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HIGH TOLERANCE OF THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*, THUNBERG) TO LOW TEMPERATURES

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ABSTRACT As an intertidal species, the Pacific oyster (*Crassostrea gigas*, Thunberg) is very tolerant to varying abiotic conditions. The temperature range for survival has, however, not been fully evaluated. Most notably, information about cold tolerance of the species is missing. As a first step toward acquiring a better understanding of the species' cold tolerance, the aim of this study was to measure mortality of groups of Pacific oysters after exposure to simulated winter conditions (–22°C) for 24, 48, and 72 h. Such conditions are not very different from what can be experienced in nature during wintertime in Scandinavia. The Pacific oyster was found to be very tolerant to low temperatures with, in general, 50% survival after 24 h. Thermal acclimatization and premature adjustment to winter conditions allowing for improved survival after cold treatment were also observed.

KEY WORDS: oyster, *Crassostrea gigas*, temperature tolerance, cold hardiness, temperature acclimatization, mortality

INTRODUCTION

The Pacific oyster (*Crassostrea gigas,* Thunberg) is an invasive species that which was first seen in large quantities along the Swedish west coast in 2007. Currently , more than 200 sites with Pacific oysters have been found in Sweden, most of which are in Bohuslän County. The harsh winter conditions experienced in this region have been suggested to be a factor that could possibly affect the continued survival and dispersal of the species (Nyberg 2010). Winter 2009 and 2010 caused high mortality of the Swedish Pacific oyster population (80–90%), yet approximately 15% of the live oysters were located at depths less than 0.3 m (Å. Strand, unpubl. data), indicating high resilience of the species to harsh winter conditions.

As an intertidal species, the Pacific oyster is very tolerant to varying abiotic conditions. The temperature range for survival has, however, not been fully evaluated. The upper thermal limit is considered to be approximately 30°C (Le Gall & Raillard 1988, Bourgier et al. 1995), but for survival at low temperatures the temperature is often referred to as ''subzero'' (Quayle 1969, Walne 1974, Diederich et al. 2005, Diederich 2006). In a few studies, mortality of the Pacific oyster is reported to be low, at temperatures of –2°C (Eklund et al. 1977), –12°C (Wa Kang'eri 2005), and –5 to –14°C (Nyberg 2010). In contrast, high mortalities were reported by Büttger et al. (2011) for oysters exposed to seawater temperatures less than 2°C and air temperatures of between 2°C and –13°C for 83 days.

As tidal fluctuations at the Swedish west coast are very small (<0.3–0.4 m), the water level is mainly regulated by climatic factors such as large-scale weather systems that, through wind and pressure changes, can cause water-level variations in meters (Swedish Meteorological and Hydrological Institute (SMHI)). Thus, sessile organisms living in shallow areas are often exposed to air for prolonged periods of time, especially during winter. Subzero temperatures during winter are also normal, and long continuous periods of freezing temperatures are common (SMHI). Knowledge about the tolerance of the species to low temperatures is thus essential for evaluation of the potential for continued survival and dispersal of the Pacific oyster in the Nordic countries. As a first step toward a better understanding of the Pacific oyster's cold tolerance, the aim of this study was to evaluate mortality of Pacific oysters after exposure to simulated winter conditions (–22°C).

