly been studied with regard to optimising hatchery production (eg Helm and Millican 1977, Nell and Holliday 1988) while few studies have attempted to determine the overall range of conditions suitable for reproduction. Knowledge of these conditions is important in determining the potential of introduced species to establish self-sustaining populations (Coleman 1986, Shatkin et al. 1997). Several authors have stated that ecological risk assessments for oyster introductions should consider the probability of establishment of wild populations (Coleman 1986, Gottlieb and Schweighofer 1996, Ruesink et al. 2005). Introduced Pacific oysters have formed wild populations in many global regions (Medcof and Wolf 1975, Ayres 1991, Dinamani 1991, Diederich 2005, 2006, Rajagopal et al. 2005) with some potential for positive impacts (eg improving water quality through filtration, Ruesink et al. 2005) but also negative impacts such as space competition with native bivalves (Diederich 2005, 2006), fouling of power station cooling systems (Rajagopal et al. 2005), and loss of public amenity through covering structures including intertidal reefs (Coleman 1986).

Pacific oysters were first introduced and naturalised in Australia in the early 1950s (Thompson 1952, 1959), and aquaculture of *C. gigas* began in South Australia in 1969 (Olsen 1994). As in other regions, a highly successful aquaculture industry has been established, with South Australian production valued at just over $16million in 2002/2003 (Wear et al. 2004). Farms are located on the West coast, Spencer Gulf, Gulf St Vincent and Kangaroo Island (Hone 1993, 1996, Madigan and Clarke 1998, Wear et al. 2004). Despite the often-stated belief that salinities in South Australia are too high to permit reproduction of Pacific oysters (Medcof and Wolf 1975, Anon 1980, Grove-Jones 1985, SA Dept Fisheries 1989, Ayres 1991, Olsen 1994, Anon 2005), spat have been discovered in limited locations over a number of seasons (Hone 1993, 1996, Vandepeer 1995,

Madigan and Clarke 1998). This shows that prior knowledge or understanding of the ecophysiological tolerances of *C. gigas* was incorrect or incomplete.

While it is now clear that *C. gigas* can reproduce in the relatively high salinities of South Australia, they have not so far established large populations such as those in New Zealand (Medcof and Wolf 1975, Dinamani 1991), Tasmania (Mitchell et al. 2000) or Port Stephens, NSW (Medcof and Wolf 1975, Ayres 1991). This is despite having been cultured in high volumes in South Australia for several decades (Olsen 1994), while elsewhere, non-native oyster populations of nuisance proportions have sometimes occurred within a few years of introduction (Ayres 1991, Diederich 2005, 2006). Clearly some factor is limiting the further spread of Pacific oysters but the limiting factors are currently unknown.

This literature review aims to summarise the existing knowledge of the ecophysiological tolerances of both adults and larvae of the Pacific oyster, particularly as they govern the potential spread of populations of this species in South Australia. It is hoped this review will provide better understanding of the known tolerances of *C. gigas* and also help identify areas where knowledge is lacking. A robust knowledge of the ecophysiological tolerances of this species is required to adequately assess and manage non-native oyster populations within South Australia and elsewhere.

2. TOLERANCES OF SPAT AND ADULT PACIFIC OYSTERS

As indicated, much of our knowledge of the tolerances of Pacific oysters comes from field observations. While not as robust as controlled experiments, useful information can still be gained from observations of Pacific oysters in the field under natural or culture conditions. Growth of oysters is affected by environmental conditions including salinity and temperature, with suboptimal conditions leading to reduced growth rates (Brown and Hartwick 1988).

2.1. Salinity

Grove-Jones (1986a,b,c) reports the growth rates and survival of Pacific oysters set out in South Australian waters of varying salinity. While results were highly variable, growth was consistently poor at sites where salinity exceeded 42‰, with the lowest growth reported at the three highest salinity sites in Baird Bay and upper Spencer Gulf, where maximum salinity exceeded 50‰ (Grove-Jones 1986b,c). The best growth was observed at sites in the Coorong North lagoon (salinity 13.5-38.3‰), Coffin Bay (maximum salinity 41.8‰) and Franklin Harbour (maximum 38.6‰) (Grove-Jones 1986b,c). However factors other than salinity clearly had an influence, as some sites with maximum salinities of around 38‰ showed low growth and survival, while others demonstrated good growth and survival (Grove-Jones 1986c). For selected sites within the

Coorong that were expected to be otherwise environmentally similar, good growth was recorded at sites with salinities ranging from 37-40‰, but little growth was recorded at sites with salinities of 42-45‰ and 45-48‰ (Grove-Jones 1986a). Nonetheless, survival was high at all sites.

These results suggest that Pacific oysters can grow at relatively high salinities, although values of salinity given by Grove-Jones (1986c) are maxima only and the salinity ranges presented by Grove-Jones (1986a and b) are for limited sites only. However, the fact that Pacific oysters can grow well at continuously high salinities up to 41‰ has been demonstrated by their successful culture in hypersaline (consistently 41‰) ponds in both South Australia (King 1977) and Eilat, Israel (Hughes-Games 1977, Shpigel and Blaylock 1991). Within South Australia, mean salinities measured in major oyster growing areas by Madigan and Clarke (1998) ranged from 34.3 to 43.2‰ with most sites having a salinity of around 36-37‰.

In laboratory experiments, Nell and Holliday (1988) tested the growth rate and survival of small (initial weight 1.1mg) and large (0.6g) spat over various salinities from 15 to 45‰. Survival was found to be 98% for small and 100% for large spat, with no influence of salinity. Small spat grew equally well from 15 to 30‰, but demonstrated significantly lower growth at 35‰ and very low growth at both 40 and 45‰. However, growth rates of larger spat were not affected by salinity (Nell and Holliday 1988).

While growth may be impaired above approximately 40‰, adult Pacific oysters can still survive above this salinity. Grove-Jones (1986a) reported that oysters survived in field trials where salinity was 45-55‰ for 12 months. In laboratory experiments, Nell (1991) also found that oysters survived at up to 55‰ (highest salinity tested) for 14 days without significant mortality. However, Grove-Jones (1986a) states that exposure to salinity of 60‰ for at least one week was usually lethal.

Despite Nell and Holliday (1988) reporting no reduction in growth of spat at 15‰, low salinity may also result in sub-optimal growth. Impaired growth of Pacific oysters was observed by Brown and Hartwick (1988) at sites within British Columbia, Canada where salinity fell below 20‰, despite the availability of abundant food. At sites where salinity was always >20‰, growth increased with food supply. Similarly, Calvo et al. (1999) found increased growth of *C. gigas* at "high" salinity sites (median salinity >25‰), compared with sites of "moderate" (15-25‰) and "low" (<15‰) salinity. Survival and condition index were also highest for the high salinity sites in that study. Pacific oysters respond to reduced salinity by decreasing shell opening and pumping activity, hence reducing their feeding capability, particularly at salinities <13‰ (Pauley et al. 1988). The optimum salinity range, where maximum pumping was observed, was between 25 and 35‰ (Quayle 1969 in Pauley et al. 1988).

