Journal of Experimental Marine Biology and Ecology 475 (2016) 108–113

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Effects of salinity on the reproductive performance of *Apocyclops royi* (Copepoda, Cyclopoida)

Yen-Ju Pan [a,b], Anissa Souissi [b], Sami Souissi [b,*], Jiang-Shiou Hwang [a,*]

[a] Institute of Marine Biology, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 202, Taiwan
[b] Univ. Lille, CNRS, Univ. Littoral Cote d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Geosciences, 62930 Wimereux, France

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form 18 November 2015
Accepted 19 November 2015
Available online 6 December 2015

Keywords:
Apocyclops royi
Salinity
Population production
Clutch production
Nauplii production

ABSTRACT

The cyclopoid copepod *Apocyclops royi* is a dominant species in inland saline ponds and estuarine areas in southern Taiwan. Although it is an ideal live prey candidate for larval fish feed because of its short life cycle, small body size, and easy maintenance, the ecology of the species is poorly documented. In this study, we conducted a series of individual and population experiments to investigate the effects of salinity on the reproductive performance of *A. royi*. In the population experiments, 8 ovigerous females were cultivated in an 800-mL culture medium at designated salinities (0, 5, 10, 15, 20, 25, 30, and 35) for 14 days, after which the population growth, composition of different developmental stages, and clutch size of adult females were quantified. Subsequently, individual experiments were conducted to test the salinity effects (0, 10, 20, and 30) on the nauplii and clutch production of 12 pairs of copepods over 14 days. The results showed that salinity significantly affected the reproduction of *A. royi*, and that 20 was the optimal salinity for attaining their highest productivity. In the population experiments, significantly lower population growth rates were obtained at the extreme salinity treatments (0 and 5, and 30 and 35). However, a reduced clutch size was revealed only at salinities 0 and 5. In the individual experiments, a significantly lower nauplii and clutch production was found at salinities 0 and 30. Salinity had varying influences on *A. royi* across its different developmental stages. Our findings revealed the reproductive patterns of *A. royi* under different salinity conditions and indicated how salinity may affect *A. royi* in their natural habitat. The results offer a crucial recommendation on the manipulated processes for larval feeding in marine and brackish aquaculture hatcheries.

1. Introduction

Copepods are major members of zooplankton, and most copepod species form important trophic linkages between primary producers and higher consumers in marine ecosystems (Lee et al., 2010; Støttrup, 2000; Turner, 2004). Copepod biomass and species composition vary significantly with the fluctuation of environmental parameters, including seasonality and climatic changes (Hsieh et al., 2005; Renz and Hirche, 2006; Speirs et al., 2006; Sullivan et al., 2007; Sun et al., 2013; Thomas and Nielsen, 1994). In particular, salinity strongly influences the ecological and biological responses of copepods. Salinity changes may place physiological stresses on copepods, and can ultimately result in copepod mortality, unsustainable population growth, and functional shifts in marine food webs (Kaartvedt and Aksnes, 1992; Soetaert and Herman, 1994). Nevertheless, euryhaline copepods have the capacity to regulate osmosis stress and survive under a wide range of salinity conditions (Schmidt-Nielsen, 1997).

Considering the different energy allocations required for physiological needs, copepods may change their biological processes depending on the salinity. A number of experimental studies have reported that the fluctuations of salinity can markedly affect euryhaline copepods. In studies on the tropical estuarine copepod *Pseudodiaptomus annandalei*, salinity treatments showed significant influences on reproduction, lifespan, and survival rate (Beyrend-Dur et al., 2009; Chen et al., 2006). A dominant estuarine copepod *Eurytemora affinis* in Europe and North America, has been reported to present different physiological responses depending on salinity, including naupliar survival and development (Devreker et al., 2004), fecundity (Devreker et al., 2009), enzymatic expression (Cailleaud et al., 2007), life cycle (Beyrend-Dur et al., 2009), and swimming behavior (Michalec et al., 2010). This remarkable salinity effect has also been revealed in studies on the different species of the genus *Acartia* worldwide. Baltic *A. tonsa* showed a variable egg hatching rate and egg production under different salinities (Holste and Peck, 2006; Peck and Holste, 2006). Chinnery and Williams (2004) reported that salinity treatments altered the egg hatching rate and naupliar survival of 4 temperate *Acartia* species collected from Southampton Water, UK. In a report from Australia, different population growth and egg hatching rates were found for the tropical copepod *Acartia sinjiensis* cultured under different salinities (Milione and Zeng, 2008).

* Corresponding authors.
E-mail addresses: sami.souissi@univ-lille1.fr (S. Souissi), jshwang@mail.ntou.edu.tw (J.-S. Hwang).

http://dx.doi.org/10.1016/j.jembe.2015.11.011
0022-0981/

The majority of these studies focused on calanoid copepods, and little is known about other copepod orders such as Harpacticoida and Cyclopoida. The copepod species *Apocyclops royi* belongs to the Cyclopoida order and can be found in estuaries and brackish aquaculture ponds in subtropical and tropical regions, and this species is used as a live food for fish larviculture (Su et al., 2005). The effect of salinity, a key fluctuating environmental factor in natural habitats, on the reproductive parameters of *A. royi* would contribute to a deeper understanding of the biology and ecology of this species. According to our preliminary observations, *A. royi* is relatively robust and can tolerate a wide range of salinities. However, quantified data on its productivity and individual reproductive pattern under different salinities have not yet been produced. Therefore, our study conducted 2 independent experiments investigating salinity effects on the population growth and individual reproductive performances of *A. royi*.

