Bull. Inst. Zool., Academia Sinica **29**(1): 57-64 (1990)

RECRUITMENT AND INDUCTION OF SETTLEMENT OF PLANKTONIC JUVENILES OF THE OYSTER FLATWORM, *STYLOCHUS ORIENTALIS* BOCK (PLATYHELMINTHES, POLYCLADIDA)[1]

CHANG-PO CHEN[2], WANN-SHENG TSAI[3], CHI-YUAN LIU[3]
and QUAN-SHIN LEE[4]

Institute of Zoology, Academia Sinica, Nankang,
Taipei, Taiwan 11529, R. O. C.[2]
The Penghu Branch, Taiwan Fisheries Research Institute,
Penghu, Taiwan 88009, R. O. C.[3]
and
Institute of Marine Biology, National Sun Yat-sen University,
Kaoshiung, Taiwan, 80424, R. O. C.[4]

(Accepted September 15, 1989)

Chang-Po Chen, Wann-Sheng Tsai, Chi-Yuan Liu and Quan-Shin Lee (1990) Recruitment and induction of settlement of planktonic juveniles of the oyster flatworm, *Stylocus orientalis* Bock (Platyhelminthes: Polycladida). *Bull. Inst. Zool., Academia Sinica* **29**(1): 57-64. The planktonic juveniles of the oyster flatworm, *Stylochus orientalis* are induced to settle by its prey species, the Pacific oyster, *Crassostrea gigas.* The two major periods of the settlement of the planktonic juveniles are fall and early summer in the field. The juveniles which settled in fall mature in the following spring and produce a new crop of juveniles which settle in early summer. The success of settlement is determined by many factors, probably including the age of the oysters, fouling organisms on the oyster-shell and the existence of adult oyster flatworms.

Key words: Inductive settlement, Oyster leech, Recruitment.

Polyclad flatworms prey on a number of marine animals (Galleni *et al.*, 1980) can cause severe damage to the oyster-industry (Pearse and Wharton 1938; Rho, 1976; Shu and Lin, 1980; Ventilla, 1984). In Penghu, Taiwan, 50% of the Pacific oyster, *Crassostrea gigas,* were preyed by the oyster flatworm, *Stylochus orientalis* (Shu and Lin, 1980; personal observation). The mortality of the oysters is positively related to the density of the flatworms in the field (Lin and Tang, 1980).

Stylochus orientalis is widely distributed in the Indo-West Pacific areas such as Taiwan Strait, Gulf of Siam and West Australia (Bock, 1913). The gravid adults spawn and brood their egg masses on the inner surface of the left shell of the oyster which has just been devoured by the flatworm. A large oyster flatworm spawns repeatly in an interval of 20 days in the summer and produces an average

1. Paper No. 329 of the Journal Series of the Institute of Zoology, Academia Sinica.
2. To whom reprint request should be sent.

of 38,000 eggs in each spawning. The fecundity is positively related to the size of the flatworm. A fertilized egg develops directly to a planktonic juvenile in 16–17 days at 33‰ S and 26°C (Shu and Lin, 1980).

Few investigations on settlement of polyclads have been reported (in Ruppert, 1978; Shinn, 1987), although early development of some polyclads has been studied (in Ruppert, 1978). Many marine invertebrate larvae select suitable habitats by responding to environmental cues often associated with their prey species (for recent reviews see Chia and Rice, 1978; Burke, 1983, 1986; Pawlik and Faulkner, 1986). Adult *Stylochus orientalis* are unable to swim and the planktonic juveniles are the major stage of dispersion. Therefore, recruitment and settlement by juveniles on the oyster-bed are of interest.

MATERIALS AND METHODS

Adult *Stylochus orientalis* were collected from the oysters in Penghu, transported to Taipei and maintained in the laboratory. They were fed with fresh oyster meat bought from local markets. When adult flatworms were brought back to the aquaria, copulation by hyperdermic insemination was observed, and most gravid adults (57.4%, $n=54$) spawned within a week.