While reduced survival was observed at salinities <25‰ and particularly at <15‰ in the field (Calvo et al. 1999), Nell (1991) observed negligible mortality over 14 days for oysters kept at

salinities as low as 5‰. However, Grove-Jones (1986a) reported mortality of *C. gigas* after one week exposure to 12‰ and Calvo et al. (1999) observed 56% mortality at one site after one month of mean daily salinities of only 3‰. A minimum mean salinity of 15‰ has been stated as being required for long-term survival of *C. gigas* (Gottlieb and Schweighofer 1996), while Shatkin et al. (1997) suggest that Pacific oysters can survive and spawn at salinities down to 10‰. Muranaka and Lannan (1984) kept adult oysters at 10‰ for 17 weeks during an experiment on gonad development and noted mortalities of between 38 and 57%. These observations are contradictory and clearly the lower salinity limit of *C. gigas* is not well established. Low salinity is not likely to restrict the spread of this species in South Australia, although it may be an important factor in other regions.

2.2. Temperature

Pacific oysters have been reported to have a very wide temperature tolerance (Vandepeer 1995, Shatkin et al. 1997), and this is also suggested by the wide range of habitats in which they are successfully cultured. These habitats range from warm water ponds in Israel where water temperatures can reach 34°C and average 28°C over summer (Hughes-Games 1977, Shpigel and Blaylock 1991), to regions where winter sea temperatures may be <3°C, such as the British Isles (Child and Laing 1998), British Columbia, Canada (Brown and Hartwick 1988) and the Dutch Wadden Sea where the mean winter water temperature is just 2.3°C (Diederich 2006). Diederich (2006) also observed no significant mortality of wild non-native oysters aged one year and above over five winters in the Dutch Wadden Sea, despite one of the years being a harsh winter with 37 consecutive days of freezing air temperatures. Pauley et al. (1988) note that oysters survive exposure to air temperatures of -4°C during periods of emersion at low tide. Shatkin et al. (1997) observed that water temperatures down to -1.8°C were recorded during the 1970s when Pacific oysters were cultured in the Gulf of Maine, and reported significant feeding activity and growth in oysters at temperatures down to 2°C. Askew (1972 in Coleman 1986) also found significant winter growth in Pacific oysters in UK waters of 2.5-10°C, although a minimum of 10°C has been reported to be needed for "rapid" growth (Coleman 1986).

While adults survive these temperatures, juvenile oysters are somewhat less tolerant. For example, Diederich (2006) observed 90% survival of wild first-year oyster spat in the Dutch Wadden Sea over a "mild" winter, but only 25% survived the "harsh" winter. In laboratory experiments, Child and Laing (1998) showed that *C. gigas* spat could survive three weeks at a temperature of 3°C and at least 11 weeks at 6°C and above. Significant mortalities occurred where juvenile oysters were exposed to 3°C for prolonged periods, with only 4% surviving to 11 weeks when fed or to 7 weeks when unfed. However, unfed spat survived at the slightly warmer

temperatures (6 and 9°C), with no mortality recorded over the 11-week study period (Child and Laing 1998). In their study, fed spat grew significantly at both 6°C and 9°C.

Optimum temperature ranges have been derived from studies of growth rates in cultured oysters. For oysters grown in warm-water ponds, higher growth rates were observed in winter when the water temperature was 11-19°C (average 14°C), than in summer, when temperatures were 25-32°C (average 28°C) (Shpigel and Blaylock 1991). However for British Columbia where temperatures were 2.5-7°C in winter and 17-22°C in summer, Brown and Hartwick (1988) found that faster growth was associated with higher temperature except at sites where food was limiting. King (1977) also observed faster growth rates in summer when temperatures were >20°C (reaching a maximum of 24°C) than in winter, when temperatures were <15°C (minimum 10.5°C).

Collectively these observations suggest that growth occurs even at very low temperatures, but increases with temperature up to an optimum of around 20-25°C whereafter it declines. For example, Shpigel and Blaylock (1991) suggest that 27°C is the maximum upper temperature for good growth, at least at the relatively high salinity (41‰) of their culture ponds.

Studies of metabolic and feeding rates have also been used to derive optimum temperatures. Both feeding and metabolic rates increase with temperature, but at higher temperatures, metabolic demands may outstrip feeding ability leading to reduced growth (Bougrier et al. 1995, Ren et al. 2000). Ren et al. (2000) found that clearance rates of *C. gigas* increased over the range 10-20.7°C then decreased at higher temperatures (maximum tested 29°C), while oxygen consumption rates increased with temperature over the range tested. Similarly, Bougrier et al. (1995) found that clearance rates were maximal at 19°C then decreased at higher temperatures, up to the maximum tested of 32°C. These studies suggest a temperature of around 20°C is optimal, which is somewhat less than was observed by Shpigel and Blaylock (1991), although the decline in feeding rate above this, while significant, was not large at temperatures of up to 26°C (Bougrier et al. 1995, Ren et al. 2000). However, at 29°C and 32°C, clearance rates declined more dramatically, falling to similar levels to those observed at 10°C and 5°C, respectively (Bougrier et al. 1995).

Bougrier et al. (1995) found that only 17 of 38 oysters survived a 10-day acclimation period at 32°C (after having been collected from waters at 23°C), and suggest this temperature is at the upper tolerable limit for *C. gigas*. A similar upper limit of 30°C is suggested by Bardach et al. (1972 in Vandepeer 1995). Even though temperatures may exceed this level occasionally in areas where *C. gigas* is cultured, it does not do so for prolonged periods. For example, in warm water ponds in Eilat the temperature may reach 34°C, but cycles through a diurnal range, falling to between 20°C and 25°C at night (Hughes-Games 1977, Shpigel and Blaylock 1991).

While prolonged exposures to temperatures above 30°C may be lethal, Pacific oysters can certainly tolerate periods at higher temperatures. As intertidal animals, Pacific oysters may

encounter exposures to elevated temperatures for several hours per day, with fluctuations of more than 15°C above ambient (Hamdoun et al. 2003). They are very tolerant of short exposures to high temperatures, and display plasticity of upper temperature limits, with non-lethal temperature shocks leading to expression of additional heat-shock proteins and increased temperature tolerance (Hamdoun et al. 2003, Rajagopal et al. 2005).

Rajagopal et al. (2005) investigated the upper thermal limits of *C. gigas* with a view to determining the feasibility of heat treatment to prevent fouling in power station cooling systems. They found that exposure to 43°C for a minimum of 1hr was required to produce 100% mortality. At temperatures between 39 and 45°C, the time to 50% mortality was greater for larger (54mm) than smaller (11mm) oysters, and decreased for both size classes with increasing temperature. After oysters were subject to a non-lethal thermal shock (1 hour at 37°C or 39°C), they survived significantly longer exposures to all temperatures from 39 to 42°C than oysters that had not been exposed to thermal shock. This induced tolerance persisted for up to 14 days after exposure to heat shock (Rajagopal et al. 2005).

Hamdoun et al. (2003) found that Pacific oysters collected in summer from higher tidal heights (and thus exposed to longer emersion periods and hence higher temperatures), had greater thermal tolerance than those from lower tidal height. Oysters from both tidal heights exhibited greater thermal tolerance after summer collection than winter collection. For *C. gigas* collected in summer, almost no mortality was observed after 65 minutes exposure to 43°C. Around 80% of high tidal height oysters and 30% of low tidal height oysters survived exposure to 44°C. A small percent of high tidal height individuals survived at up to 46°C (Hamdoun et al. 2003). It is clear from these studies that Pacific oysters can tolerate short periods of very high temperature and are very adaptable in their range of thermal tolerance.

