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A neritic species in a hypersaline lagoon; population structure of *Acartia fancetti* in relation to hyperhaline and thermal stresses

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ABSTRACT

Phenotypic plasticity and adaptation are common traits of organisms living in highly dynamic and changing environments. Several copepod species, especially estuarine dwelling species, have demonstrated high phenotypic plasticity and adaptability to variations in environmental conditions. Copepods play a critical role in aquatic foodwebs and their population structure is closely linked to water quality fluctuations, especially salinity and temperature. However, few studies have examined the response of population structure to environmental stress. Here, we examine the population structure, egg production and hatching success of *Acartia fancetti* in relation to hypersalinity (30, 40, 50 and 60) and temperature (15 °C and 20 °C). *A. fancetti* is a neritic species occupying a key niche in the hypersaline areas of the Coorong, South Australia. Our results show a significant influence of salinity and temperature on the population size, nauplii production, copepodites production and adults of *A. fancetti*. The maximum numbers of nauplii, copepodites and adults were at salinity 30 and 40, at both 15 °C and 20 °C. However, substantial population sizes were also observed at salinity 50 and 60 in 15 °C treatments. At 20 °C, no individual was observed at salinity 60. This study indicates that the population of *A. fancetti* in the Coorong is tolerant to hypersalinity at lower temperatures, but the combined effects of haline and thermal stress significantly reduces the ability of this species to survive at higher temperatures. The distribution of *A. fancetti* in the Coorong is also affected by the environmental change related to anthropogenic activities.

1. Introduction

Calanoid copepods play a critical role in aquatic foodwebs as the prevailing mesozooplankton, especially in temperate and polar regions. The population and community response of copepods to changes in environmental conditions form a crucial link between the biogeochemical impacts of environmental conditions on primary production and the foodweb that follows (Banas et al., 2016). Several copepod species respond quickly to variations in environmental factors, especially salinity and temperature. For example, copepods have been show to regulate stress gene expression, alter the production of fatty acids, produce specific enzymes to osmoregulate, and change their metabolic rates (Kimmel and Bradley, 2001; Isla and Perissinotto, 2004; Lee et al., 2017). As such, salinity and temperature are significant factors affecting the reproduction, life cycle, population and community response of copepods (Devreker et al., 2009; Horne et al., 2016; Werbrouck et al., 2016).

Copepods are ectothermic organisms, therefore their recruitment and population size tend to shift following seasonal cycles, caused by the effects of temperature on their ontogeny (Horne et al., 2016). In particular, temperature has a direct influence on survival and developmental rates of copepods, whereby increases in temperature tend to increase survival and developmental rates (Milione and Zeng, 2008; Rhyne et al., 2009). Moreover, several studies have shown differences in egg sizes, egg hatching rates, nauplii survival and reproductive output in relation to temperature changes (Rhyne et al., 2009; Hansen et al., 2009; Escribano et al., 2014; Liu et al., 2015). However, as for metabolic rates, the positive influences of temperature on overall reproductive output, developmental rates and survival of copepods diminish after a certain threshold. Although copepod ontogeny and population size are affected by changes in temperature, several studies have also shown that plasticity and adaptation to the influences of temperature play an important role in acclimation and resilience (Hansen et al., 2009; Souissi et al., 2016).

Similar to temperature, salinity tolerances of copepods are dependent on their survival and ability to reproduce under various salinity levels. The major impact of salinity on copepods is the instigation of osmotic stress (Devreker et al., 2009). Under less, or more than optimum salinity, copepods endure an energy imbalance and there is the regulation of stress gene expression to cope with osmoregulation. This,

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therefore, affects other biological traits, such as egg production, developmental time, survival and recruitment (Lee et al., 2017). As a result, copepod population and community structures are subject to substantial variations in relation to fluctuations in salinity.

Physiological stress is a common effect of changed environmental conditions. However, many copepod species are able to show certain levels of adaptation and plasticity, especially between different populations of the same species. For example, Beyrend-Dur et al. (2009) have shown clear differentiation in clutch size and longevity between two transatlantic populations of *Eurytemora affinis* under similar salinity treatments. Moreover, Schoville et al. (2012) have shown dissimilarities in temperature tolerance of different populations of *Tigriopus californicus*, whereby possible evolutionary divergences in gene expression and regulatory pathways were observed. Such differentiation in response shows the adaptability of copepods to changing habitats, and points towards the importance of understanding population-specific variations in lifecycle and population structure in relation to changes in environmental conditions. However, few studies have examined the response of overall population structure in relation to environmental stressors.