MATERIALS AND METHODS

Pacific oysters were collected at Krokesundet (58°51′41.29″ N, 11°10′26.91″ W) and at Fiskebäckskil (58°14′33.78″ N, 11°28′7.01″ W) on the west coast of Sweden on two occasions during 2010. In June, 45 oysters ranging from 6–10 cm were collected at approximately 50 cm in depth at Krokesundet and stored in a covered flow-through system using filtered deep seawater (inlet at 50-m depth) until October. The temperature of the deep water was at least 2°C lower continuously compared with the surface water temperature during the storage period. In mid October, another 45 Pacific oysters with a length of 10–15 cm were collected at Krokesundet, and 90 of the same size were collected from Fiskebäckskil, at both sites at approximately 50 cm in depth. Water temperature at the time of sample collection was 10°C and salinity was approximately 23‰. The oyster density at Fiskebäckskil was, on average, 200 individuals/m^2, and the maximum density was 450 individuals/m^2. The oysters grew in layers, thus the collected oysters were divided into 2 groups: top and lower levels, with 45 individuals in each group. At Krokesundet, average and maximum densities were 25 individuals/m^2 and 150 individuals/m^2, respectively. After sampling, the oysters were transported to the research facility (Sven Lovén Center; 58°52′28.19″ N, 11°8′48.17″ W), and all oysters (including the ones collected in June) were sorted into groups of 14–15 individuals and placed in dry, marked plastic containers in a single layer, after which the containers were placed in the cold treatment (–22°C). After 24, 48, and 72 h, 1 group each from the 4 classes (Krokesundet June, Krokesundet October, Fiskebäckskil top layer, and Fiskebäckskil lower layer) were removed from the cold treatment. The sampled oysters were then kept at room temperature (18°C) for 24 h for thawing, after which they were put into a flow-through system of filtered deep seawater with a water temperature of 4°C for another 24 h in an attempt to revive any weakened oysters. Thereafter, the oysters were judged to be dead (open shells) or alive (tightly closed shells). A few individuals were very weak

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(shells easily opened) and were subsequently classified as dead. Mortality of the oysters was expressed as percent dead oysters relative to the total number of oysters in each group.

The effect of treatment class (Krokesundet June, Krokesundet October, Fiskebäckskil top layer, and Fiskebäckskil lower layer) on mortality of the oysters (dead or alive) was tested using the Kruskal-Wallis test. The Mann-Whitney *U*-test was used as a post hoc test. The relationship between mortality and exposure time was then expressed using linear regression. Proportions (mortalities) were arcsin square root transformed before analysis.

RESULTS

Mortality of the oysters ranged from 0% (Krokesundet June, 24h) to 100% (Fiskebäckskil top and lower layers, 72h; Fig. 1). Mortality differed significantly between treatment classes (Kruskal-Wallis, $df = 3$, $P < 0.001$), with significantly lower mortality for oysters from Krokesundet June compared with the other categories (Mann-Whitney *U*-test, $P < 0.05$). No differences in mortality was found between the other categories (Krokesundet October, Fiskebäckskil top layer, and Fiskebäckskil lower layer; Mann-Whitney *U*-test, $P > 0.05$). Linear regression of all treatment categories jointly (except Krokesundet June) demonstrated a significant increase in mortality with increasing exposure time to low temperature (linear regression, $R^2 = 0.923$, $P < 0.001$, Fig. 1). The resulting regression equation was

$$y = 35 + 0.9 \cdot x$$

The corresponding equation for Krokesundet June was

$$y = -22 + 1.0 \cdot x$$

where y is mortality (measured as a percentage) and x is time (measured in hours).

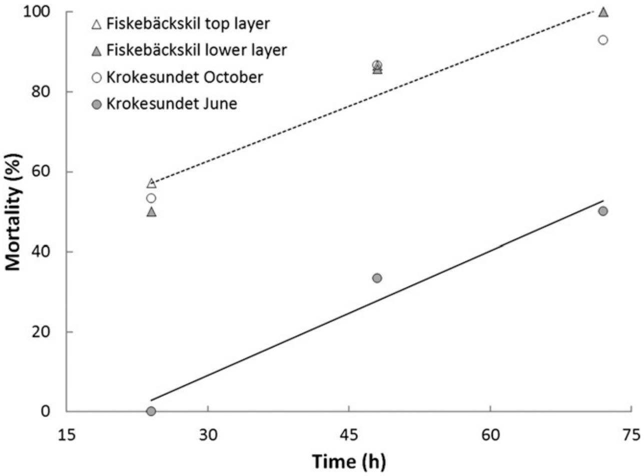

Figure 1. Mortalities (%) of the Pacific oyster (*Crassostrea gigas*) from 2 localities (Krokesundet, open and gray circles; Fiskebäckskil, open and gray triangles) in Sweden collected in October (open and gray triangles and open circles) and June (gray circles), and thereafter exposed to –22°C for 24, 48, and 72 h. The oysters collected in June were stored in darkness, with food deprivation and low temperature until the start of the experiment. The solid line represents the regression for Krokesundet June and the dotted line represents the regression of all 3 remaining classes combined.