2.3. Summary

In summary, both adult and juvenile *C. gigas* have been shown to tolerate a wide range of environmental conditions. Adults survive exposure to salinities of up to 55‰ and grow well at up to 42‰. Low salinity tolerance has not been well established but prolonged exposure to between 5 and 10‰ or below is likely to cause significant mortalities. Small spat have been shown to survive in salinities as low as 15‰ and as high as 45‰, but their tolerance to salinity outside this range has not been tested. Spat do not survive prolonged exposure to temperatures of 3°C, but can survive 11 weeks or more at ≥6°C, while adults survive periods of water temperature <0°C and prolonged exposure to <3°C. The upper temperature tolerance for long-term exposure appears to be around 30°C, however, both adults and spat can survive short (1hr+) exposures to temperatures considerably above 40°C. Tolerance to higher temperatures is mediated by expression of heat-shock proteins and varies with season, tidal height position, and prior exposure to thermal shock.

3. GAMETOGENESIS AND SPAWNING

The establishment of self-sustaining populations requires that adult oysters must not only survive, but also undergo gonad development, produce viable gametes, and spawn. Pacific oysters usually reach maturity within their first year of growth, and are protandrous hermaphrodites (Pauley et al. 1988). Spawning must be followed by successful fertilization, larval development, settlement and metamorphosis, and survival and growth of spat as discussed in Section 4.

3.1. Salinity

The effect of salinity (range 10-30‰) on gonad development was studied by Muranaka and Lannan (1984). They found that the rate of gonad development increased with salinity over this range. At temperatures <20°C no gonadal development occurred in the 10‰ salinity treatment over 17 weeks and oysters exhibited signs of poor condition and physiological stress. Effects of higher salinity on gonad development have not been specifically studied. However, Shpigel (1989) observed rapid gonad development and successive spawning in oysters kept in warm water hypersaline (41‰) ponds, and spawning has also been observed in temperate hypersaline ponds (King 1977), and South Australian Gulf waters (Olsen 1994).

3.2. Temperature

Gonad development in *C. gigas* is strongly influenced by temperature (Mann 1979, Muranaka and Lannan 1984), however, the temperature at which gametogenesis and spawning are initiated varies between regions (Shpigel 1989, Ruiz et al. 1992). Initiation of gametogenesis occurs usually in winter once water temperature begins to rise above 10°C (Shpigel 1989, Ruiz et al. 1992). In areas where water temperature does not fall below this level, gametogenesis continues year round, but a new cycle begins coincident with the lowest temperatures (Ruiz et al. 1992). The progression and rate of gametogenesis depends on both absolute temperature and time of exposure, with maturity occurring after a longer time at lower temperature (Mann 1979). Mann (1979) found that *C. gigas* took 49 days to ripen at 21°C compared with 133 days at 15°C, and extrapolated from these data that gametogenesis would cease at below 10.5°C.

While ripening occurred in oysters kept at 12-15°C in Mann's (1979) experiments, a higher temperature appears to be needed to promote spawning. Over their 19-week experiment, no spawning was observed in *C. gigas* kept at either of these lower temperatures, but at 18°C some individuals were spawning after 13 weeks. A temperature of around 18°C or above also appears to be required for spawning to occur in wild populations, with spawning beginning when temperatures reach 17°C-19°C (Shpigel 1989, Ruiz et al. 1992, Shatkin et al. 1997). However, Ruiz et al. (1992) also observed a spawning event when water temperature was only 16°C, which may have been triggered by a phytoplankton bloom.

In suitable climates, subsequent periods of gonad maturation and spawning may occur several times per year, and continue through autumn (Shpigel 1989, Ruiz et al. 1992). Conversely, in cooler climates, spawning may only occur in particularly warm years (Shatkin et al. 1997, Diederich 2006).

The effects of higher temperatures on gametogenesis and spawning have not been examined. However, mass mortalities during the summer maturation and reproductive season have been observed in France, Washington, USA, and Japan, at temperatures >20°C (Berthelin et al. 2000, Cheney et al. 2000, Patrick et al. 2006), and particularly when temperatures considerably exceed the regional averages (Cheney et al. 2000). While the exact cause of these events has not been determined, they occur only in eutrophic areas and appear to be associated with metabolic abnormalities and potentially other stressors including disease (Berthelin et al. 2000, Cheney et al. 2000, Patrick et al. 2006). Increased temperature may play a significant role in these mortality events, but is not lethal on its own as noted by Cheney at al. (2000) and demonstrated by the research of Hamdoun et al. (2003) and Rajagopal et al. (2005).

3.3. Summary

In summary, the initiation of gametogenesis requires a temperature of at least 10°C, with gonad development progressing faster at higher temperature allowing several spawning events to occur per annum in warmer climates, while spawning may be rare in cooler regions. Even though ripening may occur at cooler temperatures, a temperature of around 17-18°C is required to promote spawning. Higher temperatures may be associated with mortality events in eutrophic waters, but these mortalities are also influenced by other factors. Elevated temperature alone does not appear to have negative impacts on gametogenesis or spawning. Gametogenesis may be impaired by low salinity (<30‰), and is certainly negatively impacted by low salinity in the range of 10‰, but high salinity (up to 41‰) does not appear to be detrimental. However, no research has specifically examined potential upper or optimal limits of temperature or salinity on either gametogenesis or spawning.

4. TOLERANCES OF EARLY LIFE STAGES

4.1. Observations of natural settlement

As noted above, successful spawning does not always lead to successful recruitment, since this also requires successful larval development. This may occur over a narrower range of conditions than adult survival, as evidenced by the lack of spatfall after many observed spawning events. For example, although *C. gigas* have been observed spawning in South Australia since their

introduction in 1969, the first escaped spat were not observed until 1990 when two year classes were found (Hone 1993). An absence of spatfall was also recorded after the autumn spawning observed by Ruiz et al. (1992) and in the hypersaline experimental ponds used by Shpigel (1989) despite spawning continuing over several months.

Some established populations of introduced *C. gigas* have received particular attention, including those occurring in New Zealand (Coleman 1986, Dinamani 1991), France (Goulletquer and Héral 1991, Shatkin et al. 1997) the USA (Chew 1990, 1991), and within Australia: Port Stephens, NSW and the Tamar River estuary, TAS (Coleman 1986, Ayres 1991, Vandepeer 1995, Reid and Smith 1999). Environmental conditions in these areas have occasionally been reported. Port Stephens has an annual mean salinity of 25-28‰, but depending on location within the estuary and rainfall, instantaneous salinity may be between 10-35‰. Temperature ranges seasonally from 13-25°C (Ayres 1991, Shatkin et al. 1997). Pacific oysters first appeared in the middle to upper reaches of the estuary, and only established in the outer port after a year of exceptional rainfall that led to lower salinity (Ayres 1991). In New Zealand, oysters have established in areas with salinity ranging from 20-35‰ and temperatures of 14-22°C (Dinamani 1991). Settlement of spat is greatest in the upper reaches of estuaries where salinity is <35‰ (Coleman 1986, Dinamani 1991) and in years where the summer water temperature exceeds 22°C (Coleman 1986). Settlement also occurs in the low salinity (approximately 18‰) upper reaches of estuaries in British Columbia and Washington (Coleman 1986). Vandepeer (1995) summarises the conditions found in several areas where non-native populations occur including Canada, USA, France and New Zealand. In all these areas, oyster populations are found in waters with salinity of 17-32‰ and summer water temperatures of 16-25°C.

