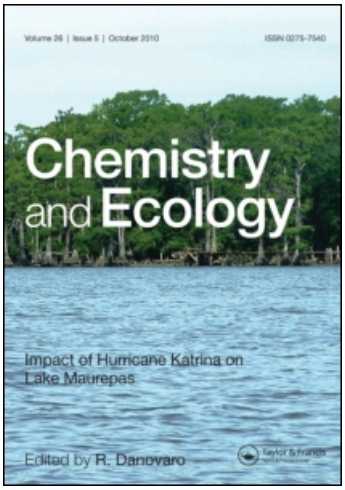

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Food type effects on reproduction of hyperbenthic calanoid species *Pseudocyclops xiphophorus* Wells, 1967, under laboratory conditions

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Food type effects on reproduction of hyperbenthic calanoid species *Pseudocyclops xiphophorus* Wells, 1967, under laboratory conditions

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Egg production, hatching success and naupliar survival were determined for *Pseudocyclops xiphophorus* pairs fed a *Skeletonema marinoi,* a flagellate (*Tetraselmis suecica*, *Pavlova lutheri* and *Isochrysis galbana*) and a mixed (*Skeletonema* and flagellates) diet. Pairs fed *Skeletonema marinoi* showed significantly lower egg production and naupliar survival than couples fed flagellates and mixed diet. But hatching success was more or less similar with the three food types. Furthermore, feeding experiments demonstrated a clear preference of *P. xiphophorus* for the flagellate food type.

Keywords: egg production; food type; hyperbenthic calanoid species; laboratory conditions

1. Introduction

Food quality has been shown to strongly affect copepod egg production, embryonic and post-embryonic development and hatching success, with some diets that are poorer than others in inducing maximum fecundity. According to Kleppel et al. [1], an increase in production is dependent on diet type so that diatoms < dinoflagellates < ciliate + dinoflagellates. The inadequacy of some diets to supply all nutritional components, such as fatty acids, amino acids and carbohydrates affects various copepod rate processes (egg production, hatching success, naupliar and copepodid development). In recent years, there has been increased scientific interest concerning the paradox of diatom-copepod interactions [2]. Traditionally, diatoms have been considered at the base of the marine food web and their blooms to initiate and support the increase of copepod populations in marine ecosystems. However, more recent evidence [3,4] has raised serious doubts that diatoms are good and harmless food items for the reproduction and development of their planktonic predators, in particular the herbivorous copepods. In fact, some studies [4,5] report that damaged cells of diatoms produce insidious compounds (α, β, γ, δ unsaturated aldehydes) with anti-cell growth activity in that they block the development of copepod embryos. Pohnert [6] showed that when diatoms are grazed by copepods there is a rapid onset of aldehyde production

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seconds after cell disruption, similar to the wound reaction in higher plants [7]. Copepod species, such as *Calanus helgolandicus*, *Temora stylifera*, and *Acartia clausi*, fed diatom and flagellate diets [3,8,9], show different effects on egg production reflecting species specific nutritional responses associated with size, taste and food assimilation rates. These studies refer only to the most common planktonic copepod species occurring in north temperate to polar regions [10]. The aim of the present paper is to show some preliminary data on the food responses of *Pseudocyclops xiphophorus*. *P. xiphophorus* belongs to a particular bentho-planktonic calanoid copepod group, including species that live at the interface between the bottom and the overlying water column. To date, studies on egg production of this species [11] revealed lower egg production rates in the field and laboratory at different temperatures than more common planktonic calanoid copepod species.

2. Materials and methods

P. xiphophorus was recently reported for the first time, in the Mediterranean Sea, in the brackish water Lake Faro (North-eastern Sicily) [12]. Before this finding, this species was discovered by Wells [13], along the Inhaca Island coasts, in the Indian Ocean. In Lake Faro, *P. xiphophorus* was found attached to submerged ropes and mooring-posts as a fouling organism. The 'fouling' samples were collected manually, about every 15 days, from November 2003 to September 2004. They were poured into a plastic container and brought to the laboratory. Specimens of *P. xiphophorus* were counted and sorted from 'fouling' washing-water by a stereoscopic microscope and transferred to an aquarium with 100 ml of filtered sea water. The aquarium was placed in a thermostatic-cell at 18°C.