DISCUSSION

In this study, the Pacific oyster was found to be very tolerant to low temperatures and to large variations in temperatures. Thermal acclimatization and premature adjustment to winter conditions allowing for improved survival after cold treatment were also observed. Although the Pacific oyster is reported to be very tolerant to varying abiotic conditions, the extreme cold tolerance observed in this study was unexpected. Lower survivability of the oysters (100% mortality) has previously been reported at higher temperatures (–18°C) after only 6 h of exposure to cold treatment (Wa Kang'eri 2005). What causes this discrepancy is not known. Because this experiment was performed during autumn, and the experiment by Wa Kang'eri (2005) was performed during winter, the differing results are not likely caused by adjustment to winter conditions, because as this would have increased the survivability of the oysters in the study performed by Wa Kang'eri (2005). It is possible that local adaptation toward higher cold tolerance is already occurring in Swedish Pacific oyster populations, but more studies are required to establish if this is the case.

Intertidal molluscs often exhibit seasonal variations in their cold tolerance and are more cold tolerant during winter (Aarset 1982, Murphy 1983, Storey & Storey 1988, Ansart & Vernon 2003). Increased cold tolerance may be attributed to temperature acclimatization, with higher cold tolerance for organisms acclimated to lower temperatures (Aarset 1982, Storey & Storey 1988, Ansart & Vernon 2003). For some intertidal species, a combination of low temperature (Murphy & Pierce 1975, Murphy 1983), low light intensity, and food deprivation increases cold resistance, even for animals collected during summer (Murphy 1983). This is in accordance with the discrepancies observed in our study. For several months before the onset of the experiment, the oysters from Krokesundet, which were collected in June, were stored in filtered deep seawater, in the dark, and were constantly exposed to lower temperatures compared with that experienced by wild populations. The oysters collected in June thus experienced a combination of low temperatures, low light conditions, and food deprivation, which may have triggered both acclimatization to low temperatures and a premature adjustment to winter conditions, thus explaining the increased survival observed in this category.

The high cold tolerance of the Swedish Pacific oyster populations makes further dispersal and establishment in the region likely. The temperature used in this study represents a realistic scenario to which the oysters at the Swedish west coast archipelago can be exposed during winter. For example, during the winter of 2009 and 2010, the oysters were exposed to long periods of low water levels (below average water level for 2.5 mo) combined with long periods (>1 month) of subzero temperatures and very low temperatures (<–20°C; SMHI). Earlier studies have shown that winter conditions in 2009 and 2010 caused high mortality of the Swedish Pacific oyster population (80–90%), yet approximately 15% of the live oysters were located at depths of less than 0.3 m (Å. Strand, unpubl. results). The results obtained in our study further support the previously perceived high resilience of the oysters to extreme temperature conditions. Thus, despite the high number of mortalities it is unlikely that natural events will be able to eradicate the species from the region. Moreover, although dramatic temperature changes (–22°C–18°C within 24 h) are not likely to occur in nature, the treatment further stresses the high resilience of the oysters to both extremely low and highly varying temperatures.

There are several strategies among invertebrates for surviving subzero temperatures, including freeze avoidance and freeze tolerance. Freeze-avoidant species cannot survive freezing of their body fluids but can endure subzero temperatures through supercooling, whereas freeze-tolerant species can tolerate formation of ice in their extracellular body fluids (Storey & Storey 1988, Marchand 1996, Ramløv 2000, Ansart & Vernon 2003). The majority of invertebrate species living in the intertidal zone are considered to be freeze tolerant (Aarset 1982, Storey & Storey 1988, Marchand 1996, Ansart & Vernon 2003). Our study was not designed to establish what mechanism is responsible for the high cold tolerance of the Pacific oyster. However, the cold hardiness observed for the Pacific oyster is in accordance with that demonstrated for other intertidal species. For example, the snail *Litorina litorea* may survive several days at exposure to –22°C (Kanwisher 1955), and 50% survival has been noted for barnacles exposed to –19°C for 18 h (Crisp et al. 1977). It is possible that the Pacific oyster can also be classified as freeze tolerant, but more studies are needed to evaluate this further and to determine what mechanisms are causing the high cold tolerance of the Pacific oyster.