Collectively, these observations suggest that lower than oceanic salinities (i.e. <35‰) favour development and setting of larvae, but other factors may also be influencing the settlement patterns observed. For example, Coleman (1986) notes that the British Columbian and Washington estuaries are both strongly stratified, and therefore the intertidal zone where Pacific oysters settle always shows reduced salinity. In a survey of Tasmanian oyster populations, Mitchell et al. (2000) found that the most important factor determining where populations proliferated were exposure and fetch, with oysters restricted to sheltered sites along high energy coastlines, but found within and outside bays along lower energy coasts.

In addition, natural spatfall in areas with salinity >35‰ has been observed several times, although often with the suggestion that larval development and spatfall occurred during periods of lowered salinity. For example, King (1977) observed several spat in a creek used as a control site for comparison of growth rates. This had a salinity range of 32-38‰, compared with a salinity of 41‰ in the experimental ponds. However, the lower salinities were mainly observed during winter, after periods of freshwater run-off, and not during the summer or autumn when spawning occurred.

Periods of lowered salinity have also been used to explain the proliferation of *C. gigas* in the Tamar river estuary, Tasmania, where mean monthly salinities may reach 37‰, but also regularly fall below 35‰ (and even as low as 13‰) over summer (Vandepeer 1995). However, spatfall has now also been recorded in South Australian waters where salinity is consistently above 35‰, including Murat Bay and Franklin Harbour (Hone 1993, 1996, Vandepeer 1995, Madigan and Clarke 1998). Over the late summer - early autumn period (February to March) in 1992, salinity in Franklin Harbour ranged from 35.9-41.7‰ and in March 1994 salinity in various regions of Murat Bay was between 36.1-40‰ (Hone 1996). In winter (June-August) 1998, mean salinities were 36.9‰ and 35.4‰ in these respective regions (Madigan and Clarke 1998) and in late autumn (May 1992) the range of salinity in Murat Bay was 38.2-40.0‰ (Hone 1996). This suggests that larvae can in fact survive and develop in oceanic salinities and above. However, Pacific oyster populations in South Australia are small compared to those in Tasmania and New South Wales (as well as to those in other regions) and no *C. gigas* spat have been found in other oyster growing areas of similar salinity (Vandepeer 1995, Hone 1996, Madigan and Clarke 1998). It is unclear from these observations if salinity is limiting these populations. In addition, the upper salinity limit for successful larval development is unknown.

Shpigel (1989) observed that larvae survived only a few days in hypersaline (41‰) warm water ponds (20-29°C during spawning season) with no resulting spatfall. However, King (1977) did find a single spat after spawning occurred in a temperate pond (maximum summer temperature of 24°C) also with salinity of 41‰, and further spatfall in these ponds was recorded in subsequent years (Hone 1993). These observations suggest that salinity >40‰ is detrimental to larval development, but may not prevent it entirely.

Larval survival in *C. gigas* is governed by a range of factors, including environmental conditions and food supply, and there may be significant interaction between these factors (Hofmann et al. 2004). Therefore, it is difficult to draw firm conclusions from observations such as those described above, which were made under a wide range of un-controlled conditions. Fortunately, several controlled studies on Pacific oyster larval growth and survival have been performed and are discussed below.

4.2. Fertilisation and hatching

Some of the earliest studies on *C. gigas* performed in its native Japan considered the impacts of salinity on early larval development. Seno et al. (1926) found that fertilised eggs developed (hatched) to shelled (D-stage) larvae at all salinities tested from 15-38‰. However, at salinities ≤18‰, all larvae were abnormal and 80% were abnormal at the highest salinity tested. Less than 10% developed abnormally at salinities of between 25-29‰. The time for development was similar

(22-25 hours) for salinities between 25 and 38‰ but was slightly longer at salinities of 18-22‰ (28-34 hours) and considerably longer at 15‰ (83 hours) (Seno et al. 1926). Amemiya (1928) carried out similar experiments but over a wider salinity range of 5.17-55.15‰. Larval development from fertilised eggs was found to occur over the range from 8.61-34.42‰, but outside this range no eggs developed. However, at salinities <15.49‰ and >32.7‰, many abnormal forms were observed. There were no abnormalities in larvae that developed at salinities of 20.65-25.82‰ and very few at salinities of 18.93‰ and up to 29.26‰ (Amemiya 1928). Theses studies used eggs that had previously been fertilised in "ambient" salinity.

The effect of differing salinities on fertilisation was studied by Vandepeer (1995), who found that fertilisation occurred at all tested salinities (25‰ and the range 34-44‰), but was optimal between 34 and 36‰. Fertilisation success declined at higher salinities to a minimum at 44‰, where many eggs disintegrated. Some eggs also disintegrated at salinities of 40 and 42‰ (Vandepeer 1995). Vandepeer (1995) also studied embryonic development or hatching at 25‰ and over the salinity range 34-44‰, and found survival was significantly different between larvae spawned from farmed (hatchery broodstock) and wild non-native oysters from Murat Bay. The greatest percentage survival to D-stage was found at a salinity of 38‰ for farmed and 36‰ for wild oyster larvae, however, both groups displayed a dramatic decline in larval survival at salinities above 40‰. Abnormal forms were noted in these higher salinities, and at 44‰ many embryos had disintegrated (Vandepeer 1995). While these results are similar in some ways to those of Seno et al. (1926) and Amemiya (1928), in that they demonstrate declining embryonic development success at higher salinities, the upper limit for optimum hatching found by Vandepeer (1995) was considerably higher at 36-38‰, compared with 29‰ in the earlier studies.

One aspect to also consider is possible interactions of salinity and temperature. Both Seno et al. (1926) and Amemiya (1928) carried out their salinity experiments at a temperature of around 23°C, which corresponds to typical water temperatures in the spawning season. Seno et al. (1926) considered the effects of temperature separately, examining development at temperatures of 11.3-31.9°C under ambient salinity (around 25‰). No larvae developed at the lowest temperatures of 11.3 or 13.6°C, or at the highest temperature of 31.9°C. The rate of development increased with temperature over the range 16.3-23.2°C, and was similar for all temperatures from 23.2-27.7°C. However, 97% developed abnormally at 29.7°C and 100% were abnormal at 16.3°C. More than half were also abnormal at 18.6°C, while less than 10% were abnormal at 23.2-27.7°C. The fastest development and no abnormalities were observed at 25.6°C. Amemiya (1928) examined development at ambient salinity at two temperatures: 15 and 25°C and found considerably faster development at 25°C. Amemiya (1928) also repeated the salinity experiments described above at a temperature of 16°C, and found that the range of salinity at which development occurred was expanded to between 12 and 29‰, with the optimum between 17 and 26‰.

Vandepeer (1995) used a factorial design, testing each salinity at each of four temperatures from 17.6°C to 26.3°C. Fertilisation success and hatching were both found to be lower at 17.5°C than at temperatures of ≥21.4°C. At the highest salinity, embryonic survival was zero for both temperature extremes, but some larvae developed at 21.4 and 23.8°C. However, no significant interaction between temperature and salinity effects was found. Additionally, examining the results found by Vandepeer (1995) for varying salinities at a temperature of 23.2°C still shows a higher than expected salinity optimum. Vandepeer (1995) suggests that since Tasmanian hatcheries from which farmed broodstock, and thus larvae, were obtained currently operate at salinities of around 35‰, this has led to acclimatisation of the oysters to reproduction at higher salinity. The alternative suggestion was that the results of the earlier studies were flawed or incomplete.