I. Inducton of juvenile settlement

Several gravid adults were placed in large beakers which were lined by a transparent plastic sheet and upon which eggs were deposited. When the color of the egg masses changed from fairly white to brown, the worms were removed and the plastic sheet was cut into small pieces and transferred into culture beakers containing filtered seawater (34‰). The planktonic juveniles, upon hatching, were collected daily and raised in non-aerated seawater under constant light (ca. 500 lux fluorescent light) at 26°C. Because aeration is lethal to the juveniles for unknown reasons (personal observation), benthic or planktonic algae were applied in the culture system for oxygen supplying.

Two sets of experiments were conducted to examine the effect of oysters on survival and settlement of the planktonic juveniles. Single classification Anova was applied for the statistic test (Sokal and Rohlf, 1981).

(1) *Survival*

Benthic algae (*Navicula* sp.) were cultured in large glass dishes (3 cm in diameter) one week before the experiment. The dishes were covered with transparent plastic sheets to prevent water evaporation. The planktonic juveniles have strong positively phototactic response and tend to gather on the wall along the water surface where they die and leave brown scars. To reduce such mortality, the margin of the dishes was covered with black plastic tape. About 200 planktonic juveniles were raised in a dish with a live oyster located in the dish. In another dish, the juveniles were treated similarly but without an oyster in it served as a control. Three replicates were conducted. The number of juveniles were counted randomly from 8 small circles (2.5 cm in diameter) on the bottom of the dishes on the fourth day of the experiment.

(2) *Settlement*

About 200 planktonic juveniles were raised in a petri dish (10 cm in diameter) containing the planktonic alga, *Isochrysis galbana* and oyster juices. The oyster juice was prepared by grinding fresh oysters. The final concentration of oyster juices in the petri dishes was 50, 30, 20 and 10 ppm. In another dish, the juveniles were treated similarly but without oyster juices in it served as a control. Three replicates were conducted. The number of settled juveniles was counted on the seventh day of the experiment. The juveniles are considered settled when they have lost their swimming ability and are dorsoventrally flattened.

II. Field observations of settlement

Oysters are cultured in open waters in the bay areas of Penghu. About 20 clusters are strung on a long rope, 20 cm apart, and the ropes are hung on buoys. The number of oysters varies in each cluster but averages in 20 individuals. The end of the ropes are free from the sea bottom. Oyster spats are transported to Penghu from the west coast of Taiwan in fall and winter.

The population of the oyster flatworm in both regular (cultured continuously on open bays) and hardened oysters (cultured in open bays in the winter as spats, transfered to intertidal beaches in spring and summer and moved to open bays again in the fall) were monitored monthly from September 1987 to August 1988 at Tsaiyuan, Penghu (Fig. 1).

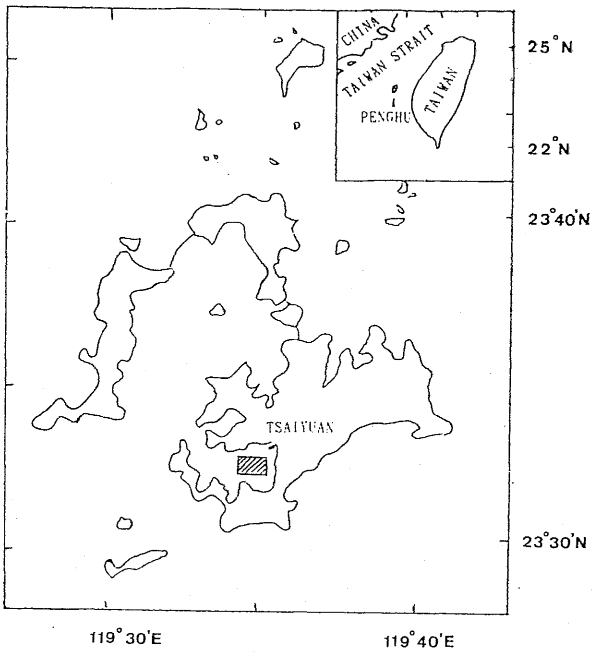

Fig. 1. Map of Penghu, showing the oyster-farming areas.

