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# PREDATION BY *Apocyclops royi* (CYCLOPOID: COPEPOD) ON CILIATES AND ROTIFERS

Raunak Dhanker and Jiang-Shiou Hwang

Key words: copepod, microplankton, algal effect, omnivory.

## ABSTRACT

We studied the prey consumption rates in *Apocyclops royi* on neonate and adult rotifers, *Brachionus rotundiformis* and ciliate, *Euplotes* sp. in the presence and absence of algae, the smaller *Isochrysis galbana* and the larger *Tetraselmis chui*. Both males and females of *A. royi* were able to successfully ingest neonate and adult rotifers and ciliate. The prey consumption rates were significantly lower in males than in females of *A. royi*. Regardless of sex and reproductive state of the copepod, prey consumption rates were higher on neonates rotifer than on adults. However, the prey consumption rates were significantly lower on ciliate than rotifer in the female copepods. Further, the prey ingestion rates did not differ statistically between ovigerous and non-ovigerous adults of *A. royi* females. The ovigerous rotifer was ingested at lower rates than either non-ovigerous or neonates rotifer by both males and females of *A. royi*. The presence of algae (no matter *I. galbana* or *T. chui*) negatively influenced the rotifer and ciliate consumption rates. The present study provides first information on predatory efficiency of *A. royi*, and attests its omnivory feeding habit. Our results suggest that *A. royi* can utilize heterotrophic food efficiently and can derive nutrients during periods of low primary production. The present study points to the role of *A. royi* in forming a link between the microbial loop and classical food chain, which expedites the flow of bacterial carbon to higher trophic levels in estuarine ecosystems.

## I. INTRODUCTION

Pelagic copepods are mainly omnivorous [28] and highly diverse [23, 43]. They feed on a wide range of food size,

such as ciliates, rotifers and crustaceans, as well as algae and transfer this carbon to higher trophic level, i.e. fish, in the estuarine and marine food webs [37, 40, 42]. The feeding efficiency of copepods on microplankton, i.e. rotifers and ciliates, is very well documented by field and empirical works [1, 2, 5, 6, 10, 12, 13, 31, 32, 49]. Copepods show tendency to switch from phytoplankton diet to heterotrophic diet, when primary producers are dominated by picophytoplankton, whereas, their propensity of feeding switches to phytoplankton diet when primary producers are dominated by medium to larger size phytoplankton [15-17, 45]. Therefore, switching of feeding modes in copepods depends on phytoplankton size composition [15]. In general, copepods are selective feeders and their feeding modes have important implications in shaping the pelagic food web structure [20, 21, 27, 44].

*Apocyclops royi* (Lindberg, 1940) is a euryhaline copepod and the only cyclopoid species being cultured commercially as live feed in aquaculture industry in Taiwan [3, 47]. This species inhibits a wide range of salinity and temperature, i.e. aquaculture ponds, estuaries, brackish ecosystems and marine caves etc. [8]. *A. royi* has a short generation time (2-5 days) that makes this species perfect model for mass culture for aquaculture [46]. *Apocyclops* species have been evaluated to feed on microalgae and cultured at very high densities to feed many fish larvae and early stages of juvenile in aquaculture [34].

The feeding mechanism of copepods is strongly influenced by the availability of various food sources [29-31] and by the swimming speed, density, size, age, reproductive stage, and abundance of prey [12, 24-26, 35, 38, 48]. Other factors are hunger level, satiation, age, and sex of the copepods [4, 12-14, 22, 29-31, 38, 41]. In addition, hydrodynamic conditions, such as turbulence considerably affected the prey predator interaction in aquatic food webs [18, 19, 33, 36]. In nature, microplankton coexists with *A. royi*, and may be preferred food source for copepods when primary production is limited. However, no information is available about *A. royi* feeding on heterotrophic diet. Therefore, we attempted to answer the following questions through this study: (a) Can *A. royi* utilize microplankton as a food source? (b) To what extent does the