In conclusion, the objective of this study was to improve the understanding of the cold tolerance of the Pacific oyster. The Pacific oyster was found to be very tolerant to low and varying temperatures, and the data indicate that the cold tolerance may be affected by temperature acclimatization and adjustment to winter conditions. More research is needed to understand more completely the relationship between survival of the species and exposure to low temperatures, and to evaluate the mechanism behind the high cold tolerance demonstrated. The results obtained may be of importance for evaluation of the species' continued dispersal in the Scandinavian region.

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LITERATURE CITED

Aarset, A. V. 1982. Freezing tolerance in intertidal invertebrates: a review. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 73:571–580.

Ansart, A. & P. Vernon. 2003. Cold hardiness in molluscs. *Acta Oceol.* 24:95–102.

Bourgier, S., P. Geairon, J. Desolus-Paoli, C. Bacher & G. Jonquières. 1995. Allometric relationships and effects of temperature on clearance and oxygen consumption rates of *Crassostrea gigas* (Thunberg). *Aquaculture* 134:143–154.

Büttger, H., G. Nehls & S. Witte. 2011. High mortality of Pacific oysters in a cold winter in the North-Frisian Wadden Sea. *Helgol. Mar. Res.* 65:525–532.

Crisp, D. J., J. Davenport & P. A. Gannott. 1977. Freezing tolerance in *Balanus balanoides*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 57:359–361.

Diederich, S. 2006. High survival and growth rates of introduced Pacific oysters may cause restrictions on habitat use by native mussels in the Wadden Sea. *J. Exp. Mar. Biol. Ecol.* 328:211–227.

Diederich, S., G. Nehls, J. van Beusekom & K. Reise. 2005. Introduced Pacific oysters (*Crassostrea gigas*) in the northern Wadden Sea: invasion accelerated by warm summers? *Helgol. Mar. Res.* 59:97–106.

Eklund, U., M. Håkansson & J. Haamer. 1977. En undersökning om förutsättningarna för ostronodling vid svenska västkusten. Report B83. Department of Geology, Chalmers University of Technology and University of Gothenburg. (in Swedish). Gothenburg, Sweden: Chambers University of Technology. 35 p.

Kanwisher, J. W. 1955. Freezing in intertidal animals. *Biol. Bull.* 109: 56–63.

Le Gall, J. & O. Raillard. 1988. Influence de la temperature sur la physiologie de l'huitre *Crassostrea gigas*. *Oceanis* 14:603–608.

Marchand, P. J. 1996. Life in the cold: an introduction to winter ecology. Hanover: University Press of New England. 304 pp.

Murphy, D. J. 1983. Freezing resistance in intertidal invertebrates. *Annu. Rev. Physiol.* 45:289–299.

Murphy, D. J. & S. K. Pierce. 1975. The physiological basis for changes in the freezing tolerance of intertidal molluscs. I. Response to subfreezing temperatures and the influence of salinity and temperature acclimation. *J. Exp. Zool.* 193:313–321.

Nyberg, C. 2010. Japanska jätteostronet (*Crassostrea gigas*) på västkusten 2009. Swedish EPA report. (in Swedish). 24 p.

Quayle, D. B. 1969. Pacific oyster culture in British Columbia. Fisheries Research Board of Canada, bulletin 169. Ottawa: The Queen's Printer. 192 pp.

Ramløv, H. 2000. Aspects of natural cold tolerance in ectothermic animals. *Hum. Reprod.* 15:26–46.

Storey, K. B. & J. Storey. 1988. Freeze tolerance in animals. *Physiol. Rev.* 68:27–84.

Wa Kang'eri, A. K. 2005. Winter mortality and freeze tolerance in Pacific oysters *Crassostrea gigas* (Thunberg). MS thesis, University of Groningen, Department of Marine Biology. 53 pp.

Walne, P. R. 1974. Culture of bivalve mollusca: 50 years experience at Conwy. Fishing news. London: Whitefriars Press. 189 pp.