The oyster samples were obtained directly from oyster farmers. Since the flatworms hid on the empty oyster shells or inside the oyster-cluster, oysters within a cluster were separated individually by hand and then examined thoroughly with naked eyes. By using this method, the flatworms as small as 5 mm in diameter can be picked up easily. In addition, oyster spats and hardened oysters which were sampled monthly from December 1987 to April 1988, were bathed in fresh-water-prepared 6% MgCl solution to find newly settled juveniles as small as 2 mm in diameter. The density of the flatworms was expressed by the number of flatworms per rope.

Each flatworm, when contracted, is round in outline and resembles a pancake. Therefore, the size of an oyster flatworm is represented by its diameter under contraction. The size of flatworms is divided into three classes; small (diameter less than 10 mm), middle (diameter from 10 to 25 mm) and large (diameter greater than 25 mm). The reproductive status of the flatworms was divided into three classes: non-reproductive (absence of eggs in the uterus), gravid (presence of eggs in the uterus) and brooding (covering egg masses).

RESULTS

I. Induction of juvenile settlement

After hatching, the juvenile was in elongated oval form with a pair of brown rudimentary tentacles and 3–4 pairs of black eye spots. No marginal or tentacular eyes were observed. The morphology of the mouth and pharynx was similar to that of the adult. The body surface was ciliated and there were two caudal tufts (Fig. 2).

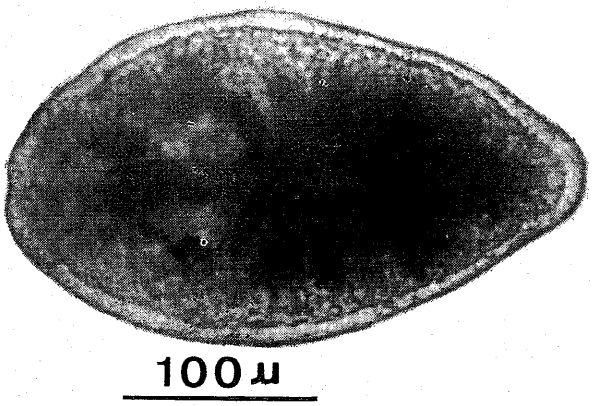

Fig. 2. A planktonic juvenile *Stylochus orientalis.*

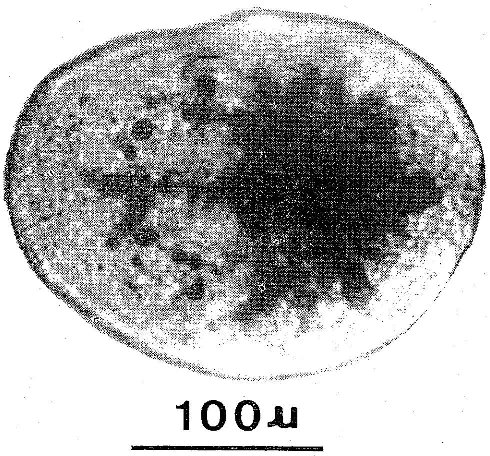

Fig. 3. A newly settled juvenile *Stylochus orientalis.*

The juvenile was positively phototactic, swimming by a rotating progression following a wide sine wave pattern. When oysters were present, the juvenile swam quickly in a small circle, the body became dorsoventrally flattened and lost its swimming ability eventually (Fig. 3).

(1) *Survival*

The number of planktonic juveniles that survived in the dishes with live oyster was significantly higher than that without live oyster (30±9 vs. 5.7±4.7; Anova, $F=17.2 \gg F_{0.05}$ (1, 4)=7.7; Table 1).

(2) *Settlement*

Planktonic juveniles settled only in

Table 1
Effect of live oyster on the survival of planktonic juvenile *Stylochus orientalis* at the fourth day of the experiment

Replicates	Number of juvenile survived	
	with oyster	without oyster
1	39	11
2	30	4
3	21	2
Mean, 1 SD	30±9	5.7±4.7

the media contained oyster juices. The mean percentage was significantly diferent among 5 groups (Anova, $F=20.7 \gg F_{0.001}$ (4, 10)=11.3). The percentage of settlement was positively correlated to the concentration of the oyster juice: 18.7, 1.9, 0.8 and 0.5 in 50, 30, 20 and 10 ppm of oyster juice, respectively ($r=0.8$, Table 2).