In the aquarium, specimens of *P. xiphophorus* were reared with a mixed diet constituted by three phytoplankton species: *Tetraselmis suecica*, *Pavlova lutheri* and *Isochrysis galbana* (diameter ranged from 3–15 mm).Three groups of five replicates were prepared with male and female couples placed individually into 50 ml crystallising dishes containing sterile sea water and one of three different food items: *Skeletonemamarinoi* diet (SKE); *Tetraselmis suecica* and *Isochrysis galbana* diet (FLA); and a mixed diet with all previous food items (MIX). Egg production and egg viability were monitored for couples incubated at 24°C for about 20 days.

Furthermore, in order to estimate food preferences of *P. xiphophorus* feeding experiments were carried out. Two crystallising dishes were prepared with 65 ml of filtered seawater and a known concentration of cell food suspension, aiming at a final concentration of approximately 10^3 cell ml^{-1}. Approximately 18–25 copepods were added to these dishes. A control dish with no copepods was treated in the same manner as the experimental dish, and an initial sample of suspension was preserved at the beginning of each experiment. All these dishes were incubated at 24°C in dark conditions for 24 h. After removal of copepods from the dishes, these suspensions were preserved with Lugol's solution, and phytoplankton cell numbers were counted using the Utermöhl method (1958). Ingestion rates were determined from differences in phytoplankton cell concentrations in initial, control and duplicate experimental dishes using the formula of Frost (1972).

3. Results

In the laboratory, *P. xiphophorus* daily mean egg production was significantly different with the three tested diets (1-way ANOVA $F = 45, 618$; $p < 0.01$) (Figure 1). Of the three diets tested, the flagellate FLA was the best in terms of fecundity, hatching success and post-embryonic development. When *P. xiphophorus* were fed on MIX and FLA, daily egg production rates