**Table 1. Experimental organisms, their body sizes and culture conditions.**

Ecological group	Taxonomic group	Species	Body size ( $\mu\text{m}$ )	Culture condition
Predator	Copepoda	<i>A. royi</i> ♀	950 $\pm$ 76	Mixture of autoclaved sea water and tap water with algae, rotifers and ciliates as food
Predator	Copepoda	<i>A. royi</i> ♂	710 $\pm$ 75	Mixture of autoclaved sea water and tap water with algae, rotifers and ciliates as food
Prey	Rotifera	<i>B. rotundiformis</i> (adult)	168 $\pm$ 22 [12]	Mixture of autoclaved sea water and tap water with <i>I. galbana</i>
Prey	Rotifera	<i>B. rotundiformis</i> (neonate)	48 $\pm$ 12 [12]	Mixture of autoclaved sea water and tap water with <i>I. galbana</i>
Prey	Ciliophora	<i>Euplotes</i> sp.	46 $\pm$ 8 [13]	Mixture of autoclaved sea water and tap water with <i>I. galbana</i>
Prey	Chromalveolata	<i>I. galbana</i>	4.16 $\pm$ 0.65 [13]	Walne's medium [50]
Prey	Chlorophyta	<i>T. chui</i>	17.35 $\pm$ 1.98 [13]	Walne's medium [50]

presence of autotrophic protists in its environment affect predation on heterotrophic diet?

## II. MATERIAL AND METHODS

### 1. Experimental Organisms

#### 1) Copepod Culture

Details of the experimental organisms are shown in Table 1. The culture of adult *A. royi* was isolated from zooplankton samples collected from a coastal brackish water pond of Taiwan. A monoculture was developed in a mixture of filtered seawater and autoclaved tap water, and inoculated into a 5-L aquarium that contained 4-L of medium. A mixture of the microalgae *Isochrysis galbana* and *Tetraselmis chui*, rotifer *Brachionus rotundiformis*, and ciliate *Euplotes* sp. was used as food for the copepods. The culture was maintained at 28°C and salinity 20 under a photoperiod of 12 h of light and 12 h of dark. The copepod culture was maintained in the laboratory for  $\geq 3$  months prior to the experiment. Moreover,  $\geq 200$  ovigerous females of *A. royi* were collected to obtain freshly hatched nauplii to perform the experiment. All experiments were conducted with *A. royi* of a known age (12 to 14 days). The culture was continuously mildly aerated to keep the food uniformly distributed in the culture tank. The culture medium was renewed twice a week.

#### 2) Rotifer and Ciliate Culture

The culture of rotifer *B. rotundiformis* was isolated from zooplankton samples collected from a coastal brackish water pond of Taiwan. Monoculture of rotifer was started from the single ovigerous rotifer of *B. rotundiformis*. The culture was maintained in 2000 ml beaker with 1800 ml water medium at salinity 22  $\pm$  1 and temperature 26  $\pm$  2°C. The microalgae *I. galbana* was provided as a food. The culture was mildly aerated continuously for keeping the food uniformly distributed in the culture beaker.

Ciliates of the genus *Euplotes* were originally isolated from the rotifer culture beaker. They were propagated and maintained in a 2-L beaker at salinity 20 and fed to the unicellular alga *I. galbana* (Table 1). The culture medium was changed on alternate days with a mixture of filtered autoclaved seawater and autoclaved tap water (salinity 20  $\pm$  1 and temperature 26  $\pm$  2°C).

#### 3) Algal Culture

Mass cultures of both algal species (*I. galbana* and *T. chui*) were established in the laboratory. Algal culture media were prepared by enriching sterile filtered seawater with macronutrients and micronutrients (Walne medium; Walne 1970) in a 2-L borosilicate glass flask (Table 1). The algal culture was maintained at 27  $\pm$  2°C and salinity 30 in 12 h light: 12 h dark photoperiodic condition. The algae were harvested in their exponential growth phase of the nutrient-replenished condition to prevent mineral nutrient limitation.