II. Field observations of settlement

Table 3 shows the field studies of population densities of the oyster flatworms from September 1987 to August 1988. The average densities of the flatworms on the regular oysters of 1986 stocks were 5, 6 and 23 ind./rope in September, October and November, respectively. The oysters sampled in September and October of 1987 were treated with freshwater in early summer to kill the flatworms. Only middle-sized and large flatworms were found in the samples of October and November. From October 1987 to March 1988, no flatworms were found on the hardened oysters of 1986 stocks examined.

In December 1987, an average of 24.5 ind./rope of newly settled juveniles were found on the oyster spats which were just transported to Penghu in October 1987. After the oyster spats were dried on the beach to kill the flatworms, the

Table 2
Effect of oyster extracts on the settlement of planktonic juvenile *Stylochus orientalis* at the seventh day of the experiment

Concentration of oyster extract (ppm)	Replicates	Number of juveniles		% of settlings	Mean (SD) of settling percentage
		planktonic	settled		
50	1	62	16	25.8	18.7
	2	41	8	19.5	(7.5)
	3	37	4	10.8	
30	1	103	1	1.0	1.9
	2	98	1	1.0	(1.6)
	3	56	2	3.8	
20	1	80	2	2.5	0.8
	2	76	0	0	(1.4)
	3	76	0	0	
10	1	93	0	0	0.5
	2	85	0	0	(0.8)
	3	68	1	1.5	
0	1	105	0	0	0.0
(control)	2	72	0	0	
	3	47	0	0	

Table 3
Monthly change of the population densities of the oyster flatworm *Stylochus orientalis* on different oyster stocks at Tsaiyuan, Penghu. Mean number and standard deviation per ropes are given when available and the number of ropes examined is given in parentheses

Stock		Date of oyster-spats transported to Penghu				
		Dec. 1986 Regular	Dec. 1986 Hardened	Oct. 1987 Regular	Dec. 1987 Regular	Jan. 1988 Hardened
1987	Sept.	5.0(8)*	—	—	—	—
	Oct.	6.0±2.6(3)*	0(2)	—	—	—
	Nov.	23.0±3.8(3)	0(2)	+	—	—
	Dec.	—	0(2)	24.5(2)#	—	—
1988	Jan.	—	0(2)	8.0(1)*	+	0(2)
	Feb.	—	0(2)	0.8±0.4(4)	1.0±1.3(7)	0(7)
	Mar.	—	0(2)	3.0±1.0(2)	0(2)	0(2)
	Apr.	—	—	0.5±0.5(2)	0(2)	0(2)
	May	—	—	1.2±1.0(5)	0(5)	—
	June	—	—	3.0±3.0(2), 3.5±2.5(2)#	28.5±0.5(2)#	—
	July	—	—	4.0±0.0(2)* 16.0±0.0(2)	4.5±3.5(2)* 23.5±1.5(2)	—
	Aug.	—	—	4.5±2.5(2)* 27.0±2.0(2)	5.0±1.0(2)* 24.5±2.5(2)	—

*: oysters were treated with fresh-water to kill the flatworms.
—: oysters were not present in the culture area.
+: no examination
#: flatworms were small, newly settled and immature.

number of flatworms dropped to 8 ind./rope in the following month, and then maintained low level of density until July. In June, a new crop of small, immature flatworms occurred with an average of 3.5 ind./rope. The population densities increased again to 16 and 27 ind./rope in July and August. The juveniles which settled on the regular oysters in December 1987 grew to about 2 cm in body diameter with a trace of ova in their uterus in February 1988. The flatworms grew continuously and spawned large amount of eggs in April. Most large worms collected after June were gravid and brooding eggs.

