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Feeding, survival, egg production and hatching rate of the marine copepod *Oithona rigida* Giesbrecht (Copepoda: Cyclopoida) under experimental conditions

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Original Article

Abstract
Marine copepods are considered as an ecologically and economically important organism in assessing the fish stock since it is serving as prime feed for most of the brackish water and marine fishes. Hence, the present attempt was made to recognize the feeding and fecundity behavior of *Oithona rigida* using four different microalgal diets viz: *Chlorella marina, Coscinodiscus centralis, Chaetoceros affinis and Skeletonema costatum.* The feeding rate of the marine copepod, *O. rigida* increased with increasing body size. Similarly, the feeding rate also proportionately increased with algal food concentration. *Oithona rigida* prefers to feed more on *C. marina* (98.53%) than other algal prey such as *C. centralis, C. affinis* and *S.costatum*. *O. rigida* has exhibited maximum survival in the highest algal concentration when fed with 50:50 combination of *C. marina:C. centralis*. The highest egg production and hatching success were seen in the larger sized copepod (0.84 mm) at an optimum temperature (26±2°C) and higher algal food concentration (15,500 cells/ml). The study indicated that combination of *C. marina* and *C. centralis* with maximum (12.5 µg/l) algal concentration might be a suitable algal food source for effective cultivation of *O. rigida*. Besides, it is clearly proved that *O. rigida* have high reproductive capacity and therefore can be considered as most suitable candidate species for mass culture of live feed for the commercial seed production of finfish and shellfishes.

Keywords: *copepod, Oithona rigida, feeding rate, survival rate, fecundity.*

Introduction
In marine food webs, copepods constitute the prominent pathways for energy transfers between primary producers and the larger predatory species of zooplankton and nekton. An understanding of quantitative trophic interactions between phytoplankton and copepods is required to elucidate the nature of marine food webs in terms of rates (Mc Allen and Brennan, 2009). The measurements of feeding activity are essential in achieving better understanding of the functioning of planktonic communities. The feeding behaviour of marine copepods can vary over periods as short as several hours, even when food concentrations are essentially constant (Calbet *et al*., 2007). The genus, *Oithona* apparently feed discontinuously and show considerable discrimination when presented with a choice of several food organisms. Several reports are available on the feeding biology of copepods for temperate waters (Prince *et al*., 2006; Calbet *et al*., 2007; Calliari *et al*., 2008; Costa *et al*., 2008). However, very few studies have been made on the feeding biology of copepods in Indian context (Shrivastava *et al*., 1999; Perumal *et al*., 2000; Santhanam, 2002).

The measurement of egg production of copepod has become a widely used tool in copepod ecology (Ianora *et al*., 2007). Knowledge of the reproductive biology is a prerequisite to understand the population dynamics and secondary production in the aquatic ecosystems (Halsband and Hirche, 2001). Reproductive characters of copepods are affected by various environmental conditions. Temperature has direct and indirect effects on egg production (Devreker *et al*., 2009). In addition to temperature, the quality and quantity of food and female size also affects the egg production (Kleepel *et al*., 1998). Several reports are available on egg production rate of different species of copepod of temperate waters (Hirche and Halsband, 2000; Halsband and Hirche, 2001). Although the copepods present in the Bay of Bengal and adjacent waters have been thoroughly studied taxonomically, virtually no information is available on the reproductive biology. Hence, in the present study an attempt was made to quantify the food requirements of a copepod *O. rigida* in different concentration of food, type of food and consumption rate related to size of copepod, besides, egg production and hatching succession in relation to temperature, food concentration and body size.