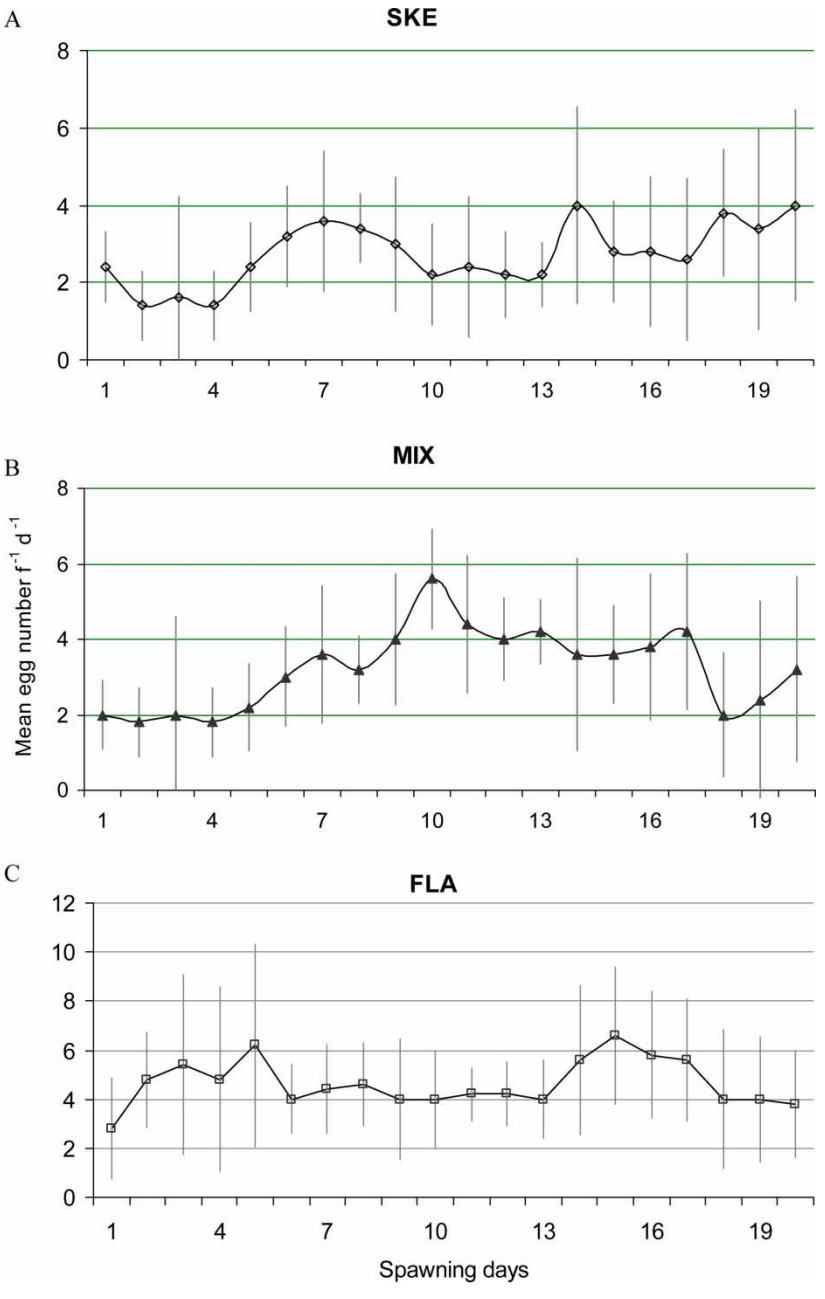

Figure 1. *Pseudocyclops xiphophorus*. Effects of three food types: (A) *Skeletonema costatum* (SKE); (B) mixed diet *Tetraselmis suecica*, *Isochrysis galbana Pavlova lutheri* and *Skeletonema costatum* (MIX); (C) flagellates diet *Tetraselmis suecica*, *Isochrysis galbana* and *Pavlova lutheri* (FLA) on daily rates of egg production.

were higher and values were not significantly different, 3.23 ± 0.6 egg female^{-1} day^{-1} and 4.64 ± 1.7 egg female^{-1} day^{-1}, respectively (1-way ANOVA $F = 4, 82$; $p > 0.05$), compared to SKE (2.74 ± 0.9 egg female^{-1} day^{-1}).

Daily egg production for females fed on SKE was significantly lower than with FLA ($F = 59, 73$; $p < 0, 01$). Hatching success (Figure 2) was more or less similar with FLA and MIX

