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Growth rates of a heterotrophic dinoflagellate *Polykrikos kofoidii* and its gamete-like organism

Hyun-Jin Cho and Joon-Baek Lee*

Marine & Environmental Research Institute. Cheju National University. Jeju-Do. 695-814. Korea

**Dept. of Oceanography. College of Ocean Sciences. Cheju National University. Jeju-Do. 690-756. Korea*

종속영양 와편모조류의 일종인 *Polykrikos kofoidii*의 성장 속도와 그 배우자 형성에 관한 실험

조 현 진 · 이 준 백*

제주대학교 해양과환경연구소, *제주대학교 해양학과

ABSTRACT

121시간 동안 200에서 700 cells ml^{-1}까지 여섯 단계의 먹이(*Gymnodinium catenatum*) 농도에서 *Polykrikos kofoidii*의 성장 속도를 조사한 결과, 가장 낮은 농도인 200 cells ml^{-1}와 가장 높은 농도인 700 cells ml^{-1}에서 0.48와 0.50 d^{-1}로 비슷한 성장 속도를 나타내었다. 이 결과는 먹이 농도가 포화 상태에 달하여 *P. kofoidii*의 성장 속도가 일정해지기 전까지의 상승 곡선에서, 먹이 농도에 따라 일률적으로 성장 속도가 증가하는 것이 아니라, 일정한 먹이 농도 사이에서는 성장 속도가 잠시 멈추었다가 다시 증가하는 가능성을 암시한다. *P. kofoidii*의 배양이 끝나갈 무렵, 먹이가 부족한 상태에서, 8개의 unit을 가진 *P. kofoidii*는 이분법으로 증식을 하는 대신 더 이상 분열을 하지 않는 4개체의 two-unit pseudocolonies를 형성하였다. 이는 *P. kofoidii*의 생활사 속에서 아직까지 정확히 밝혀지지 않은 *P. kofoidii*의 배우자로서의 가능성이 있다고 생각된다.

Growth rates of *Polykrikos kofoidii* were determined at six prey(*Gymnodinium catenatum*) concentrations of 200 to 700 cells ml^{-1} during 121 hours of incubation time. Supplementary experiments during 73 and 113 hours were conducted for 200 and 400, and 560 cells ml^{-1} prey concentrations, respectively. Even at the lower prey concentration(200 cells ml^{-1}), the growth rate of *P. kofoidii* was over 0.48 d^{-1}, while the higher (700 cells ml^{-1}) resulted 0.50 d^{-1}. The growth rates of *P. kofoidii* may not draw a simply positive increase according to prey concentration increase, but has a possibility to increase with periodical pauses until to reach the saturation of the prey concentration.

Under prey-deficient culture condition, eight-unit *P. kofoidii* divided not into two four unit pseudocolonies, but into four two-units. Although the life cycle of *P. kofoidii* has not been confirmed entirely, we suggest a possible role of a mating gamete of *P. kofoidii*, about the two-unit pseudocolonies.

Key words : *Polykrikos kofoidii*, growth rate, *Gymnodinium catenatum*, two-unit pseudocolony, mating gamete

INTRODUCTION

Grazing is one of the major factors in the decline of the blooms coupled with losses from the formation of a large number of nondividing gametees and planozygotes which eventually fell to the sediments as cysts. and advection(Anderson et al.. 1983: Lessard and Swift. 1985: Frost. 1991: Sampayo. 1998). Although typically considered to be primary producers. many dinoflagellate species are phagotrophic. capturing and ingesting other organism for nutrition(Spero and Moree. 1981). Besides of studies on grazing activities of herbivorous zooplanktons(Heinbokel. 1978: Saito and Taguchi. 1996). recently many research results reveal those of heterotrophic dinoflagellates(Jeong et al.. 1997: Jeong et al.. 1999: Cho and Matsuoka. 2000: Jeong et al.. 2001). Three different feeding ways have been discovered in heterotrophic dinoflagellates so far. They are engulfment of intact prey cells. piercing cells and sucking out their cytoplasmic contents through a peduncle. and enveloping a prey with a feeding veil(Jacobson and Anderson. 1986: Hansen. 1992: Naustvoll. 1998). Among them whole prey cell is ingested by engulfment. while only parts of prey ingested by using peduncle or feeding veil. These heterotrophic dinoflagellates include thecate species *Oblea rotunda*(Strom and Buskey. 1993). *Diplopsalis lenticula*(Naustvoll. 1998) and *Protoperidinium* spp. (Jeong and Latz. 1994). and naked species *Gymnodinium* sp.(Storm. 1991) and *Gyrodinium spirale*(Hansen. 1992).

