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NEGATIVE EFFECT OF γ-AMINOBUTYRIC ACID (GABA) ON THE SETTLEMENT OF LARVAE OF THE SMALL ABALONE, *HALIOTIS DIVERSICOLOR SUPERTEXTA* LISCHKE[1]

LI-LIAN LIU, LIH-SING CHU, KUN-HSIUNG CHANG

National Sun Yat-Sen University

AND

FU-SHANG CHIA

Faculty of Graduate Studies and Research
University of Alberta

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Li-Lian Liu, Lih-Sing Chu, Kun-Hsiung Chang and Fu-Shang Chia (1985) Negative Effect of γ-Aminobutyric Acid (GABA) on the Settlement of Larvae of the Small Abalone, *Haliotis Diversicolor Supertexta* Lischke. *Bull. Inst. Zool., Academia Sinica* **25**(1): 1-5. This paper reports a negative effect of using γ-aminobutyric acid (GABA), a settlement-inducing agent, on the small abalone of *Haliotis diversicolor supertexta.* The experimental results show that a high GABA concentration (10^{-3}, 10^{-4} M) might stimulate the settling rate at 24 hours but will kill all abalones after 72 hours. The treatments with lower GABA concentration, 10^{-5} and 10^{-6} M, has no effect on the settling as the same as the contrast experiments of 0 M. The larvae begin settling after 12 hours of fertilization and exceed 90% settling after 48 hours.

There are many substances which have been found to be able to induce the settling and metamorphosis of the larvae of red abalone, *Haliotis rufescens*, ex. γ-aminobutyric (GABA), γ-hydroxybutyric acid, ϵ-amino-*n*-caproic acid. δ-amino-*n*-valeric acid, red algae (*Lithothamnium* sp. and *Lithophyllum* sp.) (Morse, 1979), algal extracts and cyanobacteria crude extracts etc. (morse and morse, 1984; morse *et al.*, 1984). Among these chemicals, GABA, a neurotransmitter, is the one used most frequently to study its effect on many mollusks. Those organisms which show positive reactions to the GABA include: *Haliotis* spp. (Morse, 1984); *Trochus* spp., *Aplyia* spp., *Mopalia* sp. (Morse, 1982), and *Katharina tunicata* (Rumrill and Cameron, 1983) etc. The only exception is the *Conus* sp. which has been proved unresponsive to the GABA and its congeners (Morse, 1979). Since to increase the settlement rate of *H. diversicolor supertexta*, an economical important species in Taiwan, can improve the productivity of this small abalone significantly, it is important to study whether the settlement-inducing chemicals can react on this local abalone species or not.

On the development of *H. diversicolor supertexta*, at the temperature of 23-24°C, the trochophore hatch within 10 hours after fertilization, and start to swim on the surface water. The veliger settle to the bottom within 36-40 hours after fertilization which lasting

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4-6 days and then begin to metamorphosis. (Chu, 1984).

In the natural environments, the small abalone is often found to inhabit crytic sites under the boulders which dispersing on sandy bottom from low tide level up to a depth of about 20 meters. It usually feeds the algae at night. Chiang and Lai (1976) have indicated that the small abalone does not have specific preference on the species of algae. They can eat different kind of algae, such as brown, green or red algae.

MATERIALS AND METHODS

Mature broodstock of small abalones were collected from shallow cultural ponds constructed on the intertidal rocky shore on the mid-eastern Taiwan.

Spawning induction was accomplished by exposing mature abalones in the air about 40 minutes and then adding UV-treated seawater with increasing the water temperature from 21°C to 26°C (at the rate of 1°C/h). The male and female abalones were held in separate containers during the about procedures.