In this study, the aim was to identify the reproductive response and variations in population structure of *Acartia fancetti* when exposed to salinity and temperature fluctuations. *A. fancetti* is a neritic calanoid copepod. *A. fancetti* was first observed and described in Westernport and Port Phillip Bays, South Eastern Australia (McKinnon et al., 1992). It is an allopatric sibling species of the *Acartia* genus. This species has been observed in typical marine waters of South Eastern Australia (McKinnon et al., 1992). However, only recently, this species has also been encountered in the hypersaline regions of the Coorong (South Australia), a coastal lagoon undergoing health restoration from past degradation. The species has been observed at nauplius, copepodite and adult stages in salinities ranging from 40 to 65 at different abundances (Hemraj et al., 2017a), which are much higher than the salinities in Port Phillip Bay (Lee et al., 2012). The Coorong is a 110 km long, shallow (1–4 m deep) inverse estuary located at the mouth of the Murray River (Australia's longest river) and listed in the RAMSAR convention for wetlands due to its ecological importance. It has a high salinity gradient, temporarily and spatially varying from salinity 1 to 90 (Hemraj et al., 2017a). Freshwater input in this system is anthropogenically controlled by barrages and released into the system in bursts, usually during colder months when freshwater volumes in the Murray River are higher (Hemraj et al., 2017b). This creates swift changes in the water quality of the Coorong throughout the year, especially salinity that usually decreases rapidly with freshwater release, but increases promptly during warmer months when freshwater release has ceased and the system is subjected to high evaporation rates. In turn, this creates significant changes in the plankton communities in the system (Hemraj et al., 2017b). Here, we look more specifically at the egg production and population dynamics of *A. fancetti* under a variety of hypersaline and temperature manipulations to gain a better understanding of the effects of seasonal and anthropogenic derived changes on the population structure. Salinities lower than marine were not used as a treatment as this species has not previously been observed in these salinity levels. We hypothesize that the population structure and size of the species will be significantly affected and reduced at higher temperatures and salinities, which are representative of harsher conditions in the Coorong. Such information provides insight into the possible explanations for a change in copepod population in the Coorong, which is highly important for managing water release and the health of the system. This study also provides a comparative base for understanding variations in the reproductive response between the Coorong and Port Phillip copepod populations. Moreover, this study provides information for understanding the temporal changes in copepod populations in other hypersaline estuaries, such as the Sine Saloum, Senegal.

2. Materials and methods

2.1. Copepods and microalgae

For this study, *A. fancetti* nauplii, copepodite and adults were collected from the Coorong at Parnka Point (35.9017° S, 139.3961° E, South Australia) in November 2016. The water salinity and pH at time of sampling were 52.45 and 8.33, respectively. Quantitative copepod samples were taken using a plankton net (35 μm meshed). *A. fancetti* has been observed in high abundance at Parnka Point at salinity 40 to 60 (Hemraj et al., 2017a). Copepods were cultured in 10 L Nalgene bottles (Thermo Fisher Scientific, Australia). Cultures were conducted in a culture room at 20 °C, with a photoperiod of 12:12 h light and dark cycle. After 5 days, cultures were mixed with COMBO medium (Kilham et al., 1998) to achieve a ratio of 1:3 (COMBO: Coorong water). This was repeated every five days, each time reducing the amount of Coorong water (1:1 and 3:1), until copepods could be reared in 100% COMBO medium. The COMBO medium was used as it is a good culture medium, supporting robust growth and reproduction for both zooplankton and algae, and, especially, due to the difficulty of obtaining freshly filtered Coorong water at salinity 50. Preparation of COMBO medium at salinity 50 was done by mixing 10 μm-filtered seawater (salinity ~37) with sodium chloride (Chem Supply Pty Ltd) to achieve the desired salinity. A 25% change of the culture volume was performed every three days to provide fresh conditions. Copepods were fed with 100 mL of *Isochrysis galbana* at stationary growth stage (maximum cell density; 176 ± 49 × 10^5 cells/mL), cultured in f/2 medium at salinity 37.

2.2. Experimental conditions and procedure

Experimental conditions included four different salinities (30, 40, 50 and 60; measured with a Metler Toledo probe; Table 1) and two temperatures (15 °C and 20 °C), representing temporal variations in environmental conditions at Parnka Point, where individuals of *A. fancetti* were collected in the Coorong. Prior to experimentation, male and female copepods were separated from the stock cultures and acclimated for three days at different conditions. Thirty males and 30 females were acclimated at each of the eight different conditions, summing to a total of 480 individuals. Copepods subjected to salinity 30 were previously placed in salinity 40 for three days and then transferred to salinity 30 for three days to reduce sudden high osmotic shock. For all other treatments, copepods were directly transferred from the stock culture and acclimated for three days. During acclimation, no mortality or disparities in swimming and feeding behaviour were observed. To examine the population structure under each condition, 6 males and 6 females were placed in 2 L of COMBO medium of the relevant salinity. This density was chosen to match the general population density of adults in the Coorong when there are no peaks in abundance. Moreover, a lower density was used to allow for significant population growth in treatments that provided adequate conditions, and avoid

Table 1
Measured salinity and pH values of prepared COMBO for experimental procedure.

	Actual salinity	pH
Treatment 15 °C		
30	30.83 ± 0.09	8.29 ± 0.06
40	40.85 ± 0.36	8.31 ± 0.05
50	52.84 ± 2.73	8.37 ± 0.09
60	60.01 ± 0.33	8.25 ± 0.02
Treatment 20 °C		
30	30.73 ± 0.05	8.38 ± 0.11
40	41.60 ± 0.4	8.28 ± 0.04
50	52.45 ± 0.2	8.31 ± 0.03
60	62.25 ± 1.11	8.25 ± 0.07