Material and methods

Feeding rate

The zooplankton samples were collected from the Vellar estuary (11° 29′ N; 79° 46′ E) using plankton net (158μm). From these, the healthy adults of *O. rigida* were picked up by using a fine capillary tube. The isolated copepods were kept overnight in 250 ml beakers containing filtered seawater (1μm) of ambient salinity (34‰) with vigorous aeration for starving prior to the experiment. The monocultures of microalgae viz: *Chlorella marina (C.m), Coscinodiscus centralis (C.c), Chaetoceros affinis (C.a)* and *Skeletonema costatum (S.c)* were cultured separately in the laboratory using mixed culture medium (Gopinathan, 1996). The experiment on effect of animal size and algal concentration on feeding rate of copepod was followed by the method of Spiros and Gerard (1990). For the effect of animal size on feeding rate, the experimental set-up consisted of 500 ml flasks containing 250 ml of algal cell suspension were used. A single healthy adult copepod of different size ranges were introduced into separate flask. One ml of cell suspension was removed for cell counts at an hourly interval for 7 hours from each flask. The hourly feeding rate was estimated by subtracting the density from the values obtained for the previous hours.

For estimation of effect of algal concentration on feeding rate, the mixed microalgae were given in the concentrations of 2.5; 5.0; 7.5; 10.0 and 12.5 μg/l to feed the copepod. The experiments were conducted in triplicates and one control (algal suspension without copepod). The flasks were covered with black cloth to avoid photosynthesis and further cell divisions. One healthy adult copepod was inoculated into each experimental flask. The flasks were gently shaken every 30 minutes to avoid settling of algal cells. The feeding rate of copepod was measured by subtracting the final Chl 'a' concentration from the initial value. The experiments were conducted at room temperature of 28±1°C. The method adopted by Perumal *et al.* (2000) was followed for the assessment of effect of algal type on feeding behaviour of copepod. 250 ml of algal cell suspension was added to 1000 ml flasks containing 500 ml of filtered seawater of ambient salinity for each algal species. Initial chlorophyll 'a' concentration was determined by Spectrophotometric method (Strickland and Parsons, 1972). The hourly difference in algal population was estimated by removing 10 ml of cell suspension at an hourly interval for 7 hours. The hourly feeding rate was estimated by subtracting the Chl 'a' from the values obtained for the previous hour.

Survival rate

The method described by Shrivastava *et. al* (1999) was followed to study the survival experiment on copepod. Ten individuals of *O. rigida* were stocked in 100 ml glass bowls containing filtered seawater with 34‰ salinity. Four different combinations of live microalgae in different concentrations were prepared using mixed cultures of species viz. *C. marina*, *C. centralis, C. affinis* and *S. costatum* (Table 1). Ten individuals were maintained separately in each bowl for each feed combination. The daily water exchange was given using freshly filtered seawater and feed. The daily mortality was recorded carefully. The experiment was maintained at 28±1°C till the death of all animals.

Table 1 Different algal feed combination used for *O. rigida* survival experiment

Feed Type	*C.marina* (%)	*C.centralis* (%)	*C.affinis* (%)	*S.costatum* (%)
A	40	40	10	10
B	10	10	40	40
C	35	35	15	15
D	0	0	50	50
E	25	25	25	25
F	50	50	0	0

Egg production rate

The effect of body length, temperature and algal concentration on fecundity of copepod was determined by the method of Koski and Kuosa (1999). For the temperature experiment, a single female of healthy adult *O. rigida* were placed in pyrex test tubes (2.5 cm mouth diameter; 15 cm deep) containing 20 ml of the algal culture consisting of *C. marina, C. centralis, C. affinis* and *S.costatum*. *Oithona rigida* were incubated at

15±1, 18±2, 23±1, 26±2 and 30±1°C. The temperature below and above 30°C was maintained using air conditioning and water bath respectively. An immersion heater in the water bath can be used to control temperature ambient using the temperature fixer in water bath. The water was stirred using an air stone so that a layer of heated water does not form at the surface and overheat the animals. For the effect of algal feed concentration on fecundity, single female of *O. rigida* was placed in pyrex test tubes provided with mixed algal food *(C. marina, C. centralis, C. affinis* and *S.costatum)* at the concentration of 1000, 2500, 5000, 10,000 and 15,000 cells ml/1 with one control containing filtered seawater without algae at constant temperature of 26±2°C. During the experiment, a stirrer agitated the water in the beakers at 2-6 h intervals. The beakers were covered with black cloth to avoid illumination. For the experiment on effect of body length on egg production, the different size ranges of adult females were stocked in the test tubes containing filtered seawater fed with saturated algal feed and constant water temperature (26±2°C). The body length of the copepod was measured as described for the effect of animal size on feeding rate. Eggs produced in the various above mentioned experiments were placed on a watch glass and counted under binocular microscope.