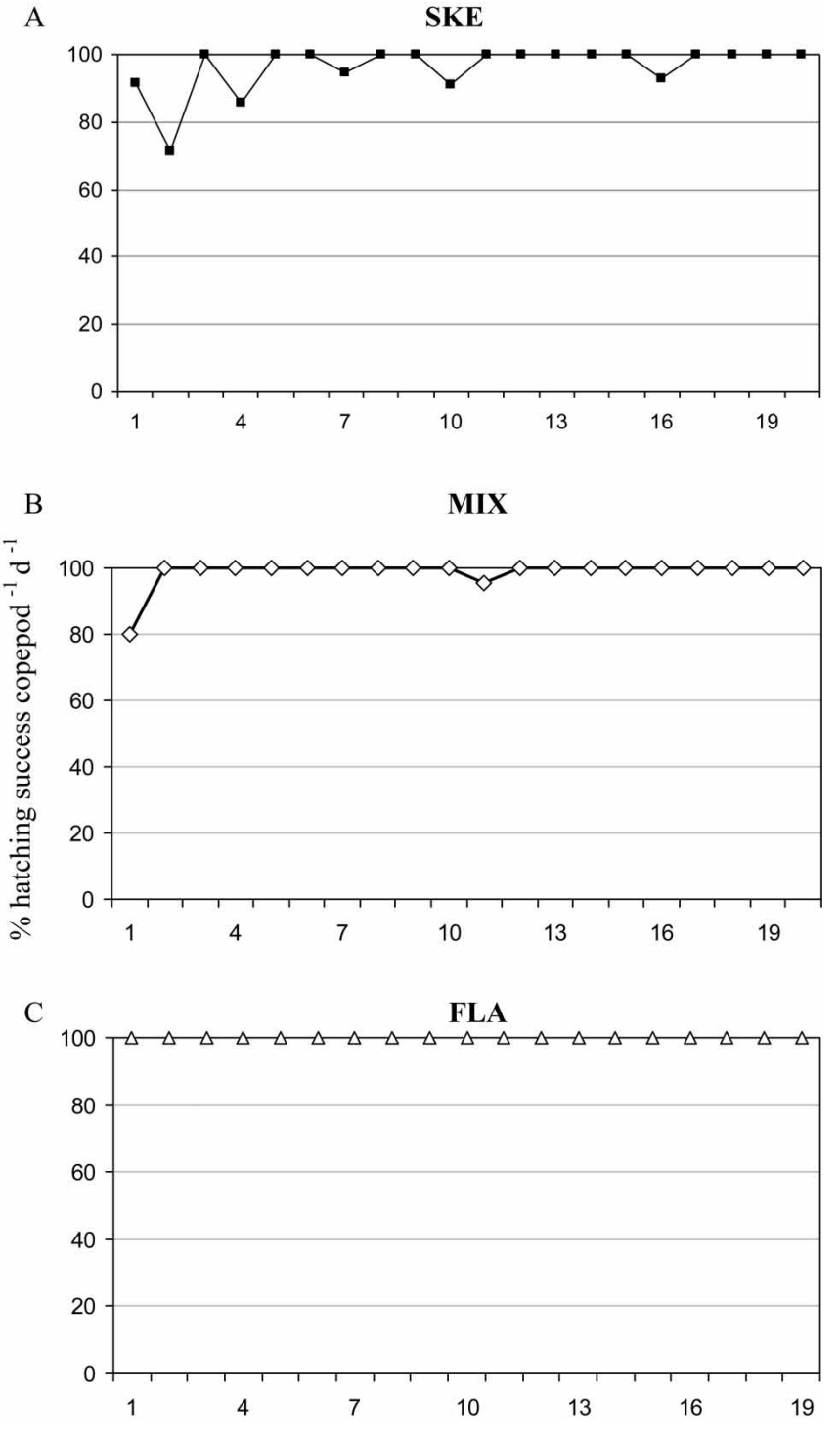

Figure 2. *Pseudocyclops xiphophorus*. Effects of three food types: (A) *Skeletonema costatum* (SKE); (B) mixed diet *Tetraselmis suecica*, *Isochrysis galbana Pavlova lutheri* and *Skeletonema costatum* (MIX); (C) flagellates diet *Tetraselmis suecica*, *Isochrysis galbana* and *Pavlova lutheri* (FLA) on daily percent of hatching success.

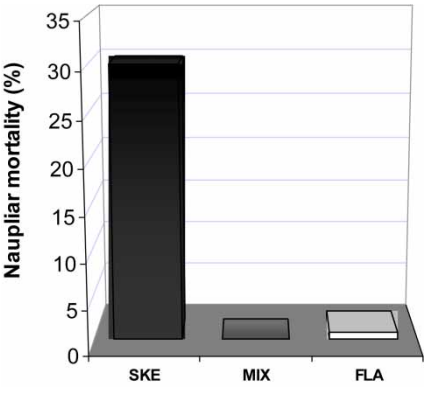

Figure 3. *Pseudocyclops xiphophorus*. Percent of naupliar mortality of the early stages in copepods fed *Skeletonema costatum* (SKE), mixed diet *Tetraselmis suecica*, *Isochrysis galbana Pavlova lutheri* and *Skeletonema costatum* (MIX), flagellates diet *Tetraselmis suecica*, *Isochrysis galbana* and *Pavlova lutheri* (FLA).