### 2. Experimental Protocol

Predation rates on ciliate (*Euplotes* sp.) and rotifer (neonates, nonovigerous adults and ovigerous adults) by males and ovigerous and nonovigerous females of *P. annandalei* were examined in the presence and absence of an algal diet. The experimental protocol included the following: (a) prey (either rotifer or ciliate) alone, (b) prey (either rotifer or ciliate) with *I. galbana* and (c) prey (either rotifer or ciliate) with *T. chui*. Known-age individuals of *A. royi* were collected from stock culture and transferred to a bowl containing 40-mL of medium 3-h prior to the experiment. *A. royi* was deprived of food for 3-h prior to the experiment. Subsequently, 40 cells of *Euplotes* sp., neonate rotifer and adult rotifer were introduced into each respective bowl, and five bowls were used for each treatment. The number of prey consumed was recorded after 60-min. *A. royi* was removed from the experimental bowl at the end of the test, and all remaining live prey from each bowl were carefully counted under a stereo zoom microscope (Olympus SXZ 16)

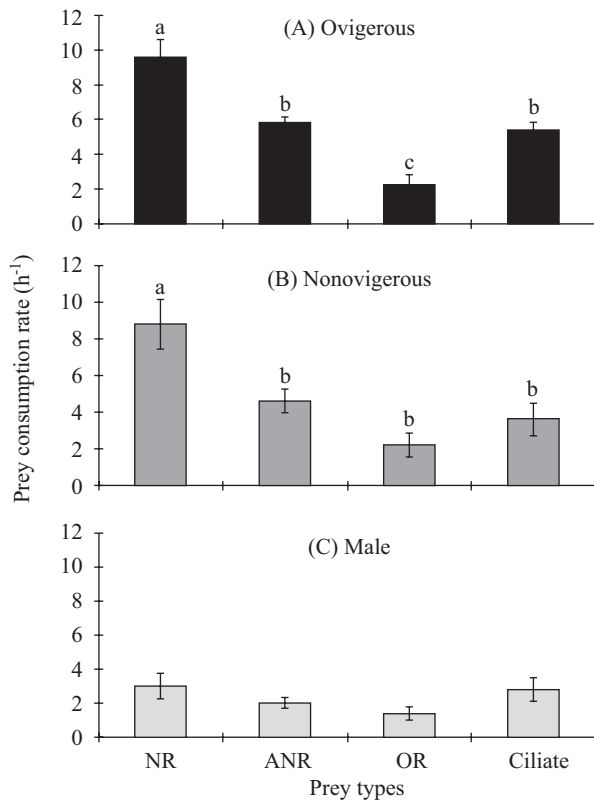


Fig. 1. Prey consumption rates (mean  $\pm$  SE) of ovigerous and non-ovigerous ♀s and ♂s *A. royi* preying on the rotifer *B. rotundiformis* (neonates, non-ovigerous and ovigerous) and ciliate *Euplotes* sp. The superscripts with different letters denote significant difference within each gender. NR = neonate rotifer, ANR = adult nonovigerous rotifer, OR = ovigerous rotifer.

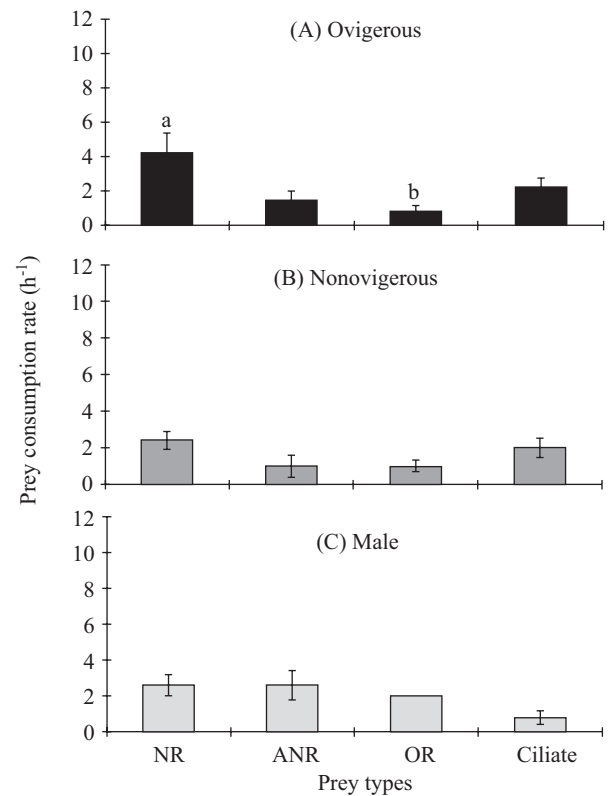


Fig. 2. Prey consumption rates (mean  $\pm$  SE) of (A) ovigerous, (B) non-ovigerous ♀s and (C) ♂s of *A. royi* preying on the rotifer *B. rotundiformis* (neonates, non-ovigerous and ovigerous) and ciliate *Euplotes* sp., in the presence of algae, *I. galbana*. The superscripts with different letters denote significant difference within each gender. NR = neonate rotifer, ANR = adult nonovigerous rotifer, OR = ovigerous rotifer.