4.2. Larval Growth and Survival

4.3.1. Temperature and salinity

Studies of larval development in *C. gigas* have examined development of larvae already at the trochophore or D-stage and later, so it is difficult to directly compare their results to those of Seno et al. (1926), Amemiya (1928) and Vandepeer (1995).

Helm and Millican (1977) studied the hatchery rearing of *C. gigas* larvae over a range of temperatures and salinities. They examined growth rates and survival of D-stage larvae at temperatures between 20 and 32°C, and salinities of 15 to 30‰, plus ambient salinity. Ambient salinity varied between trials, being 30.9, 32.7, and 34.0‰ for the first, second and third trials respectively. At all salinities, optimum growth was found at 28°C. Growth rates increased with temperature up to 28°C, but were considerably less at 32°C. At temperatures of 24 and 28°C best growth was found at a salinity of 25‰. In the two trials where ambient salinity was >32‰, reduced growth rates were found at this higher salinity. Survival was generally high (at least 60% over 6 to 10 days), and was >95% in all salinities at temperatures ≤28°C. Lower than 90% survival was observed in a number of batches at 32°C without a clear pattern of salinity impacts, although the lowest survival (60%) was observed in the ambient salinity of 32.7‰ at 32°C. Survival was not assessed in the final series of trials when ambient salinity was even higher (Helm and Millican 1977). Helm and Millican (1977) also note that low growth and reduced survival at 32°C may have resulted from disintegration of food items at this temperature.

Nell and Holliday (1988) examined the effects of salinity over the range 15-39‰ on growth and survival of *C. gigas* D-stage larvae at a constant temperature of 26°C. Growth was best over the range 19-27‰ with considerably lower growth at 15‰, and a marked decrease also at 35-39‰. However, survival was very high over the 6-day period (average 96%), with no influence of salinity

(Nell and Holliday 1988). Hence, while these data suggest the optimum salinity for larval growth is near to or below 30‰, they do not demonstrate reduced survival at higher salinity.

His et al. (1989) considered the additional variable of whether larvae were fed or unfed. Their experiments on *C. gigas* D-stage larvae were carried out over four salinities between 20 and 35‰ and four temperatures from 15-30°C. Survival over 7 days was >95% in nearly all treatments. The exception was unfed larvae at a salinity of 35‰, where survival was considerably lower than other treatments, although still good at 60-75%. For fed larvae, growth increased with temperature over the experimental range, and with respect to salinity was greatest at 30‰, although there was little difference in growth over the range 25-35‰. There was no interaction of temperature and salinity effects. Unfed larvae showed a similar pattern, although with much reduced growth. Unfed larvae showed a greater reduction in relative growth at 35‰ salinity compared with 30‰ than did fed larvae (His et al. 1989). Similarly to the results found by Nell and Holliday (1988), His et al. (1989) found that survival of D-stage larvae was not adversely affected by salinity, at least when fed. The optimum salinity for growth suggested by His et al. (1989) is slightly higher than that found by Nell and Holliday (1988), and the decline in growth found at salinity above this optimum was only very slight. However, it should be noted that the decline in growth observed by Nell and Holliday (1988) between 31 and 35‰ was not large (a decline of ~12%), while the growth rate of *C.* gigas larvae at 39‰ was less than half that at 35‰.

In combination, the studies of Helm and Millican (1977), Nell and Holliday (1988) and His et al. (1989) suggest that the optimum salinity for growth of D-stage *C. gigas* larvae is between 25 and 30‰, with some impairment of growth at 35‰ and greater impairment at higher salinities. Larvae survive and grow (though less well) at salinities down to 15‰, but survival and growth of D-stage larvae at lower salinities than this have not been tested. Survival over periods of 6-10 days is not adversely affected by salinities of up to 39‰ (Nell and Holliday 1988). However, none of these studies examined larval survival over longer time periods.

A study by Tan and Wong (1996) on larval development in *Crassostrea belcheri* found that a similar percentage of larvae survived at a higher than optimal salinity (30‰, compared with the optimum of 12-24‰) for 12 days, but thereafter suffered significantly greater mortalities. Under favourable conditions (30°C, 30‰, feed supplied), the larval period for *C. gigas* is between two and three weeks (His and Seaman 1992, Rico-Villa et al. 2006), a similar length to that of *C. belcheri* (Tan and Wong 1996). Therefore, a study carried out over 6 days may underestimate the impact of salinity on larval survival in *C. gigas*. Interestingly, Tan and Wong (1996) also found that embryonic development success was greatest at a higher salinity (24-30‰) than was optimal for larval growth, and was poor at the salinities of 12-18‰ that proved best for larval growth and settlement success. Whether *C. gigas* embryonic, larval development and later larval growth have different optimal

salinity ranges is unclear from the data presented above, particularly since very few studies have examined salinities outside the range 15-35‰.

4.3.2. Nutritional effects

Nutritional status is an important component of larval survival, and may be influenced by or interact with environmental factors (His and Seaman 1992, Bochenek et al. 2001), and a number of studies have considered the effects of nutritional status on larval development.

His and Seaman (1992) reared larvae under two sets of conditions: "favourable" (30°C with 30‰) and "adequate" (22°C with 25‰), with starvation periods of 1 to 6 days after hatching, followed by supply of feed for 15 days. Starvation periods of up to 3 days caused negligible mortality in *C. gigas* kept under "favourable" conditions, but there was considerable mortality in batches not fed for 4-5 days, and 100% mortality after 6 days without food. However, under "adequate" conditions, unfed larvae survived longer, with only 20% mortality recorded after 8 days starvation. In both sets of conditions, larvae starved for up to 3 days were able to resume growth and suffered little to no mortality after the resumption of feeding. Those that were unfed for 4 days or more showed a lag in growth after feed was offered, and suffered significant mortality (12-80%) after 15 days feeding, although those that survived showed normal development. However, larvae reared in "adequate" conditions that survived 7 or 8 days starvation did not grow once food was offered, and 100% mortality resulted within the subsequent 15 days. While the comparison was not made by His and Seaman (1992), larvae under favourable conditions grew considerably faster than those in the equivalent "adequate" treatments. They do note that, after resumption of feeding, larvae under "favourable" conditions which suffered short starvation periods were able to match growth rates of fed larvae, while in the "adequate" conditions, growth rates were always less for larvae that had undergone starvation periods than for fed controls.

Clearly there are complex interactions between feeding and environmental conditions on larval growth and survival, but while there are a number of studies on the impact of feeding on larval development, most have been carried out at a single temperature/salinity combination. Additionally, not all support the finding of His and Seaman (1992) that larvae do not survive (or fail to recover from) more than 6 days without feeding. For example, Moran and Manahan (2004) found around 75% survival of C. *gigas* larvae that were not fed for up to 14 days, and 15% survived 23 days. After feeding delays of up to 17 days larvae were able to resume feeding and obtain growth rates similar to fed controls, however at 8 days after resumption of feeding, larvae that had been unfed for 17 days had suffered almost 75% mortality. Measurements of biochemical composition and metabolic rates of fed and unfed larvae suggest that unfed larvae were not surviving on reserves

but must have been utilising an exogenous energy source, most likely dissolved organic matter in the filtered natural seawater that was used (Moran and Manahan 2004).