Egg hatching rate

To estimate the hatching success, a single gravid female from wild samples were incubated in test tubes along with filtered seawater for 96 hours and after that the hatched-out nauplii were counted using a binocular microscope. The effect of temperature on the hatching rate of copepods was determined by incubating individual egg carrying female copepod in a test tube and maintaining various temperatures viz., 15±1, 18±2, 23±1, 26±2 and 30±1°C. The method adopted for maintaining different temperatures is the same as described for egg production rate. The triplicates were made for each experiment. The hatched out nauplii from individual test tube were placed in petriplates and counted under binocular microscope. In an another experiment, a single individual of adult female with egg sacs were placed in test tubes with filtered seawater. The food was given at the following concentrations: 1000, 2500, 5000, 10,000 and 15,000 cells/ml of mixed microalgae with one control containing copepods without algae. Triplicates were made for each feed concentration.

Results and discussion

Feeding rate of O. rigida

The hourly feeding rate of *O. rigida* was found to increase with increasing body length. Minimum feeding rate of 3511.5 cells/animal was obtained in 0.68 mm copepod whereas the maximum (4949.1 cells/animal) was seen in 0.84 mm copepod (Table 2). Body length and feeding rate was found to be highly significant ($P<0.001$). Similarly, minimum feeding rate of 2.41 µg/l/h and maximum of 12.49 µg/l/h was observed at lowest (2.5 µg/l) and highest (12.5 µg/l) algal concentration respectively (Table 3) which was highly significant at $P< 0.001$. *O. rigida* preferred more (99.33%) *C. marina* than other feeds used viz., *C. centralis* (75.20%), *C. affinis* (60.53%) and *S.costatum* (48.26%). The maximum feeding efficiency (1.96 µg/l/h) was noticed with *C. marina* at 5th hour. However the minimum feeding (0.15µg/l/h) was obtained with *S.costatum* at 2nd hour of experiment. The feeding rate of copepod showed an initial decrease up to 2nd hour and steady increase up to 5th hour and declined thereafter (Table 4) which supported with and the relation was highly significant ($P<0.001$).

Table 2. Effect of female body length on feeding rate (cells / animal) of *O. rigida*

Hours of feeding	Body Length (mm)				
	0.68	0.72	0.75	0.80	0.84
1	429.0 ± 1.63	469.6 ± 1.69	509.6 ± 1.69	585.6 ± 1.24	608.6 ± 2.86
2	332.3 ± 2.05	355.0 ± 3.74	412.6 ± 2.35	496.6 ± 1.24	515.0 ± 1.47
3	659.0 ± 0.81	678.6 ± 0.94	697.6 ± 0.47	790.3 ± 3.39	827.0 ± 4.32
4	784.6 ± 3.29	808.3 ± 1.24	834.3 ± 1.69	946.6 ± 1.24	989.6 ± 1.24
5	813.6 ± 1.24	838.6 ± 2.86	908.0 ± 2.16	1096.3 ± 3.09	1139.0 ± 0.81
6	304.0 ± 2.16	328.3 ± 1.69	406.6 ± 2.62	449.6 ± 0.47	508.3 ± 1.69
7	189.0 ± 2.94	216.6 ± 1.69	256.0 ± 0.81	314.0 ± 0.47	361.6 ± 0.47
Total	3511.5	3695.0	4025.7	4679.0	4949.1

Survival rate of O. rigida

It is clear that survival of *O. rigida* showed positive proportion over food combinations and concentrations (Fig.1). In low algal concentration, 100% survival was noticed for about 5-9 days. Among the feed tested, feed F (Cm50: Cc50) showed 100% survival up to 9th day and total mortality obtained