to obtain an estimate of the number consumed. All experiments were conducted at salinity  $19 \pm 0.5$  and at a fixed temperature ( $28^\circ\text{C}$ ) in a BOD incubator. The same sized of rotifers, ciliate and algal prey were used for the experiment that was used in our previous published studies [12, 13].

### 3. Statistical Analysis

Data were analyzed by one-way and two-way analysis of variance (ANOVA). When significant differences were found among treatments, Turkey's post hoc test was used to test specific differences among treatments. SPSS (Statistical Program for Social Sciences) software version 17 was used for all statistical analysis.

## III. RESULTS

### 1. Prey Consumption Rate

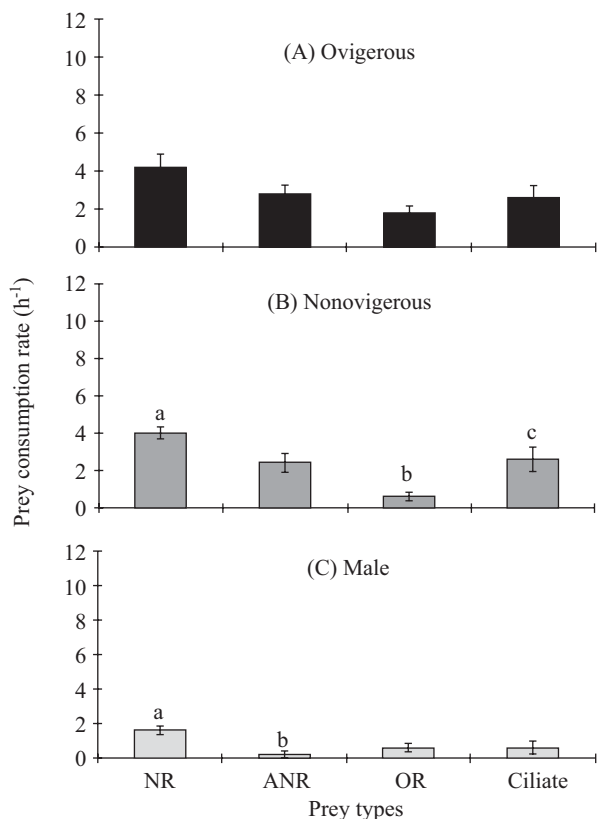
Prey consumption rates in *A. royi* have been shown in Fig. 1. The prey consumption rates in *A. royi* were significantly lower in males than those in females for both rotifer (neonate and adults) and ciliates ( $p < 0.001$ , two-way ANOVA). Within ovigerous and nonovigerous females, the prey consumption

rates were non-significant regardless of prey size and type in the environment ( $p > 0.178$ , Fig. 1). Furthermore, the rotifer ingestion rates were significantly higher on neonates than adults of *B. rotundiformis* ( $p < 0.01$ , two-way ANOVA) and *Euplotes* cells in female copepods.

The prey ingestion rates were significantly different in female regardless of type and size of prey ( $p < 0.0001$ ). The highest and lowest consumption rate was recorded for neonate and ovigerous rotifer respectively in female copepods compared to nonovigerous rotifer and ciliate ( $p < 0.001$ , one-way ANOVA). However, the consumption rate did not differ statistically between ciliate and nonovigerous rotifer ( $p > 0.974$ ). In contrast, the ingestion rates did not differ statistically among all tested prey in male *A. royi* ( $p > 0.421$ ).