Sampayo(1998) reported that during a paralytic shellfish poisoning(PSP) episode due to *G. catenatum* on the Lisbon coast. a bloom of the heterotrophic dinoflagellate *P. kofoidii* was detected. Some of those *P. kofoidii* cells were seen attached to *G. catenatum* chains breaking them. and the latter declined rapidly in abundance. Although they reported a case of grazing of *P. kofoidii* on *G. catenatum*. there was no evidence of the predation of *P. kofoidii* on *G. catenatum* except the fact that *G. catenatum* which had been increasing in abundance before the detection of *P. kofoidii*. decreased sharply. ending its bloom phase. Thereafter. the feeding process of *P. kofoidii* was observed by Matsuoka et al.(2000): the predator used nematocysts to pull the prey into its body through the posterior sulcus. and finally engulfed it whole. We also observed high numbers of *P. kofoidii* were associated with a bloom of *G. catenatum* in the sea around the Amakusa Island. southwestern Japan in January 1998. In the case of *P. kofoidii*. it fed on preys by the method of engulfment. This observation gave us the idea of measuring the growth rate of *P. kofoidii* on *G. catenatum*. the notorious red tide dinoflagellate for a causative species of PSP. In additon. we will describe gamete-like organisms of *P. kofoidii*. which occur under prey-deficient culture conditions.

MATERIAL AND METHODS

Preliminary Experiments

Seawater samples containing *P. kofoidii* were collected from Isahaya Bay in western Kyushu. Japan in November 1998. during a red tide period whose causitive species was *Fibrocapsa japonica*. Raphidophyceae. *P. kofoidii* isolated were naked. ellipsoidal to cylindrical in shape. The organismas consisted of four or eight units with the same number of girdle which was displaced by twice of its own width. The sulcus was deep. narrow and slightly curved. running from apex of the first unit to antapex of the last one. There were also other heterotrophic dinoflagellates(e.g. *Protoperidinium* spp.) and zooplankters in the sample to feed on the red tide species for the prey.

We screened colored seawater gently through a 20 ㎛ mesh plankton net. collected samples for microscope and observed 2 ml from the sample with

an Olympus IMT-2 Inverted Microscope at ×100 and 400 magnifications. immidiately after coming back to the laboratory. *P. kofoidii* were isolated by a capillary pipette from the sample. and each individual was kept in Falcon Multiwell Tissue Culture Plates filled with *G. catenatum* suspension. whose concentration was 700 cells ml^{-1}. And then *P. kofoidii* were adapted for 5 days to the experimental conditions prior to experiments. We have cultured *G. catenatum*. a naked and causative species of PSP obtained from the outbreak of the species around Amakusa Island. Kumamoto Prefecture. southwestern Japan in January 1998. Isolating *G. catenatum*. we have cultured them in the incubator at 20℃ and 27psu with SW II medium(Iwasaki. 1961). The experiments were run at constant light condition.

Experimental Procedures

All experiments were carried out with clones of *P. kofoidii* adapted to the prey of *G. catenatum* for reliable experiments. Growth rates of *P. kofoidii* were determined at six prey concentratons of 200 to 700 cells ml^{-1}. We made each prey concentration by the dilution of the stock culture(1060 cells ml^{-1}) with SW II medium. Individual *P. kofoidii*(four-unit) was inoculated into each plates filled with food species suspension. whose volume was 2 ml. Two tissue culture plates containing *P. kofoidii* and *G. catenatum* were established for each food concentraton. Because the risk could be considered that several individuals of predator inoculated into each prey plate would die to mislead underestimation of the growth rates. we transferred only one individule of *P. kofoidii* into each plate. The experiments lasted 121 hours and terminated with adding 1% formalin. Supplementary experiments during 73 and 113 hours were conducted for 200 and 400. and 560 cells ml^{-1} prey concentrations. respectively(Table 1). Growth rates of *P. kofoidii* were calcualted utilizing the equations cited from Jeong and Latz(1994):

$$\text{growth rate } (\mu \cdot d^{-1}) = \ln (N_t/N_0)/t$$

t = incubation time (d)