Table 3. Effect of algal concentration on feeding rate (μg/l/h) of *O. rigida*

Hours	Feed concentration (μg/l.)				
	2.5	5.0	7.5	10.0	12.5
1	0.37 ± 0.012	0.69 ± 0.016	0.86 ± 0.024	0.96 ± 0.012	1.37 ± 0.012
2	0.29 ± 0.008	0.47 ± 0.016	0.65 ± 0.035	0.76 ± 0.008	0.87 ± 0.016
3	0.40 ± 0.008	0.75 ± 0.028	0.80 ± 0.008	1.27 ± 0.016	1.57 ± 0.016
4	0.43 ± 0.012	0.87 ± 0.012	1.32 ± 0.024	1.67 ± 0.016	1.96 ± 0.016
5	0.52 ± 0.016	1.6 ± 0.094	1.94 ± 0.029	2.45 ± 0.032	2.89 ± 0.008
6	0.27 ± 0.012	0.60 ± 0.035	1.06 ± 0.099	1.65 ± 0.028	2.18 ± 0.021
7	0.13 ± 0.026	0.45 ± 0.028	0.82 ± 0.021	1.21 ± 0.026	1.65 ± 0.016
Total	**2.41**	**4.89**	**7.45**	**9.97**	**12.49**

Table 4. Effect of algal type on feeding rate (μg/l/h) of *O. rigida*

Hours of feeding	Feed type			
	C. marina	*C. centralis*	*C. affinis*	*S. costatum*
1	0.7 6 ± 0.096	0.72 ± 0.021	0.57 ± 0.012	0.55 ± 0.008
2	0.59 ± 0.012	0.46 ± 0.016	0.28 ± 0.018	0.15 ± 0.008
3	0.86 ± 0.016	0.76 ± 0.021	0.54 ± 0.016	0.34 ± 0.016
4	1.28 ± 0.021	0.86 ± 0.008	0.77 ± 0.012	0.51 ± 0.014
5	1.96 ± 0.016	1.24 ± 0.016	0.94 ± 0.016	0.77 ± 0.016
6	1.08 ± 0.023	0.85 ± 0.028	0.75 ± 0.024	0.64 ± 0.016
7	0.92 ± 0.012	0.75 ± 0.028	0.69 ± 0.012	0.66 ± 0.021
%	**99.33**	**75.20**	**60.53**	**48.26**

only after 15th day onwards whereas the feed D (Ca50: Sc50) resulted poor survival where copepod showed 100% survival only up to 5th day and complete mortality on 11th day onwards (Fig. 1). The 100% survival was noticed up to 10th day of the experiment at high algal concentration. Here feed F was found more suitable than other feeds tested presently. It is inferred that the copepod survival extended for one more day at high concentration of feed F where 100% survival extended up to 10th day of experiment followed by 53% on 13th and total mortality on 17th day. But the least survival was observed in feed D, where 100% survival was reported only up to 5th day followed by 39% and 0% survival on 8th and 11th days respectively (Fig. 2).

Egg production rate of O. rigida

The small sized females (0.74 mm) of *O. rigida* could produce 11.6 clutches/female and 442.3 eggs/female whereas the larger sized females (0.84 mm) produced 21.0 clutches and 605.3 eggs/female (Table 5). Correlation between egg production and body length was highly significant at ($P<0.001$). However, the clutch production was not found to be significant with body length. The females behaved in an irregular manner in the