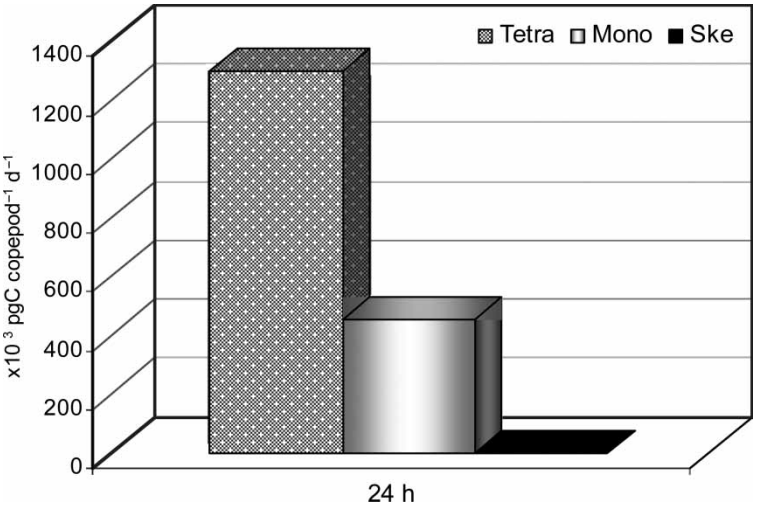

Figure 4. *Pseudocyclops xiphophorus.* Ingestion rates of copepods offered three phytoplanktonic species (*Tetraselmis suecica*, *Pavlova lutheri* and *Skeletonema costatum*).

(98.8 e 99.5%, respectively). With SKE, hatching success was slightly reduced (96.9%). SKE also induced higher naupliar mortality (30.0%) three and four days after eggs hatched compared to FLA and MIX (99.1% and 100.0%) (Figure 3). Under satiated food conditions and with all available phytoplankton species, *P. xiphophorus* seems to prefer *Tetraselmis suecica* and *Pavlova lutheri* to *Skeletonema marinoi*, which was not fed upon by *P. xiphophorus*, as demonstrated by ingestion rates reported in Figure 4.

4. Discussion

Food type is known to affect egg production in copepods (e.g. [1]). The present study demonstrates that *P. xiphophorus* egg production and hatching success strongly depend on the diet. Furthermore, *P. xiphophorus* may have been exhibiting size-selective feeding, ingesting more of *Isochrysis galbana* indicated that this diet did not contain any noxious compounds that deterred feeding by adult

females. *Skeletonema marinoi* was not fed upon by copepods when they are able to select among different food types. Although *P. xiphophorus* egg production was strongly affected by diatom diet, values for egg production remained constant together with hatching success for more than 20 days. In *Temora stylifera* fed the same diatom, hatching success was reduced to 20% and egg production was blocked, with females dying after 3–4 day incubation [14]. This reflects species specific nutritional responses and suggests that *P. xiphophorus* is better capable of metabolizing toxins derived from diatoms than planktonic copepod species through more advanced detoxification mechanisms. This could be an adaptation of this species to balance its lower specific fecundity rate [11] than planktonic copepods. *P. xiphophorus* nauplii hatched by females fed on *Skeletonema marinoi* had lower survival rates. This could be attributed to cell size, when cells form colonies and are therefore not easily handled by nauplii, or to anti-mitotic effects that block cell division and the normal development of these larval stages. Recent studies demonstrated that production of abnormal nauplii was determined by maternal food effects and the longer females fed on diatoms, the higher the number of teratogenic larvae [15]. Ianora et al. [16] observed the higher production of abnormal nauplii, with strong teratogenic defects, in *C. helgolandicus* females fed on *Skeletonema marinoi*. Feeding of females and nauplii on this phytoplanktonic species induced 100% larval mortality. Development stage reached by juveniles was a function of exposure time of mothers to *Skeletonema marinoi* diet. The same species tested on *P. xiphophorus*, caused 30% mortality in the early naupliar stages again denoting detoxification mechanisms in this copepod. According to the traditional view that early naupliar stages of copepods (N1 and/or N2) are non-feeding stages that rely on maternal yolk reserves [17], their survival should thus be strongly dependent on the past feeding history of females. Laboratory rearing studies have in fact shown that maternal diet is extremely important in promoting high hatching success and larval fitness and survivalship [18–20].

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