Title: An oviposition alarm system for the crab *Eriocheir japonica*

Name of author: Tai Hung Lee

Postal address: Laboratory of Aquaculture Genetics and Genomics
Faculty of Fisheries Sciences
Hokkaido University
3-1-1 Minato-cho
Hakodate, Hokkaido
041-8611, Japan

Abstract

This paper describes an electronic system for obtaining mature, unfertilized eggs from decapods. The system was a by-product of the author's many years of research on *in vitro* artificial fertilization in crabs (brachyurans). Based on the results of multiple tests, the system has proved to be highly utilizable for research involving the use of mature, unfertilized eggs. The greatest advantage of this system is that it saves researchers a tremendous amount of time and trouble, as the collection of mature, unfertilized eggs is an extremely exhausting procedure that requires 24-hour monitoring over a period of days or weeks. Although the system was originally designed for research with the fresh water mitten crab *Eriocheir japonica*, it can be used with other decapods with slight modifications.

Keywords: Oviposition; alarm; artificial fertilization; crab; decapod; *Eriochier*

Introduction

Success in *in vitro* artificial fertilization in crabs (brachyurans) was first reported in the Chinese freshwater mitten crab *Eriocheir sinensis* (Lee and Yamazaki, 1989). *In* *vitro* artificial fertilization research of any species involves the collection of mature, unfertilized eggs, which is an extremely exhausting procedure as it requires 24-hour monitoring of the oviposition of female crabs over a period of days or weeks. To resolve this problem, the author invented an electronic alarm system. Although it was originally designed for research with the fresh water mitten crab *Eriocheir japonica*, the system can be used with other decapods with slight modifications.

Design, materials and procedure

This alarm system serves two functions: to inform researchers of the occurrence of oviposition and at the same time to keep almost all of the ovulated eggs inside the ovary of the female crab *Eriocheir japonica* until they are taken out for various research purposes.

The system mainly consists of a wireless transmitter/receiver set (Noatek's Yobidashikun NE-755 or similar models) and an electronic switch sensor (Fig.1). The transmitter/receiver set is an ordinary beeping device (Fig.2) available at do-it-yourself stores for people who are in need to send signals for help. The electronic switch sensor is a simple circuit as those described in general books on electronic appliances. The circuit (Fig.3) is connected to the main switch of the transmitter. When the poles of the switch sensor come into contact with an electric conductor such as the eggs of a crab, the switch will automatically go on and the transmitter will immediately send a signal to the receiver. When the receiver catches the signal, it will turn on the alarm system to make a sound and emit light. One may also attach a timer and a telephone to the receiver so that when the receiver catches the signal, it will start the timer and at the same time dial the phone number pre-entered by the researcher. This will allow the researcher to be immediately informed when oviposition occurs even if he or she is away from where the experiment is being conducted.

The most important part of this system is the complex of the poles and its attachment to a gonopore of the female crab. The complex consists of the poles of a sensor, a pocket made by cutting off the opening part of a rubber balloon (Fig. 4) and a piece of cushion. The poles are inserted into the rubber pocket through its cut end. The cut end is then completely sealed with instant adhesive and the poles are fixed inside the pocket also with instant adhesive (Fig. 5). Then the pocket was coated with a layer of synthetic rubber (polychlroprene) to increase its water-proofness. Prior to adhering the opening of the pocket to the gonopore, a cushion made of waterproof soft rubber sheet is attached to the gonopore with instant adhesive (Fig. 6). Without the cushion, it will be difficult to firmly attach the open end of the pocket to the gonopore because of the uneven surface of the area around the gonopore. A hole, which is smaller than the opening of the rubber pocket, is made at the center of the cushion for the passage of the extruded eggs. The opening of the pocket is attached to the cushion with instant adhesive (Fig. 7).

Several points should be noted here. First, when attaching the opening of the pocket to the cushion, the air inside the pocket must be squeezed out in order to make room for the extruded eggs to flow into the pocket and come into contact with the poles. Second, the attachment of the complex of the poles must be waterproofed, which requires utmost care and skills in using the instant adhesive. Finally, the female crab must be placed in a fixed position to ensure that the attaching procedure goes smoothly.

In order to prevent extrusion of eggs from the other gonopore during oviposition, the other gonopore is sealed by attaching a small piece of soft rubber sheet upon it with instant adhesive (Fig. 6).

After attaching the complex of the poles to one of the gonopores and sealing the other gonopore, the transmitter and the switch sensor compacted in a waterproof plastic case is then attached to the back of the female crab (Fig. 8). Because the surface of the back of the crab is uneven, a cushion made of soft rubber sheet is attached between the transmitter and the back of the crab.