### 2. Effect of Larger and Smaller Algae on Prey Consumption Rates

The presence of algae in the environment of *A. royi* elicit significant effect on rotifer and ciliate consumption rates ( $p < 0.009$ , Two-way ANOVA, Fig. 2 and Fig. 3) except the consumption rates of ciliate and ovigerous rotifer in nonovigerous female ( $p > 0.33$ , one way ANOVA) and neonate consumption



**Fig. 3.** Prey consumption rates (mean  $\pm$  SE) of (A) ovigerous, (B) non-ovigerous ♀s and (C) ♂s of *A. royi* preying on the rotifer *B. rotundiformis* (neonates, non-ovigerous and ovigerous) and ciliate *Euplotes* sp., in the presence of algae, *T. chui*. The superscripts with different letters denote significant difference within each gender. NR = neonate rotifer, ANR = adult nonovigerous rotifer, OR = ovigerous rotifer.

rate in male copepods ( $p > 0.25$ ). We observed almost similar ingestion rates on ciliate and rotifer (neonate and reproductive states) by *A. royi* (gender and reproductive states) in the presence of smaller, as well as larger sized algae except the cases of ciliate and ovigerous rotifer by nonovigerous female copepod in the presence of *T. chui* ( $p > 0.05$ , one way ANOVA). In the presence of larger algae *T. chui*, the higher number of ciliates were ingested than ovigerous rotifer by nonovigerous *A. royi* females ( $p < 0.037$ , one way ANOVA).

#### IV. DISCUSSION

*A. royi* actively ingested 1.5 to 9.6 number of the rotifer and 2.8 to 5.4 number of the ciliate. Although, the presence of algal diets in the medium resulted in 14-56% reduction of the rotifer ingestion and 51% reduction in the ciliate ingestion in the males and females of *A. royi*. In consequence, our results clearly indicate that *A. royi* is omnivorous and microplankton can be the essential part of the diet of this species, when the autotrophic food production is limited in its habitat. The rotifer and ciliate genus used in this study are widely distributed

(cosmopolitan) such as several other microzooplankton. In nature, microplankton coexists with copepods and constitutes an important link in the food chain. They are the preferred prey of many copepods [7, 12, 13, 29, 39, 49]. Dhanker *et al.* [12, 13] investigated that *Pseudodiaptomus annandalei* fed efficiently on the rotifer *B. rotundiformis* and ciliate *Euplotes* sp. even in the presence of alternate algal food.

The hunger level, satiation, age, and gender of the copepods have been investigated to influence predatory behavior of copepods [4, 12, 13, 29, 30, 31, 41]. In our previous studies, male copepods have been noted as less-efficient predator compared to their female counterparts [12, 13]. Longer searching and handling times, and lower ingestion and higher rejection rates of prey were recorded for male *P. annandalei* in comparison to female [12, 13]. Similarly, 21-50% and 34-49% higher consumption of rotifer and ciliate were observed in females of *A. royi* respectively in comparison to males in the present study.

Other factors that influence predation interactions between copepods and their prey are the density, swimming speed, size, age, reproductive stage, and abundance of prey [4, 12, 13, 29, 30, 31, 38, 41]. In the present study, we found that *A. royi* ingested the lower number of ciliate than rotifer. Prey size and mobility may attribute different feeding modes in the copepods [13].

In nature, phytoplankton food is not adequate to support normal growth and to realize actual reproductive performance in some copepod species [11, 25]. In such cases, microplankton may be more beneficial food compared to phytoplankton due to their higher dietary value [9]. Copepods can produce more eggs on a mixed diet of microplankton and algae than algal diet alone. *Acartia tonsa* produced 25% more eggs when fed ciliates and rotifers than when this was fed only with algal diet [45]. However, the influence of the experimental prey on reproductive performance of *A. royi* has not been discussed in our study.

In conclusion, the perennial abundance in natural habitats and efficiency of utilizing autotrophic and heterotrophic foods, suggest that feeding habits of *A. royi* are highly adaptive and it can derive nutrients during periods of low primary production. The present study points to the role of *A. royi* in forming a link between the microbial loop and classical food chain, which expedites the flow of bacterial carbon to higher trophic levels in estuarine ecosystems. Laboratory experiments such as those in this study are important for estimating ingestion rates at specific prey concentrations and determining what factors influence those rates. However, better estimates of natural microplankton concentrations, size and permanence of patches are necessary before such studies can be used to quantitatively measure mortality from predation in nature.

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