In the regular oysters of which the spats were transported to Penghu in December 1987, the population density of the flatworms was low, about 1 ind./rope or none from January to May 1988, then increased to 28.5 ind./rope in June. All flatworms were small and immature, indicating a new crop of settlement. The flatworm population densities were the same when the oyster were not treated (i. e., 23.5 and 24.5 in June and August 1988, respectively), but the population dropped to 4–5 ind./rope when the oysters were treated with freshwater. From January to April 1988, no flatworms were found on the oysters which were transported to Penghu in January 1988.

Two facts are revealed from these field data, (1) the two major periods of the settlement of planktonic juveniles were in the fall and early summer, and (2) the small settled flatworms of the fall matured and spawned in late spring of the following year, contributing to the juvenile population of the early summer.

DISCUSSION

The settlement of the planktonic juveniles of *Stylochus orientalis* is induced by oysters, the prey species of the adult flatworms. However, the success of settlement *in situ* is related to a number of factors. In the fall of 1987, small flatworms were found only on the oyster spats in October 1987, but not in hardened oyster in 1986 (see Table 3; 24.5 vs. 0 ind./rope). This suggests that the success of settlement is influenced by many factors and the age of oysters may be one of them. In addition, in June 1988, small flatworms occurred significantly more in regular oysters of December stock than in those of October stock (see Table 3; 28.5 vs. 3.5 ind./rope, Anova $F=96.1 \gg F_{0.05}(1, 2)=18.5$), but large flatworms occurred a little more in October stock than in December stock (3 vs. 0 ind./rope). Hyman (1951) mentioned that the worst enemies of the tubellarians are other tubellarians because they are highly cannibalistic, the larger ones tend to eat the smaller ones. Moreover, fouling organisms were more abundant on the oysters in October stock than on those in December stock (unpublished observation). Fouling organisms such as simple tunicates, small crabs and polychaetes may eat newly settled juveniles. Therefore, it is reasonable to infer that the presence of adult flatworms and/or fouling organisms may influence the success of recruitment.

Results from laboratory experiments revealed that the planktonic juveniles survive only within 3 weeks (Shu and Lin, 1980). In other words, the juveniles must settle down during this brief period. Because the planktonic juveniles are induced to settle down by the oysters, the population size of the oyster becomes a major control factor to the population size of the flatworms. Thus, the outbreak of oyster flatworms occurred in Penghu areas after ten years of commercial operation. At that time the average number of flatworms per rope was 25 in August, 1978 (Lin and Tang, 1980). Moreover, two strong typhoons struck Penghu in August-September 1986. The majority of the oyster buoys and oyster ropes were swept to land or to the sea bottom. The population of oysters decrease sharply. Before the typhoon, the average number of flatworms per rope was about 100 in June 1985 (Lin, personal communication) and 30 in June-July 1986 (personal observation). After the typhoon, the population densities of oyster flatworms dropped to 1 in June 1987 (personal observation) and 12 in June 1988 (this study). These suggest that the population density of the oyster flatworms is highly variable and may be easily affected by the availability of it prey, the behavior of settlement and the climate.

The prey organisms of the genus *Stylochus* consist of oysters, mussels, clams, gastropods and barnacles (Galleni *et al.*, 1980). *Stylochus mediterraneus* preys on oysters and mussels. Ferrero *et al.* (1980) showed through the choices in a Y-maze system, that individuals of *S. mediterraneus* were attracted chemically towards its prey and those individuals collected from mussel beds had no significant preference for water conditioned by the oyster, *Ostrea edulis*, and even significantly avoided water which had percolated through *Crassostrea gigas*. Thus, this predator-prey interaction may arise as a result of "ingestive conditioning." More studies are required to detect whether or not this ingestive conditioning starts as early as the inducing settlement.

Acknowledgements: The authors thank the Council of Agriculture, Republic of China for financial support (76 ARDP-8.1-FID-19F, 77ARDF-7.1-FID-23E, 78ARDP-7.1-FID-05P) and Drs. F.-S. Chia and John M. Lawrence for their suggestions and comments.