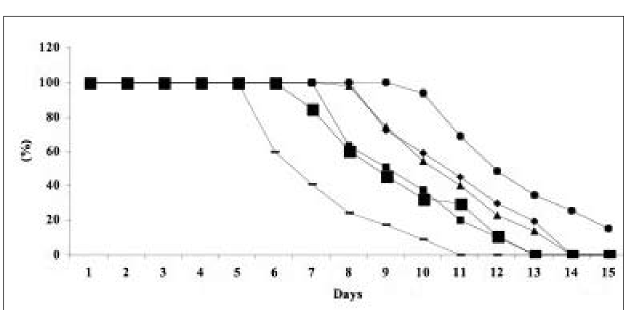

Fig. 1 Survival of *O. rigida* at low algal food concentration

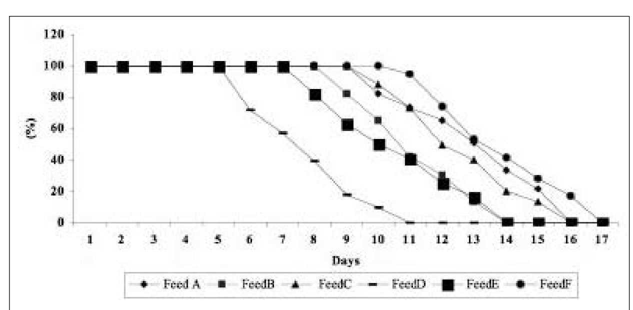

Fig. 2 Survival of *O. rigida* at high algal food concentration

Table 5. Effect of female body size on fecundity of *O. rigida*

Body Length (mm)	Clutch production (clutches/female)	Egg production (eggs/female)
0.75	11.6 ± 1.24	442.3 ± 2.05
0.78	12.3 ± 1.24	482.0 ± 4.24
0.80	13.3 ± 1.69	523.6± 2.05
0.82	16.3 ± 1.24	565.0 ± 2.94
0.84	21.0 ± 1.63	605.3 ± 6.23

clutch and egg production in respect to temperature (Table.6). Here, the minimum clutch (2.3± 0.47 clutches/female) and egg production (26.6±2.49 eggs/female) were observed at 15±1°C whereas the maximum clutch (11.3±0.94 clutches/ female) and egg production (564.0±14.38 eggs/female) were acquired at 26±2°C. At 30±1°C temperature, the clutch and egg production slightly decreased to 7.33 clutches/female and 404.0 eggs/female respectively (Table 6). The clutch and egg production was inversely correlated with temperature. In the present experiment, the clutch and egg production rate have increased with increasing food concentration (Table 7). The clutch (13.3 clutches/female) and egg production was high (617.6 eggs/female) at 15, 500 cells/ml algal concentration whereas the low clutch (2.0 clutches/female) and egg production (23 eggs/female) were achieved at 1000 cells/ml concentration ($P < 0.001$).

Egg hatching succession of O. rigida

The egg hatching was low (20.2%) at 15±1°C while the maximum hatching (99.8%) was obtained at the optimum temperature of 26±2°C (Table 6). Likewise, the lowest (18.8) and highest (99.9 %) hatching was perceived at minimum (1000 cells/ml) and maximum 15000 cells/ml algal cell concentrations respectively (Table 7) as evidenced by highly significant ($P<0.001$) relation between hatching rate and food concentration.

It is clear that the feeding rate of *O. rigida* increased with increasing body length. The larger sized copepod fed efficiently on algal particles than smaller one which might be due to wider mouth and high digestibility nature of larger copepods owing to presence of digestive enzymes which digest the food particles more quickly as agreed by Costa and Fernandez (2002), Olsen *et al*. (2000) and Costa *et al*. (2008). *O. rigida* prefer *C. marina* than rest of the feeds used presently ascribed to small mouth parts of copepod which is capable of capturing only smaller microalgae. In addition, *O. rigida* have a less overwhelming ability on chain forming diatoms because the mouth parts are not facilitating the capture of larger food organisms so that copepod fed less on *S. costatum* which has chain forming structure (Perumal *et al*., 2000). The feeding rate of *O. rigida* showed direct proportion to algal concentration as it increased with increasing food concentration as supported by statistical analysis ($P< 0.001$) as in accordance with the earlier reports (Stottrup and Jensen, 1990; Prince *et al*., 2006; Calbet *et al*., 2007; Costa *et al*., 2008). Copepod showed least survival at low algal concentration because of the insufficient food supply. However, maximum survival was procured at high algal concentration due to ease of sufficient food which might have stimulated the organisms to survive for longer period as agreed by Davis (1984) and Corkett and Mc Laren (1978). It is inferred that the morphology of algae perhaps