2. Material and methods

2.1. Copepod and microalgae stock culture

The copepod *A. royi* was obtained from the Tungkang Biotechnology Research Center (Tungkang, Taiwan). A starter culture of the marine microalgae *I. galbana* (Haptophyceae) was obtained from Roscoff Culture Collection (Roscoff, France). Stable cultures were established in the LOG-Marine Station of Wimereux, France for the experiments. Copepods were cultivated in 20-L polycarbonate carboys with salinity-adjusted seawater at salinity 20 (made from a mixture of distilled water and Whatman GF/F-filtered natural seawater). The cultures were placed in a photoperiodic room (12:12 h light–dark cycle), and thermostatic heaters (EHEIM thermocontrol 50 W, EHEIM GmbH, Germany) were used to maintain the water temperature at 28 °C.

Batch cultures of *Isochrysis galbana* were performed in 2-L flasks containing Whatman GF/C-filtered and autoclaved natural seawater supplied with a Walne medium (Walne, 1970). The algal culture used for copepod feeding was in its exponential growth phase (3–4 days after inoculation). The algae was introduced every 2 days at an approximate cell concentration of 80,000 cells/mL into the copepod culture water; this food concentration was determined to be sufficient because of the existence of live algal cells before each inoculation. The copepod culture water was completely exchanged every 7 days.

2.2. Precultivation and salinity acclimation

A precultivation was conducted to standardize the copepods used in our experiments and to ensure that they were of similar age. Nauplii were isolated from the stock culture tank and transferred to a new culture tank under the same conditions. The culture was checked every day, and copepods were collected one day after the first appearance of ovigerous females. To avoid the mortality caused by acute salinity shock, we conducted a salinity acclimation 1 h prior to every trial: approximately 300 adults were collected from the precultivation tank and placed in a 500-mL beaker with 50 mL of the original culture water at salinity 20. Filtered natural seawater (salinity 35) or distilled water (salinity 0) was gradually added into the beaker until the salinity approached (±1) the designed salinities (0, 5, 10, 15, 20, 25, 30, and 35). After salinity acclimation, live individuals were randomly selected for population and individual experiments.

2.3. Population experiments: population growth and clutch size

Eight ovigerous females were randomly collected from acclimation beakers and transferred to 1-L beakers with 800 mL of water at the designed salinities (0, 5, 10, 15, 20, 25, 30, and 35). The cultures were kept in a thermostatic incubator (MLR-351H, SANYO, Osaka, Japan) programmed to 28 °C with a 12:12 h light–dark cycle. To maintain stable salinities, the algal diets were centrifuged (4000 rpm for 1 min) to remove the original culture medium. Water at different salinities was added to suspend the algal cells, and the prepared algal diet was then introduced to the experimental cultures at the approximate density of 80,000 cells/mL. Four replicates were examined for each salinity treatment. All experiments lasted 14 days; copepods were then collected and fixed in 4% buffered formalin for subsequent counting and analysis. The number of copepods at different developmental stages (nauplii, copepodites, males, females, and ovigerous females) was counted using a stereomicroscope (SZX9, OLYMPUS, Tokyo, Japan). After the counting process, 20 to 30 ovigerous females carrying complete egg sacs were randomly sorted from the replicates. These ovigerous females were placed on a petri dish, and the egg sacs were carefully dissected to examine the clutch size (eggs per clutch).

2.4. Individual experiments: nauplii and clutch production

Twelve pairs of adult *A. royi* were sorted from the acclimation beakers and cultivated on 6-well culture plates at designed salinities (0, 10, 20, and 30). The cultures were placed in the thermostatic incubator programmed to the same conditions as those in the population experiments. Nauplii and clutch production were recorded from daily stereomicroscopic observations of the culture wells for 14 days. After each observation, the pairs of copepods were transferred to new culture wells containing fresh culture water and algal diets.

2.5. Data analysis

Data analyses for this study were conducted using the SPSS program (Version 17.0), with a significance level set at $p = 0.05$. Salinity effects in the population and individual experiments were analyzed using one-way ANOVA test to compare the mean values of the productive traits. Because significant differences were detected for all treatments ($p < 0.05$), a Tukey multiple comparison test was used to analyze specific differences between pairs of treatments.

3. Results

3.1. Population experiments: population growth and clutch size

The effects of salinity on the population growth and composition of *A. royi* are shown in Figs. 1 and 2, respectively. The significantly ($p < 0.05$) largest final population was found at salinity 20 (1917.25 ± 316.5 individuals), and the smallest populations were found in both low- and high-salinity treatments (salinity 0: 67.25 ± 12.8, salinity 5: 229.25 ± 58.6, salinity 30: 237.25 ± 23, and salinity 35: 85.25 ± 38 individuals). The salinity significantly affected the

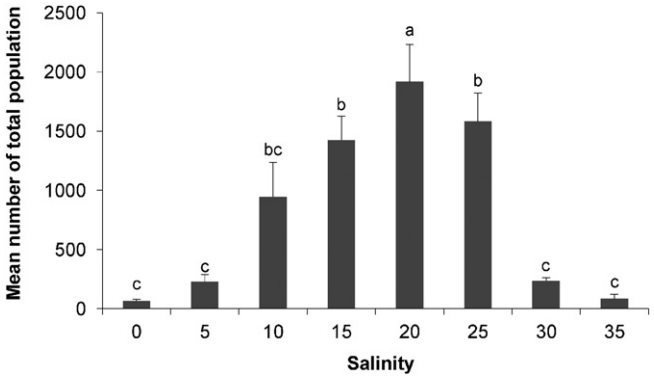

Fig. 1. Mean total number of the final population after a 14-d cultivation in various salinity treatments. Data were averaged from 4 replicates and presented as the mean ± standard deviation. The different letters (a, b, c) above each bar indicate the significant differences identified by Tukey multiple comparison test ($p < 0.05$).