A number of studies of feeding in *C. gigas* larvae have aimed to identify the optimal diet for hatchery rearing (eg Thompson and Harrison 1992, Rico-Villa et al. 2006) and not surprisingly, these show that increased food quality leads to increased growth. Fewer studies have considered the impact of varying food quantity, which may be more relevant for the survival of larvae in the wild. Gerdes (1983) examined the feeding behaviour and growth of Pacific oyster larvae fed two different cell concentrations (25 x 10^6 cell/l and 100 x 10^6 cells/l) and found that larvae fed the lower food ration had increased filtration activity, although this still resulted in a lower overall clearance rate. After 12 days, growth rate of these larvae was approximately half that of larvae fed the higher food ration, and for the first six days was only marginally greater than that of unfed larvae (data for unfed larvae not presented after >6 days). Whether larvae can continue to grow and develop on such a reduced feed ration is unclear since the fate of the larvae after the 12-day period is not discussed. Another batch of larvae fed 100 x 10^6 cells/l of the mixed diet subsequently shown to give the best growth rates (Rico-Villa et al. 2006), demonstrated feeding activities and growth rates that were not constant throughout development. For the first 5 days, larvae fed and grew little, but from day 6 to 15 filtration activity and growth rates increased, before decreasing again once larvae reached approximately 300 μm, at which point they are competent to metamorphose (Gerdes 1983).

4.4. Settlement and Metamorphosis

Studies of nutrition on later larval periods and spatfall have shown a strong influence of nutritional status on successful completion of metamorphosis. For example, Laing and Earl (1998) found that percentage spatfall was correlated with lipid content in larvae and similarly, Laing (1995) found that percentage spatfall correlated with daily feed supply when competent larvae were fed varying rations. Laing (1995) also found that complete withdrawal of feed prior to metamorphosis reduced settlement success, with the success rate of spatfall decreasing with duration of starvation; and Rico-Villa et al. (2006) found that algal diets which promoted the fastest larval growth also resulted in greater settlement success. However, none of these studies considered other environmental impacts.

Combined impacts of temperature and salinity on settlement success in the tropical oyster species, *Crassostrea iredalei* were studied by Devakie and Ali (2000). They found a significant influence of temperature and salinity on mean percentage settlement of *C. iredalei* larvae, and also a significant interaction of temperature and salinity effects. At low salinity (5‰), settlement success was low regardless of temperature, while at moderate salinities (15-25‰) there was a pronounced optimum temperature at 30°C. Similar experiments have been carried out on *C. gigas* larvae.

Henderson (1983 in Devakie and Ali 2000) found that setting percentage of *C. gigas* was greatest at 30°C and 30‰, but the range of temperatures/salinities tested and by how much settlement success was reduced outside this optimum, were not described. Chew (1983 in Pauley et al. 1988) found that remote setting (i.e. eyed larvae removed from hatchery conditions and placed in ponds for settlement) of *C. gigas* was influenced by the temperature and salinity of settlement ponds. Settlement success increased with temperature from 15-30°C and with salinity from 15-30‰, but declined sharply at 35‰ at temperatures >25°C and <20°C. At 20 and 25°C, settlement success was only slightly lower at 35‰ than at 30‰. At 35°C, settlement was lower for all salinities than at 30°C, and the lowest settlement success of all treatments occurred at 35°C/35‰. Overall, the greatest settlement success found in their study was also at 30°C /30‰.

4.5. Biochemical modelling

It is apparent from the results presented in this review that there may be significant interactive effects of temperature and salinity throughout larval development, and combined stresses may have significant impacts on larval survival and development. These stresses may also be ameliorated or exacerbated by nutritional status (His and Seaman 1992, Bochenek et al. 2001). The results found by His et al. (1989), in which larvae reared under adverse salinities were more affected by withholding of feed, lend additional support to this. Similarly, both Helm and Millican (1977) and Vandepeer (1995) found the lowest values for larval and embryonic survival where high salinity was combined with temperature extremes. However, Amemiya (1928) found embryos developed over a wider salinity range at sub-optimal temperatures and His and Seaman (1992) found lower mortality in unfed larvae under "adequate" rather than "favourable" growing conditions. Our understanding of salinity, temperature and nutrition interactions on larval growth and survival is clearly lacking, particularly regarding longer-term larval survival, growth and metamorphosis.

To attempt to resolve some of these questions, Bochenek et al. (2001) developed a biochemically-based model of larval growth and survival which was used in their study as well as subsequent studies by Powell et al. (2002) and Hofmann et al. (2004) to predict growth rates and cohort survival (% eggs which successfully complete metamorphosis) of *C. gigas* larvae under various environmental conditions. Interestingly, their model predicts that above 30°C, mortalities due to metabolic imbalances will increase (Hofmann et al. 2004), which tallies with the observations of Helm and Millican (1977). However, the model also suggests that survival will be zero below 23°C for most food rations, which is not supported by the fact that *C. gigas* larvae clearly survive through to settlement in many regions where temperatures stay below this level, eg Tasmania (Vandepeer 1995), New Zealand (Dinamani 1991) and the Dutch Wadden Sea (Diederich 2006). Nonetheless, this discrepancy does not necessarily mean that all outputs of the model are invalid.

With respect to the interactions of temperature, salinity and food supply, the model (as implemented by Hofmann et al. 2004) predicts that an increased food ration expands the range of temperature and salinity combinations that lead to cohort survivals of greater than 6%. When food was not limiting, cohort survival was predicted to be >6% over a wide salinity range so long as temperature was >25°C, with a temperature of 27.5°C resulting in >6% survival at all salinities >20‰ to the maximum used (35‰). The range of salinities giving cohort survival >6% narrowed at temperatures outside this range (Hofmann et al. 2004). However, when food ration was low, not only was the range of suitable temperatures and salinities restricted, but the optimum (which still gave < 2% cohort survival) was shifted to a lower temperature and some successful settlement was predicted at temperatures down to 21°C, although for only a narrow salinity range around 25‰. While these results have clearly not been tested, they do further demonstrate the need for studies that incorporate nutritional status as well as abiotic factors over the entire larval life cycle, since optimum temperature and salinity may be affected by food supply.

Another point made by Pauley et al. (1988), Bochenek et al. (2001) and Hofmann et al. (2004) is that where the larval life span is extended due to suboptimal growth conditions, non-metabolic sources of mortality such as predation will increase. Additionally, Davis and Calabrese (1964) and Arakawa (1990) observe that a prolonged larval period may increase the risk that larvae are removed from suitable settlement sites by the action of tides and currents.

The length that the larval lifespan of *C. gigas* will extend under various conditions is unknown. However, Tan and Wong (1996) found that settlement in *C. belcheri* occurred an average of 6 days later (day 23 compared with day 17) at a higher than optimal salinity. Similarly, Davis and Calabrese (1964) found that a reduction in temperature from 30 to 20°C led to *Crassostrea virginica* larvae settlement beginning at day 36 compared with day 10 at the higher temperature. Arakawa (1990) presents data from the Hiroshima City Fisheries Division (1972) that suggests *C. gigas* larvae obtained a suitable size for metamorphosis in as little as 11 days at 26-28°C, but at 22-24°C they had not reached this size by day 20. Given that Nell and Holliday (1988) found that growth rates of larvae were more than halved by a salinity change from 35‰ to 39‰, it can be expected that the larvae will take twice as long (or more) to reach a suitable size for metamorphosis (Bochenek et al. 2001).