Table 6. Effect of water temperature on fecundity of *O. rigida*

Temperature (° C)	Clutch production (clutches female^{-1})	Egg production (eggs female^{-1})	Hatching Rate	Hatching (%)
15 ± 1	2.3 ± 0.47	26.6 ± 2.49	5.3 ± 0.47	20.2
18 ± 2	4.6 ± 0.94	169.0 ± 3.74	71.6 ± 3.29	42.7
23 ± 1	6.16 ± 1.57	388.0 ± 5.88	33.6 ± 7.13	86.1
26 ± 2	11.3 ± 0.94	564.0 ± 14.38	546.6± 14.8	99.8
30 ± 1	7.33 ± 0.47	404.0 ± 12.56	342.3 ± 17.1	84.7

Table 7. Effect of algal feed concentration on fecundity of *O. rigida*

Phytoplankton Concentration (cells ml^{-1})	Clutch production (clutches female^{-1})	Egg production (eggs female^{-1})	Hatching rate	Hatching (%)
1000	2.0 ± 0.81	23.0± 2.44	4.3 ± 0.47	18.8
2500	4.3 ± 0.94	139.3 ± 13.2	57.3 ± 10.49	38.3
5000	5.3 ± 1.24	203.0 ± 6.48	146.6 ± 6.23	72.2
10000	8.6 ± 1.24	428.0 ± 12.83	400.0 ± 29.40	90.2
15500	13.3 ± 0.94	617.6 ± 5.79	617.3 ± 5.73	99.9

influenced the survival of copepod so that the maximum survival was noticed in feed F (Cm 50: Cc 50) those which have comparatively smaller size than other algae as opined by Shrivastava *et al*. (1999).

In the present study, highly significant relationship was found between body size and clutch and egg production of *O. rigida* ($P<0.001$). Smaller sized copepod produced less number of clutches with minimum eggs whereas the larger copepods produced more number of clutches and eggs since it has more metabolic energy than smaller one as coincided with the findings of Hirche (1992); Ohman *et al*. (1996) and Koski and Kuosa (1999). It is understood that the egg production of *O. rigida* was influenced by temperature since more clutch and eggs were produced at the optimum temperature while low number of clutch and eggs were procured at low temperature. In contrast, egg production decreased at high temperature as reported earlier by Devreker *et al*. (2009). In the present experiment, the temperature increase up to $26\pm2^{o}C$ have resulted the maximum egg production indicating its adaptation to optimum temperature as similar to earlier reports (Hirche and Halsband, 2000) but disagrees to Kiorboe and Nielsen (1994). From the present study, it could be concluded that the clutch and egg production increased with increasing food concentration. *O. rigida* seemed to have given good results in egg production with high food concentrations as supported by statistical approaches ($r=0.966547$ and 0.997528 respectively). In the present study, maximum clutch and egg production coincided with high algal concentration while in low food concentration, the low clutch and egg production might be due to lack of energy which was not favouring for reproduction and egg laying due to shortage of food as agreed earlier by Hirche (1990) and Hirche *et al*. (1997).

In our experiment, the lowest egg hatching was noticed at low temperature and low food concentration whereas the high hatching was observed at the stable temperature and high food concentration. It is inferred that the insufficient food supply and unfavourable temperature might be the probable reason for low hatching as supported by Koski *et al*. (1999). Further, it is understood that the copepod *Oithona rigida* can survive more if it is reared at maximum density of *C. marina* and *C. centralis*.

The findings of the present study on feeding performance and survival of *O. rigida* provide us a realistic basis for formulating ecological principles that govern food chains in the coastal and marine environment. Besides, present observation also indicated the levels of food concentration and suitable food required for copepods in hatchery condition. The fecundity experiment on *O. rigida* helped us to understand the maximum production with high survival if it is cultivated at water temperature of $26\pm2^{o}C$ with maximum algal concentration.

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