N_t = the final abundance(individuals ml^{-1}) after time t

N_0 = the initial abundance(individuals ml^{-1}) of the predator

We fully discussed two different literatures of Jeong et al.(2001) and Morey-Gaines and Ruse (1980) comparing the results with ours about growth rates of *P. kofoidii* on *G. catenatum* and two-unit pseudocolonies produced under prey-deficient culture conditions. respectively.

RESULTS AND DISCUSSION

The predatory dinoflagellate *Polykrikos kofoidii* is additionally complex becaus it is pseudocolonial: that is. made up of several individuals(subunits) but without typical separations(cell boundaries) between them(Morey-Gaines and Ruse. 1980). According to Jeong et al.(2001). *P. kofoidii* grew on *Lingulodinium*

Table 1. Growth rates(μ) of *Polykrikos kofoidii* according to the actual initial concentrations(cells ml^{-1}) of prey, *Gymnodinium catenatum*, in 2 ml wells, during three different incubation times. Only one four-unit pseudocolony(from a clone) of *P. kofoidii* was inoculated into each prey well

Expt	Prey species	Incubation time (h)	Prey density (cells ml^{-1})	μ (d^{-1})
1	*G. catenatum*	121	200. 300. 400. 500. 600. 700	0.47/0.50. 0.49/0.51. 0.52/0.51. 0.53. 0.55/0.47. 0.50/0.51
2		73	200. 400	0.57/0.55. 0.48
3		113	560	0.48/0.49

polyedrum, *Scrippsiella trochoidea*, *Ceratium furca*, *Gymnodinium catenatum*, *Gyrodinium impudicum*, *Prorocentrum micans* and *Amphidinium carterae*, of which the maximum specific growth rate of *P. kofoidii* was 1.12 d^{-1} for *G. catenatum* diet whose concentration ranged 31~3555 cells ml^{-1}. Table 1 shows the growth rates of *P. kofoidii* on *G. catenatum* for prey concentrations ranging from 200 to 700 cells ml^{-1}. Even at the lower concentration (200 cells ml^{-1}), the mean value of the growth rate of *P. kofoidii* was over 0.48 d^{-1}, while the higher prey concentration(700 cells ml^{-1}) resulted 0.50 d^{-1} of the *P. kofoidii growth* rate. The growth rates obtained from other incubation times of 73 and 113 hours also revealed not so significantly different mean values: 0.56 and 0.48 d^{-1} for 200 and 400 cells ml^{-1}, and 0.48 d^{-1} for 560 cells ml^{-1} prey concentration. These similar values implied the growth rates of *P. kofoidii* were not significantly affected by prey concentration in this study.

However, specific growth rates of *P. kofoidii* increased rapidly with increasing density of *G. catenatum* before saturating around 1000 cells ml^{-1} in Jeong et al.(2001). The prey concentrations experimented here must not be saturated for *P. kofoidii* predation as we know from the results in Jeong et al.(2001), where further prey concentrations over our maximum(700 cells ml^{-1}) were used. The growth rates of *P. kofoidii* may not draw a simply positive increase according to prey concentration increase, but has a possibility to increase with periodical pauses until to reach the saturation of the prey concentration.