4.6. Impacts of broodstock acclimation

Vandepeer (1995) raised the question of whether broodstock acclimation influences larval tolerances, and there is some evidence to indicate it does. Helm and Millican (1977) acclimatised broodstock at 26‰ and 31‰ for 40 days then reared larvae at salinities between 15 and 30‰. At up to 21‰ the two groups showed a similar response, with growth rates increasing with salinity. However, at higher salinities the larvae from the lower salinity broodstock displayed considerably

reduced growth compared with larvae from the higher salinity broodstock. Muranaka and Lannan (1984) studied embryonic development and larval survival at a single salinity of 29‰ using gametes from adults conditioned at 20‰ and 30‰. They found that a lower percentage of embryos from the 20‰ conditioned parents successfully developed, and larvae had substantially lower survival. Even a short exposure of the adults to 20‰ prior to spawning led to reduced embryonic and larval success at 29‰, due to osmotic shock rather than acclimation effects (Muranaka and Lannan 1984). This has implications for experiments in which spawning takes place at a different salinity to experimental treatments, as is the case in nearly all the studies described in this review.

4.7. Summary

In summary, the effects of salinity and temperature on early life stages have still not been comprehensively determined. Effects on fertilisation have been considered in only one study by Vandepeer (1995), that showed that fertilisation could take place at salinities up to 44‰, but was greatly reduced above 40‰. Studies on embryonic development (hatching) have suggested different optimum ranges of salinity. Seno et al. (1926) and Amemiya (1928) both suggest that embryonic development requires a salinity of 18 to 32‰ with highest successful development between 20 and 29‰. However, Vandepeer (1995) found that embryos develop into larvae at salinities up to 44‰ (though with significant impairment at above 40‰) with optimal development (defined as % success) at between 34 and 38‰. For D-stage larvae, salinities from 15-39‰ do not cause significant mortalities over periods of 6-10 days, but growth is optimised by salinities between 20 and 30‰ (Helm and Millican 1977, Nell and Holliday 1988, His et al. 1989). Effects of salinity outside this range, or over longer period, have not been studied. Settlement success appears to increase with salinity up to 30‰, but becomes impaired by higher salinity (Henderson 1983 in Devakie and Ali 2000, Chew 1983 in Pauley et al. 1988). However, this has not been studied at salinities above 35‰.

With respect to temperature, embryonic development appears to be most successful at between 16 and 28°C, and to be severely impaired by temperatures outside this range (Seno et al. 1926, Amemiya 1928). Vandepeer (1995) found lower fertilisation success at 17.5°C compared with that at temperatures between 21 and 26°C. Growth rates of D-stage larvae increase with temperature from 15°C (lowest tested) to 30°C, but decline at higher temperatures, and mortalities may result where both temperature and salinity are high (Helm and Millican 1977, His et al. 1989). Settlement success shows a similar pattern, increasing from 15 to 30°C, and being less at 35°C, especially at high salinities (Henderson 1983 In Devakie and Ali 2000, Chew 1983 In Pauley et al. 1988).

While a combination of high temperature and high salinity appears particularly detrimental, a low temperature may extend the range of salinities over which successful development occurs

(Amemiya 1928), and a combination of lower than optimal temperature and salinity led to better survival of D-stage larvae during periods of starvation (His and Seaman 1992). Biochemical models (Hofmann et al. 2004) suggest that the range of suitable temperature and salinity combinations for successful larval development will be greater as food supply increases. However, this has not been experimentally tested and knowledge of the performance of larvae at varying food rations is lacking.

Since it is known that salinity and temperature outside the optimal ranges reduce growth rates (Helm and Millican 1977, Nell and Holliday 1988, His et al. 1989) it can be expected that the larval period will be extended under these conditions and hence mortalities due to predation will increase (Hofmann et al. 2004). A longer larval period may also increase the chance that larvae are removed from suitable settlement areas by tides and currents (Davis and Calabrese 1964, Arakawa 1990). However, the length of the larval period of *C. gigas* has not been specifically studied outside standard hatchery conditions.

Acclimation of broodstock may influence the performance of larvae. In particular it is indicated that larvae from lower salinity broodstock do not grow as well at high salinity as those from higher salinity broodstock (Helm and Millican 1977, Muranaka and Lannan 1984), although larvae from high salinity broodstock were not impaired by lowered salinity during development (Helm and Millican 1977). However, these experiments were carried out over limited salinities, and more data are needed before firm conclusions can be drawn.

5. CONCLUSIONS

Figures 5.1 and 5.2 summarise our current understanding of the temperature and salinity tolerances of the Pacific oyster, *Crassostrea gigas*, based on the information presented in this review.

As presented earlier, the optimum temperature range for spawning and larval development is more restricted than for adult growth. However, the tolerated minima and maxima for fertilisation, larval survival and settlement have not been established, nor has the maximum temperature for spawning. The optimal temperature for growth of spat has also not been reported, although it is likely to be similar to that of adults. The upper temperature tolerance of very small spat is also unclear, but even quite small juveniles (11mm) were shown to tolerate short exposures to above 40°C (Rajagopal et al. 2005). At temperature extremes, adults can survive considerably colder conditions than spat, and longer periods of high temperature exposure.

The optimum temperature for all life stages appears to be between 23 and 30°C. Mortalities of larvae and embryos may occur when exposed to temperatures above 30°C. Spawning requires a temperature of at least 16°C but usually 18°C or more, although gametogenesis occurs at temperatures down to 10°C. Embryonic development also requires a temperature of around 18°C.

Larval growth is slowed by lower temperatures, but has not been studied at <15°C. It is unknown whether lower temperatures are lethal or simply reduce growth further (eg as assumed by Hofmann et al. 2004).

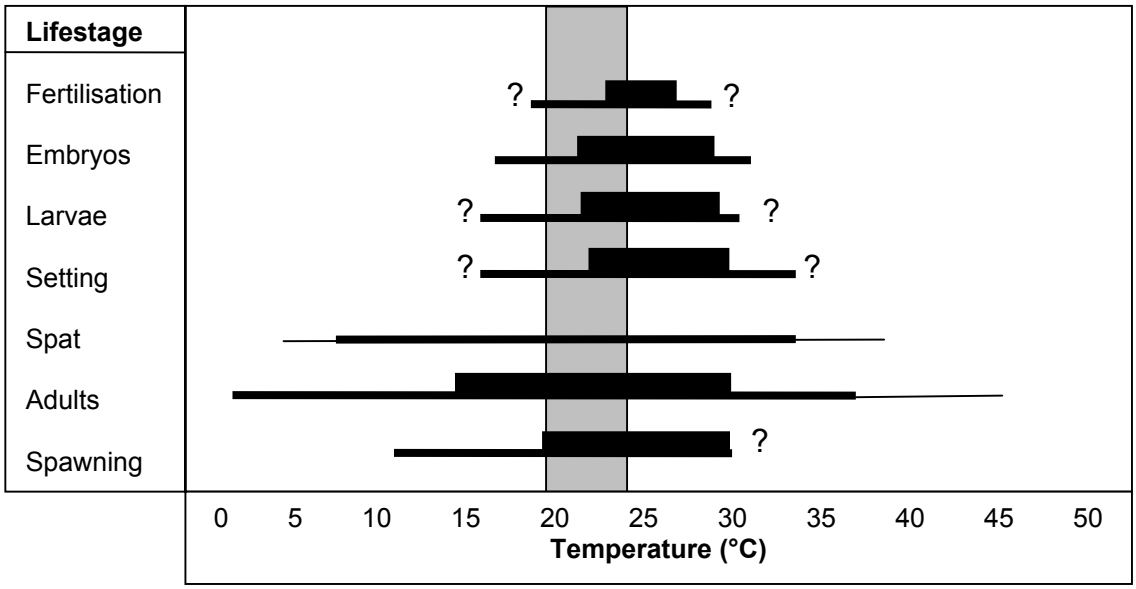

Figure 5.1: Tolerated and optimal temperature ranges for the Pacific oyster.

