Journal of Experimental Marine Biology and Ecology 458 (2014) 39–45

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Impact of short-term salinity stress on larval development of the marine gastropod *Crepipatella fecunda* (Calyptraeidae)

J.A. Montory [a,c,*], O.R. Chaparro [a], J.A. Pechenik [b], C.M. Diederich [b], V.M. Cubillos [a]

[a] Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile
[b] Biology Department, Tufts University, Medford, MA 02155, USA
[c] Programa de Doctorado en Ciencias Mención Ecología y Evolución, Universidad Austral de Chile, Valdivia, Chile

ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form 10 April 2014
Accepted 5 May 2014
Available online 24 May 2014

Keywords:
Clearance rate
Larvae
Larval growth rate
Oxygen consumption rate
Salinity stress

ABSTRACT

Shallow-water coastal environments suffer frequent reductions in salinity due to heavy rains. This creates stressful conditions for the organisms found there, particularly for the early stages of development, including pelagic larvae. Freshly hatched larvae of the gastropod *Crepipatella fecunda* were exposed to different levels of salinity stress (32 (control), 25, 20 and 15) for a single 6 h period. Subsequently, all veligers were maintained at normal control salinity (32) through metamorphosis. Periodic measurements were made of mortality, larval growth, and larval behavior. In particular, we measured changes in velar surface area, swimming velocity, clearance rate, oxygen consumption rate, shell growth rate, larval mortality, time to metamorphosis, and size at metamorphosis. The short exposure to salinity stress decreased subsequent mean growth rates at normal salinity and mean size at metamorphosis, but increased the duration of the planktonic period and the extent of larval mortality. It also reduced the rate at which the velar surface area increased relative to shell growth, and reduced mean larval swimming velocity. Mean oxygen consumption rates and clearance rates were also significantly lower for larvae that had been stressed early in larval life, compared with values obtained for control individuals. Exposure to low salinity for even a short time early in larval life can clearly have a substantial impact on the rest of larval development.

1. Introduction

Organisms living in intertidal or shallow water areas are periodically exposed to fluctuations in temperature, dissolved oxygen concentration, salinity, and food availability (Allen et al., 2012; Amado et al., 2011; Dahlhoff et al., 2002; Diederich and Pechenik, 2013; Diederich et al., 2011; Lohrer et al., 2000; Lowell, 1984; Richmond and Woodin, 1996; Sampath-Wiley et al., 2008; Underwood, 1979), among other stressors.

Salinity reductions can have particularly strong effects on distribution, behavior, and survival in such environments (Berger and Kharazova, 1997; Bodinier et al., 2009; Kinne, 1971; Sameoto and Metaxas, 2008; Spicer and Strömberg, 2003; Torres et al., 2006; Yen and Bart, 2008), due to the effects of changes in solute concentrations on the efficiency of metabolic processes (Kinne, 1966). Such salinity declines are common in shallow coastal waters, estuarine areas, tidepools, and areas of river or glacial input, or where heavy local rains during substantial low tides can strongly reduce ambient salinity, sometimes to almost freshwater conditions (Stickle and Denoux, 1976; Toro and Winter, 1983). In extreme situations, prolonged exposure to low salinities may kill many individuals (Génio et al., 2008). However, shorter duration exposures may lead to situations of physiological stress (Aarset and Aunaas, 1990; Sameoto and Metaxas, 2008; Tedengren et al., 1988) that have negative but sublethal impacts (Pechenik, 1983; Roller and Stickle, 1985, 1989, 1993). Sublethal effects caused by low salinities can be especially pronounced in free-living organisms, particularly during the early stages of development (e.g. pelagic larvae, Bas and Spivak, 2000). Larvae may find themselves in tide pools (Moulin et al., 2011) or in other shallow waters during periods of heavy rain, where they are potentially exposed to short-term but extreme hyposaline events. Such salinity stress can prolong larval swimming times and delay the process of metamorphosis (Diederich et al., 2011; Giménez, 2002; Zimmerman and Pechenik, 1991), potentially leading to increased larval mortality (Giménez, 2002). Moreover, sublethal stresses experienced in the early stages of development often lead to later effects on individuals (Pechenik, 2006), such as decreased growth rates, food clearance rates, and oxygen consumption rates (e.g. *Balanus amphitrite*, Pechenik et al., 1993; *Hydroides elegans*, Qian and Pechenik, 1998; *Haliotis iris*, Roberts and Lapworth, 2001; *Crepidula fornicata*, Pechenik et al., 2002; *C. onyx, C. fornicata, C. fecunda*, Diederich et al., 2011), either later in larval development or even in more advanced stages after metamorphosis (Diederich et al., 2011; Marshall et al., 2003; Pechenik, 2006; Pechenik