The most common mode of *P. kofoidii* reproduction in culture was simple transverse fission of the entire chain which posseses eight units, and then it formed two four-unit daughter pseudocolonies(Fig. 1). In the general life cycles of cyst-forming dinoflagellates, motile planktonic vegetative cells divide(by mitosis) to form chains, producing two types of gametees, pairs of which fuse to give a planozygote(Blackburn et al., 1989). Under prey-deficient culture condition,

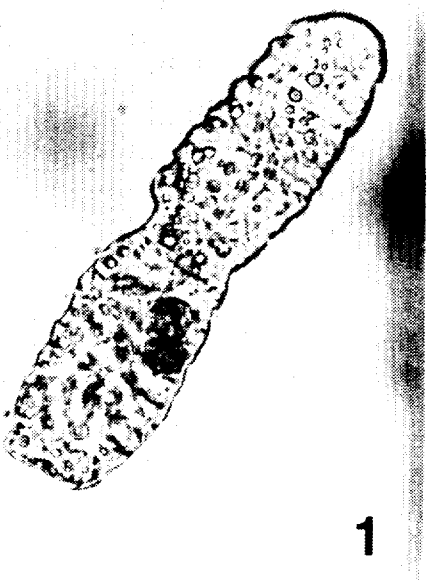

Fig. 1. Transverse fission of *Polykrikos kofoidii* which possess eight units.(76 ㎛ in length, 19 ㎛ in width) Then it forms two four-unit daughter pseudocolonies.

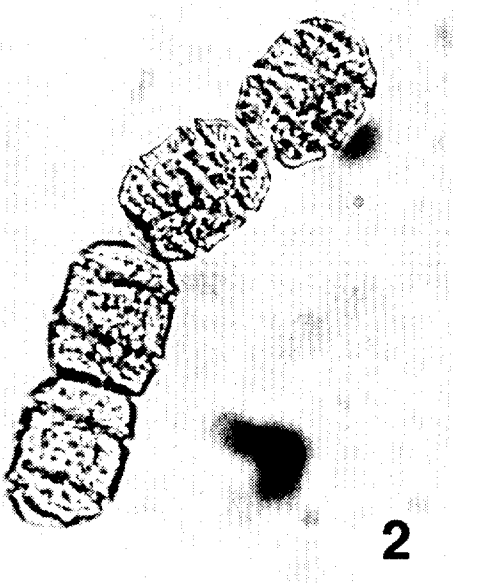

Fig. 2. Eight unit *Polykrikos kofoidii* divided not into four-units, but into four two-unit pseudocolonies(20 ㎛ in length, 15 ㎛ in width) under prey-deficient culture condition.

eight-unit *P. kofoidii* divided not into two four-units. but into four two-unit pseudocolonies(Fig. 2). In spite of the presence of three or four nematocysts in the body. these two-unit pseudocolonies were too small (20 ㎛ in length. 15 ㎛ in width) to engulf *G. catenatum*. Morey-Gaines and Ruse(1980) observed this kind of freed subunits in the *P. kofoidii* culture. which were 20~33 ㎛ long and 12~21 ㎛ wide and possessed all the features of a *Gyrodinium* species. and they proposed that these free subunits of *P. kofoidii* have been mistakenly identified as *Gyrodinium pellucidum*. Morey-Gaines and Ruse (1980) descripted that the subunits were fully capable of surviving independently for an indefinite period. reproducing by oblique longitudinal fission. and they can also return to the pseudocolonial stage. though the details of this process have not yet been observed.

On the contrary. the two-unit pseudocolonies explosively produced in this experiment at the end of culture(under prey-deficient condition. not lack) could not reproduce by dividing in two. but swam around over one day without any food particles in the body. Eventually. the individuals died to be destroyed rapidly at the bottom of the culture paltes. The above result was shown in all the culture plates. In the encystment experiment in natural seawater sample. Matsuoka and Cho(2000) reported *P. kofoidii* produced cysts with reticulate ornaments. Although the authors could not confirm the life cycle of the species. two-unit pseudocolonies of *P. kofoidii* were also observed from the natural seawater sample. One of the possible reasons of non-producing cyst of *P. kofoidii* in our sample may be due to utilization of a cultured clone of *P. kofoidii*. Moreover. morphologies of two-unit pseudocolonies were different between that in Morey-Gaines and Ruse(1980) and that in this experiment. We. however. suggest a possibility of two different types from each other as mating gametes(+/-) of *P. kofoidii*. Further study is needed to clarify the life cycle of *P. kofoidii* in order to understand the role of these two-unit pseudocolonies.

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