Testing and discussion

The system was tested eleven times. It succeeded ten times in informing the occurrence of oviposition by different female crabs. Eggs collected from the ovaries of the female crabs were used for *in vitro* fertilization and the fertilized eggs were later observed under light microscopy (Fig. 9). In these tests, the average rate of fertilization, which was based on cleavage rates, was over 99%. The only time the system failed and caused a false alarm was because the attachment of the complex of the poles was not completely waterproofed. In addition, this system did not quicken or delay oviposition. Based on the results of the testing, it can be concluded that the alarm system is highly utilizable.

Even though this system was originally designed for monitoring the oviposition of the crab *Eriocheir japonica*, it has great potential to be utilized for other decapods as well with slight modifications such as making the system lighter or smaller, or using a different kind of poles. For example, the fact that the operculum (Hartnoll, 1968; Lee and Yamazaki, 1990) in lobsters, prawns or crabs such as the family *Portunidae* is absent at the gonopore may allow mucus from the vagina of the female to flow into the pocket and come into contact with the poles, resulting in a false alarm. In such cases, one may consider replacing electric conductor-driven poles with poles driven by the mechanical pressure caused by the extrusion of eggs.

References

Hartnoll R. G., 1968, Morphology of the genital ducts in female crabs. J. Limn. Soc. (Zool). 47, 279-300.

Lee T H., Yamazaki F., 1989. Cytological observations on fertilization in the Chinese fresh-water crab, *Eriocheir sinensis*, by artificial insemination (*in vitro*) and incubation. Aquaculture 76, 347-360.

Lee T H., Yamazaki F., 1990, Structure and function of a special tissue in the female genital ducts of the Chinese freshwater crab *Eriocheir sinensis*. Biol. Bull. 178, 94-100.

Figure captions

Fig. 1. Schematic illustration of the oviposition alarm system.

Fig. 2. Wireless transmitter and receiver set available at do-it-yourself stores.

Fig. 3. Schematic illustration of the wireless transmitter and part of the electronic switch sensor compacted in a waterproof plastic case.

Fig. 4. Rubber pocket made by cutting the opening part out of a rubber balloon.

Fig. 5. The structure of the poles complex. (A) Poles of the sensor made by exposing the copper wires at the tops of the electric wires; (B) Poles inside the rubber pocket.

Fig. 6. Attachment of a cushion on one gonopore and sealing the other gonopore by attaching a piece of soft rubber sheet upon it. C=cushion rubber sheet; G=gonopore with operculum; S=sealing rubber sheet

Fig. 7. Attachment of a poles complex on the cushion. (A) Extended abdomen exposing the ventral thorax; (B) Abdomen covering the ventral thorax.

Fig. 8. Attachment of a waterproof plastic case contained the wireless transmitter and the electronic switch sensor on the back of a crab. C=cushion rubber sheet; Ca=case; W=electric wire of the poles.

Fig. 9. *In vitro* fertilization using the eggs collected with the help of the oviposition alarm system described in this study. (A) The entering of a spermatozoon into an egg; (B) Cleavage of a fertilized egg. C=cleavage furrow; F= fertilization membrane; Fc=fertilization cone; S=spermatozoon. Bar(A)=50 μm, Bar(B)=150 μm

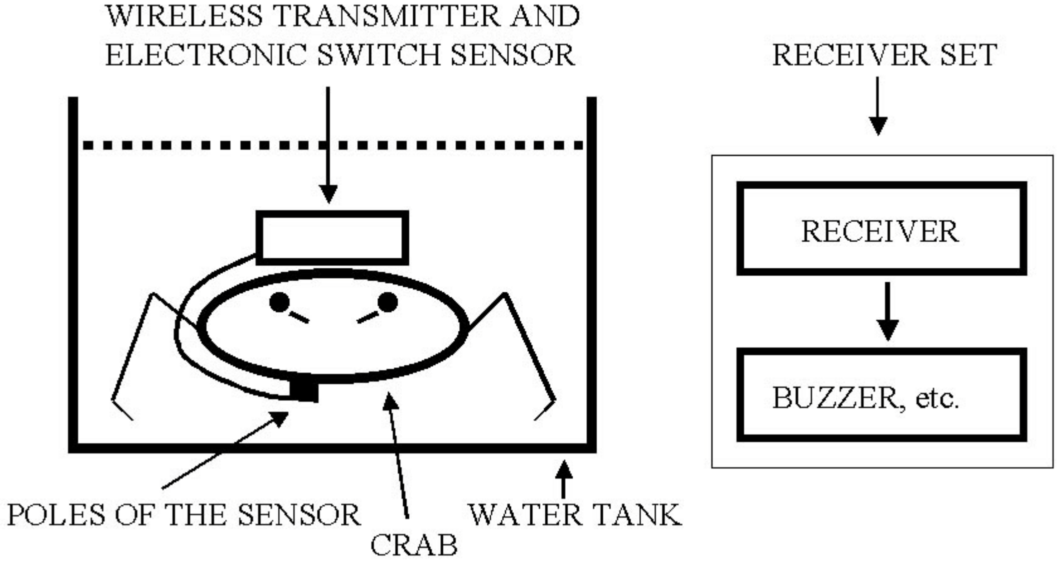
WIRELESS TRANSMITTER AND
ELECTRONIC SWITCH SENSOR
RECEIVER SET
RECEIVER
BUZZER, etc.
POLES OF THE SENSOR
CRAB
WATER TANK