After spawning, the ova were collected by siphon and put into 2-liter beaker. Fertilization occurred within 30 minutes after spawning. Excess sperms were removed by gently washing the eggs six times. Following the last decantation, the culture container was refilled by the 0.7 μm filtered, UV-treated, 33‰ and 75 ppm dihydrostryptomycin (Sigma Co., USA) seawater. Finally, they were incubated in the waters of 24±1°C for 10 hours to develop the larvae to early veliger stage.

After hatches, these veliger larvae will ascend to the culture surface.

To determine the effects of GABA on larval settlement, experiments were carried out by using 10 hours larvae. Treatments are divided into six conditions: 0, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}M of GABA (filtered nature seawater adding antibiotics) and 0M GABA (artificial seawater prepared according to standard methods [Rand, *et al.,* 1976] and adding antibiotics). Each treatment had duplicate and was arranged in a total of twelve acrylic containers (6×9×2.5 cm) each of which filled with 100 ml seawaters conditioned as mentioned previously. Finally, 208±5 larvae were added into each container.

The numbers of settling larvae were recorded in a 6-hour intervals from 12 to 48 hours. The X'-independence test was used to analyze the collected data (Yeh, 1977).

RESULTS

The results of the settlement rate experiments are shown in Fig. 1. Larvae began to settle after 12 hours of fertilization at all cultures and exceeded 90% settling after 48 hours. There were no significant differences found amoung the cultures of GABA 10^{-5}, 10^{-6}, 0M and artificial seawater from 12 hours to 48 hours (Table 1). And the use of pairing *t*-test indicated that there is no significcant difference within duplicate ($p>0.01$).

TABLE 1

χ^2-independence test on the settling larvae of *Haliotis diversicolor supertexta*

* $p<0.01$

Source	Hours						
	12	18	24	30	36	42	48
Artificial seawater vs. 0 M	0.16	6.11	0.04	0.02	0.04	0.21	0.01
Artificial seawater vs. 10^{-6} M	1.34	0.09	1.25	1.69	0.66	1.47	4.54
Artificial seawater vs. 10^{-5} M	0	4.89	2.61	1.01	3.07	1.75	0.61
Artificial seawater vs. 10^{-4} M	5.55	0.68	47.60*	11.68*	3.67	5.12	0.02
Artificial seawater vs. 10^{-3} M	3.14	0.12	61.62*	2.85	0	5.97	0.02

(settling larvae: sum of duplicate)

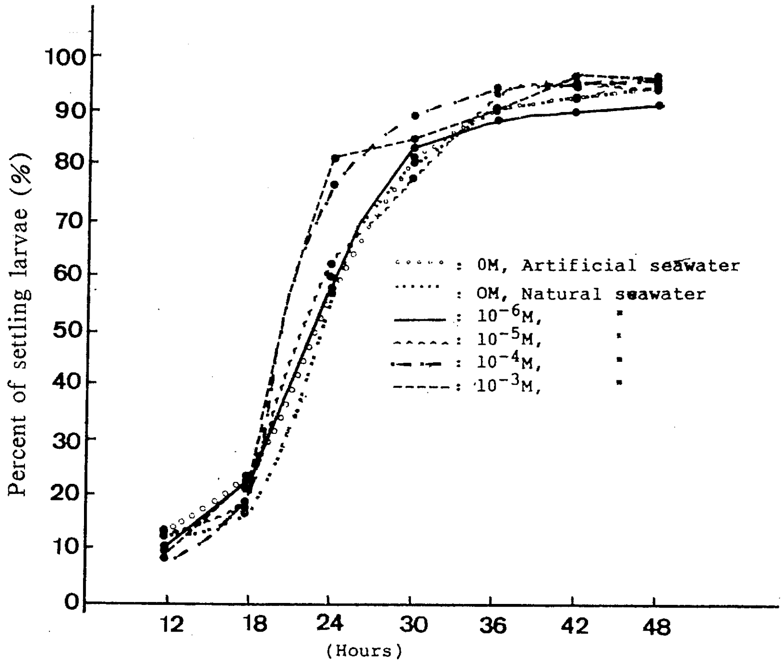

Fig. 1. Effects of GABA on mean settling rate of *Haliotis diversicolor supertexta* larvae.