* Corresponding author at: Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile.
E-mail address: jaimemontory@gmail.com (J.A. Montory).

http://dx.doi.org/10.1016/j.jembe.2014.05.004
0022-0981/

et al., 1993; Pechenik et al., 2002; Qian and Pechenik, 1998; Takami et al., 2002; Wendt, 1996; Wendt and Johnson, 2006).

The gastropod *Crepipatella fecunda* (Gallardo, 1977, 1979) is especially appropriate for studies on salinity impacts. The species is common in intertidal and shallow subtidal areas along the coast of southern Chile, where salinities can drop after heavy rains during the breeding season. Females incubate their egg masses within the mantle cavity beneath the shell for about 4 weeks before releasing feeding veliger larvae into the plankton (Chaparro et al., 2005; Mardones et al., 2013). Although females may retain the veligers within the mantle cavity for a time before releasing them into the plankton, in this paper we will refer to release into the plankton as "hatching". Veliger larvae emerge from the female at approximately 350 μm shell length (Gallardo, 1979) and continue to feed and grow in the plankton for about another 15 days before settlement and metamorphosis (Chaparro et al., 2005). During those several weeks, planktonic veligers of *C. fecunda* may be exposed to decreases in environmental salinity. Although the impact of prolonged larval exposures (12–48 h) to reduced salinities on larval survival, larval growth, and juvenile survival and growth has been reported for this species (Diederich et al., 2011), the possibility that even short-term exposures to sublethal low salinities can affect subsequent larval performance has not been previously considered, nor were any physiological effects considered. In this investigation we studied the after-effects of temporary low-salinity stress on larval survival, physiology, and swimming behavior after the veliger larvae were permitted to continue their development at normal salinity levels. Because Klinzing and Pechenik (2000) showed that velar lobe size varies with food level and diet for larvae of the related species *Crepidula fornicata*, we also tested to see if velar lobe size in *C. fecunda* was affected by short-term exposure to water of reduced salinity.

2. Material and methods

Adult specimens of the gastropod *C. fecunda* (35–45 mm shell length) were collected from Pelluco beach, Puerto Montt (41°28′S; 72°56′W) between September and December 2012 and transferred to the laboratory. The individuals were kept in aquaria with circulating unfiltered seawater (salinity of 32) at 14 °C with constant aeration. Feeding was supplemented with microalgae cultures of *Isochrysis galbana* until females released veliger larvae into the surrounding seawater. Those larvae were then used in the experiments described below.

2.1. Later effects of stress: mortality, duration of pelagic life, and size at metamorphosis

Aquaria (200 mL capacity) were filled with filtered seawater (0.5 μm) at 4 different levels of salinity: 32 (control), 25, 20 and 15. Reduced salinities were obtained by adding distilled water to filtered seawater. We included 4 replicate aquaria for each salinity level, with 200 newly hatched veligers (350 ± 40 um shell length) per replicate. Newly-hatched veligers were maintained at each salinity for 6 h. The veligers from each salinity treatment were then transferred to separate aquaria with filtered seawater (0.5 μm) at a salinity of 32. Seawater was changed daily, any dead larvae were removed, and remaining larvae were fed with microalgae (*I. galbana*, 50,000 cells mL^{-1}). Once metamorphosis occurred, the number of settled juveniles was quantified; this information was used to estimate the larval mortality rate during the study, by comparing the initial number of individuals in each aquarium with the number of settled and metamorphosed individuals. For each treatment, the duration of pelagic life was estimated from the time elapsed between the day of hatching and the day when at least 50% of the surviving individuals had metamorphosed in each aquarium. Metamorphosis was identified through loss of the larval swimming organ, the velum (Pechenik, 1984).