REFERENCES

Bock, S. (1913) Studien uber Polycladen. *Zool. Bidrag.* **2**: 31-344.

Burke, R. D. (1983) The induction of metamorphosis of marine invertebrate larvae: stimulus and response. *Can. J. Zool.* **61**: 1701-1719.

Burke, R. D. (1986) Phermones and the gregarious settlement of marine invertebrate larvae. *Bull. Mar. Sci.* **39**: 323-331.

Chia, F. S. and M. E. Rice 1978. *Settlement and metamorphosis of marine invertebrate larvae*, Elsevier, New York. 290pp.

Ferrero, E., P. Tongiorgi, L. Galleni, U. Salghetti and P. Salvadego (1980) Chemical attraction of *Stylochus mediterraneus* Galleni, 1976 (Turbellaria: Polycladida) toward its prey *Mytilus galloprovincialis* L. *Mar. Biol. Let.* **1**: 213-224.

Galleni, L., P. Tongiorgi, E. Ferrero and U. Salghetti (1980) *Stylochus mediterraneus* (Turbellaria: Polycladida), predator on the mussel *Mytilus galloprovincialis*. *Mar. Biol.* **55**: 317-326.

Hyman, L. H. (1951) *The Invertebrates: Platyhelminthes and Rhynchocoela, The acoelomate Bilateria.* Vol. II, McGraw-Hill, New York.

Lin, Y. S. and H. C. Tang (1980) Biological studies on cultured oyster in Penghu. *Bull. Inst. Zool., Academia Sinica* **19**: 15-22.

Pawlik, J. R. and D. J. Faulkner (1986) Specific free fatty acids induce larval settlement and metamorphosis of the reef-building tube worm *Phragmatopoma californica* (Fewkes). *J. Exp. Mar. Biol. Ecol.* **102**: 301-310.

Pearse, A. S. and G. W. Wharton (1938) The oyster "leech" *Stylochus inimicus* Palombi, associated with oysters on the coast of Florida. *Ecol. Monogr.* **8**: 605-655.

Rho, S. (1976) Studies on the polyclad Turbellaria of Korea. 1. Studies on the damage of cultured oysters in Yeosu coast by predation of polyclads, *Stylochus* sp. and *Pseudostylochus* sp. *Bull. Fish. Res. Dep. Ag. Pusan*, **15**: 111-124.

Ruppert, E. E. (1978) A review of metamorphosis of turbellarian larvae. *In: Settlement and metamorphosis of marine invertebrate larvae* (F. S. Chia and M. E. Rice, eds.). Elsevier, New York. pp. 65-81.

Shinn, G. L. (1987) Phylum Platyhelminthes with an emphasis on marine Turbellaria. In: *Reproduction and development of marine invertebrates of the Northern Pacific coast.* (M. F. Strathmann ed.). University of Washington Press, Seattle and London. pp. 114-128.

Shu, J. U. and Y. S. Lin (1980) Biological studies on the oyster predator, *Stylochus inimicus. CAPD Fisheries Seried No. 3, Reports on Fish Disease Research (III)*: 39-51, Taiwan, R. O. C.

Sokal, R. R. and F. J. Rohlf (1981) *Biometry, 2nd edition.* W. H. Freeman and Company, San Francisco. 859pp.

Ventilla, R. F. (1984) Recently development in the Japanese oyster culture industry. *Adv. Mar. Biol.* **21**: 1-57.

牡蠣扁蟲 *Stylochus orientalis* Bock 浮游稚蟲的附著及補充

陳章波 蔡萬生 劉繼源 李坤瑄

牡蠣扁蟲 *Stylochus orientalis* 的浮游稚蟲受其成蟲所捕食的牡蠣 *Crassostrea gigas* 之誘引而附著。在海域中，秋天及早夏爲二個主要附著期，秋季附著的稚蟲在次年春發育成熟產卵、孵化之稚蟲於同年早夏附著。附著是否成功受到牡蠣之年齡、牡蠣殼上之附著生物量及扁蟲成體之影響。