Although, the settling rates of high GABA concentration cultures (10^{-3}, 10^{-4}M) were significantly higher than that of artificial seawater cultures after 24 hours. (Fig. 1 and Table 1: $X^2=61.62$ for 10^{-3}M and $X^2=47.60$ for 10^{-4}M, $p<0.01$). The settling larvae all died after 72 hours. Other cultures still have 60%–70% survival rate and begin to metameorphose and to grow peristomal shell after 4–6 days. This result suggests that the GABA doesn't induce the settling of small abalone successfully since their toxic effect when using higher dosage.

DISCUSSION

Morse (1979) have indicated that the larvae of chiton *Mopalia muscosa*, red abalone *H. refuscens* (herbivorous molluscks) are all induced to settle and metamorphose by GABA and by intact *Lithothamnium* and *Lithophyllum* spp. The larvae of *Conus* sp. (carnivorous mollusck) is the only exception that has been proved unresponsive to these algae and GABA or its congeners. With the results he mentioned that the lock-and-key mechanism of sterochemically specific induction of settling and the related interactions between abalones and their crustose coralline red algal hosts apparently reflect the intimate and adaptive coevolution of these planktonically dispersed animal herbivores and their algal substrates.

The time for the veliger of small abalone to reach swimming stage only about 12–36 hours (Fig. 1), but took after settling to the bottom, it elapsed about 4–6 days to metamorphosis at temperature of 23–24°C (Chu, 1984). The larvae of *H. gigantea* swim incessantly 7–10 days then settle and metamorphose at the temperature of 16–18°C (Marayama, 1935). In *H. discus*, larvae settle and metamorphose at 10 days after swimming about 4–7 days at 16–18°C. The *H. sieboldii* has 6–10 days swimming stage, then, settles and metamorphose after 10–11 days (Ino, 1952). Iwata (1980) also showed that of swimming stage, then the *H. discus hannai* has 5–6 days, settles and metamorphoses about 10 days at temperature 20°C. The negative reaction to the GABA of the *H. diversicolor supertexta* contrasts to the other positive experiments results of other abalones might be due to its different evolutionary strateqies reflected on the physiological ecological traits. For example, a comparison of the rate of settlement and metamorphsis among different species of *Haliotis* indicates that the small abalone has a short swimming and a long creeping periodl.

The characters of shorter swimming stage and faster settling of the small abalone than other species, might be related to its special adaption to the local environments. On the east coast of Taiwan, the slope of sea bottom drops drastically to leave a very narrow shelf area. Most rocks or boulders, which makes the shelters for the small abalone, are even distributed locally and closely to the shore line within about 500 meters. Beyond this range, the sea bottom is all sand. In this narrow zone, most macroalgae flourish seasonlly on the rocks to form a verdant "algae bed" on the sea floor (Peng *et al*, 1984). Therefore, a relatively shorter pelagic larva stage might be favorable to this species of abalones to live in this kind of *r*-selected environment when compare to the other big abalones.

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γ- 丁氨酸 (GABA) 對九孔苗附着之負效應

劉莉蓮　朱麗馨　張崑雄　賈福相

本研究報導一種附着誘導劑，γ- 丁氨酸，(γ-amino butyric acid, GABA), 並無誘使九孔苗 (*Haliolis diversicolor supertexta*) 附着之效果。本實驗結果顯示高濃度 GABA (10^{-3},10^{-4}M) 確能在 24 小時內促進九孔苗之附着，但却會在 72 小時後殺死所有的九孔。而 GABA 在低濃度 10^{-5} 和10^{-6}M 時，其作用一如 OM 一般，完全不具誘導作用。本種九孔之幼苗係在受精後 12 小時開始着苗，然後在 48 小時後達到 90%以上。