Newly hatched larvae and metamorphosed juveniles were both photographed using an inverted microscope Olympus BX41 at 40× magnification. A calibrated slide was also photographed and used to determine actual larval sizes. The images were later processed using an image analysis program (Scion image pro) that allowed us to estimate the respective sizes.

2.2. Later effects of stress on velar surface area and larval swimming speeds

Veligers of *C. fecunda* were stressed as described above and then maintained at normal salinity (32). They were examined 5 and 11 days after the low-salinity stress treatment ended. From each experimental aquarium, 6 veliger larvae were transferred to a small glass chamber with filtered seawater (0.5 μm, salinity of 32). Larval swimming was videotaped at 35× magnification using a magnifier- equipped Leica EZ4 with a video camera. The film obtained allowed us to determine the distance traveled by the veligers and the time it took to travel that distance. With this information, we were able to estimate the displacement velocity (mm s^{-1}) of each individual. The distance was measured in a straight line, but in short distances which reduced the problem of any non-linear movements made by the veligers. Measurements were made using a reference slide that was also videotaped at the same magnification.

Larvae were videotaped at 100× magnification using an inverted microscope. Still pictures were obtained from the videos at times when the larvae had the velum fully extended and frontally exposed to the observer. Using standard image processing software (Scion image pro), the velar lobe surface area (mm^2) was then determined for each of the veligers, by tracing the border of the extended velum (Chaparro et al., 2002b; Cubillos et al., 2007). An index that relates the length of the shell (an indicator of larval biomass) and the surface area of the velum (which moves the larval biomass through the water) was also estimated for each individual, again using still frames from the videos. This "propulsion index" was calculated for larvae examined 5 and 11 days after they experienced the 6 h low-salinity stress.

2.3. Later effects of stress on larval physiology and growth rates

2.3.1. Oxygen consumption rate (OCR)

Twenty-four aquaria with seawater of different salinities (450 mL per aquarium) were prepared by mixing filtered seawater (0.5 μm) with appropriate amounts of distilled water. The final salinities in aquaria were 32 (control), 25, 20 and 15, with 6 replicates for each salinity. Newly-hatched veligers of *C. fecunda* (350 ± 40 μm shell length, $N = 500$) were added to each aquarium and maintained under the conditions described for 6 h. The veligers from each treatment were then removed to new aquaria containing filtered seawater (0.5 μm) at a salinity of 32. Thus larvae were stressed for only 6 h, and then reared under control conditions for the rest of the study, as before. Larvae were fed daily with pure cultures of the microalgae *I. galbana* at 50,000 cells mL^{-1}. After 5 days, 50 veliger larvae were collected at random from each aquarium and placed in 10 mL syringes, with each syringe containing larvae from a single treatment and also from a single original aquarium. Each syringe contained 3 mL of filtered seawater (salinity of 32, 0.5 μm filtered), which was previously saturated with oxygen by continuous bubbling. The syringes used were maintained in a thermostated water bath at 14 °C. A Microx TX oxygen sensor was introduced through the tip of each syringe; oxygen concentration was measured at time 0 and after 2–4 h, to subsequently calculate mean rates of oxygen consumption per individual for each syringe. We repeated these measurements at 11 days post hatching, using the same procedure.

2.3.2. Clearance rate (CR)

CR measurements were made at a salinity of 32, 5 and 11 days after the 6 h salinity treatments. Into each of 24 syringes (6 syringes per treatment salinity), we added 5 veligers of *C. fecunda*. Each syringe was filled