(—)Temperatures tolerated for short periods, (▬) temperatures tolerated long-term, (■) optimal temperature range, (?) not tested beyond this point. Grey shaded range represents typical South Australian summer water temperatures.

Overall, the typical summer temperature of South Australian waters, while lower than the absolute optimum, is within the range expected to be conducive to spawning, larval development and spat fall. Winter water temperatures rarely fall below 12°C except for very short periods (Madigan and Clarke 1998) and so remain comfortably within the range suitable for spat and adult survival. Hence it is highly unlikely that temperature is restricting the spread of *C. gigas* in South Australia. However, interactions of temperature and salinity may be important.

As with temperature, adults demonstrate wider optimal ranges of salinity than do early life stages. Again, tolerated maxima and minima of salinity have not been established for all life stages. While growth rates are decreased by salinity above 30‰, no significant mortality was observed in either larvae or spat kept at salinities up to 39‰ and 45‰ respectively (Nell and Holliday 1988). Settlement success appears to be less at 35‰ than 30‰, but has not been tested at higher salinities. Fertilisation success has received little attention, but Vandepeer (1995) found it was high at up to 36‰ then declined with increasing salinity; it was extremely low at the highest salinity tested (44‰). Results for embryonic development are mixed, with Vandepeer (1995) finding a higher salinity optimum (and overall tolerance range) than Seno et al. (1926) and Amemiya (1928). Adults survive

long exposures to salinities up to 55‰, though growth is reduced at salinities >42‰. Lower salinity tolerance is less well established. Prolonged exposure to salinities below 10-15‰ is detrimental and may cause mortality. Certainly continual exposure to salinities <10‰ has negative impacts, but one study (Nell 1991) suggests that periods up to two weeks at 5‰ may be tolerated.

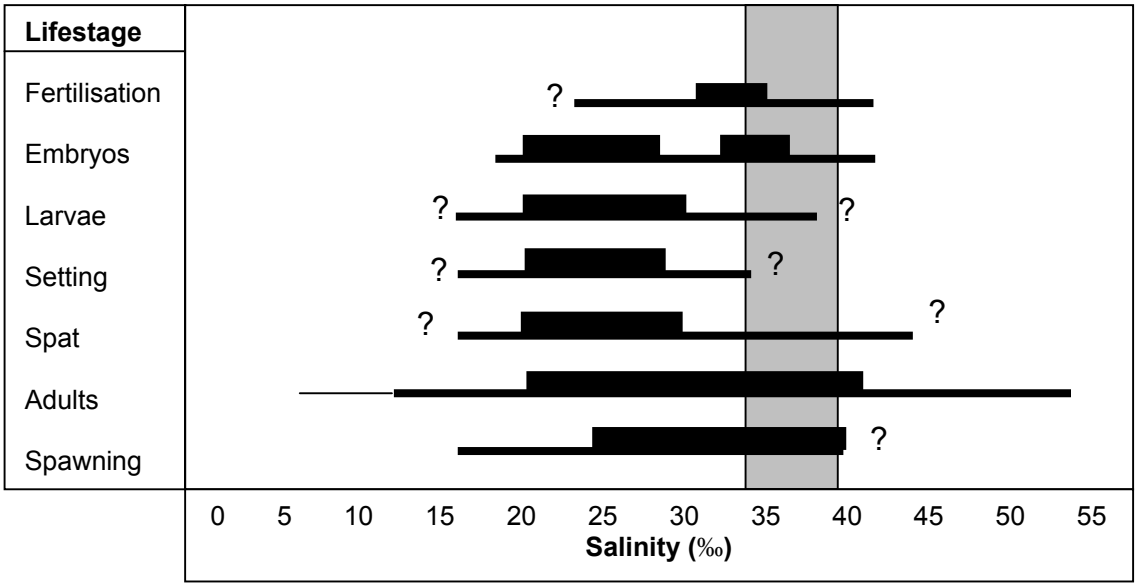

Figure 5.2: Tolerated and optimal salinity ranges for the Pacific oyster.

(—) Salinity tolerated for short periods, (▬) salinity tolerated long-term, (■) optimal salinity range, (?) not tested beyond this point. Grey shaded range represents typical South Australian salinities year round.

The typical salinity of South Australian waters is higher than the optimal salinity stated for many life stages but will not necessarily prevent reproduction. As stated, little mortality has been observed at the highest salinities tested so far, although these experiments were of limited duration. Pacific oyster larvae can obviously survive through to settlement at salinities around 35‰ and above as spat have been found in areas of South Australia where salinity is consistently high, however, the numbers of spat are relatively low and they have not occurred at all possible sites. There are a number of possible reasons for this and it is highly likely that many factors influence the patterns observed. At extremes of salinity, lack of feed may have a greater negative impact than at optimal salinities (His et al. 1989). Hone (1993) noted that Chlorophyll *a* levels in South Australian waters were low (0-4 mg/l) compared with the optimum for *C. gigas* larvae (>12mg/l). At low feed levels, biochemical models predict that very few temperature and salinity combinations will result in successful larval survival through to settlement (Hofmann et al. 2004). Additionally, the extended larval period that results from suboptimal conditions exposes larvae to greater predation pressure (Hofmann et al. 2004), and to the risk of being removed from suitable

settlement areas (Arakawa 1990). Most simply, greater mortality (though clearly <100%) may result when larvae are exposed to high salinities for longer periods.

There is a lack of firm data to support any of these theories, with no studies examining longer term larval survival at temperature or salinity extremes or at decreased feed rations, and very few considering interactions of feed, temperature and salinity. The possibility of acclimation is also unclear, but it cannot be ruled out as a factor that may increase the tolerance of larvae spawned from adults that are cultured and ripen under high salinity conditions. Certainly there is no evidence to suggest that high salinity impairs gametogenesis or spawning.

Our current knowledge of the ecophysiological tolerances of Pacific oysters confirms that adults have a very wide range of tolerance with respect to salinity and temperature. Suitable conditions for reproduction are more restricted, but while we have information on optimal conditions for growth, our knowledge about the absolute tolerance limits of early life stages is lacking. In particular, mixed results for the optimum range for embryonic development have been found, longer term impacts of high salinity on larval growth and mortality are unknown, interactions of salinity, temperature and nutrition have not been fully investigated, and whether acclimation of adults pre-disposes larvae to better development at high salinity is unclear. Further understanding of these issues is required in order to determine the potential for *C. gigas* populations to spread within South Australia and elsewhere.

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