Fig. 1

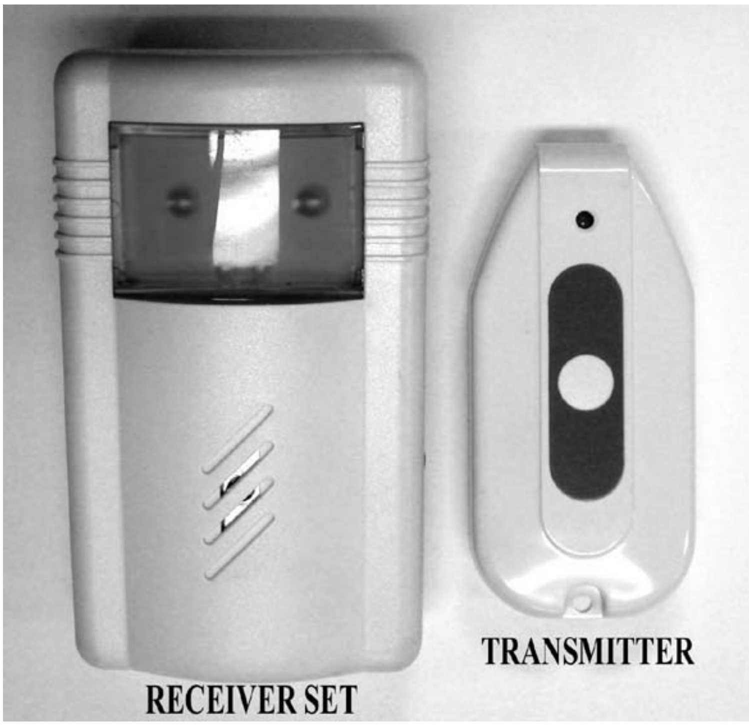
TRANSMITTER
RECEIVER SET

Fig.2

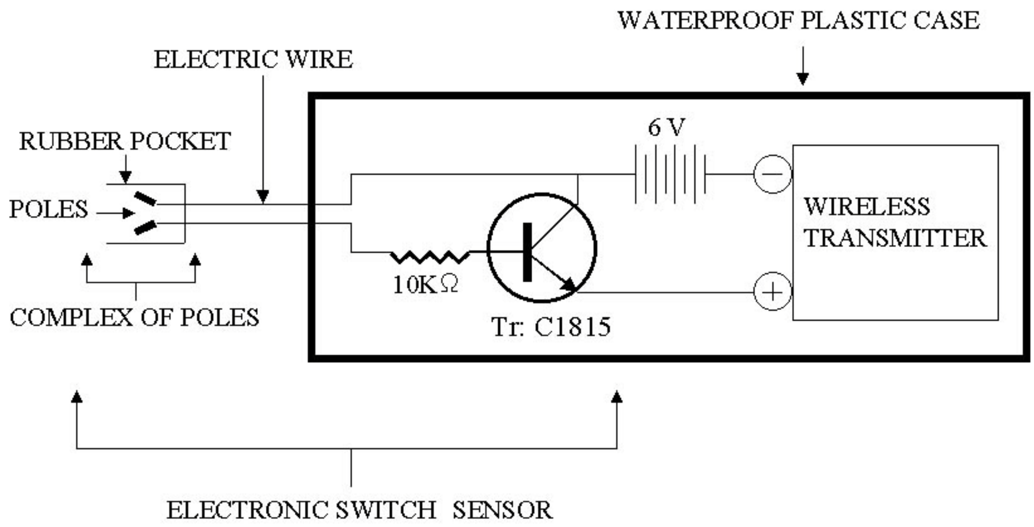
WATERPROOF PLASTIC CASE
ELECTRIC WIRE
RUBBER POCKET
POLES
6 V
WIRELESS
TRANSMITTER
10KΩ
Tr: C1815
COMPLEX OF POLES
ELECTRONIC SWITCH SENSOR

Fig.3

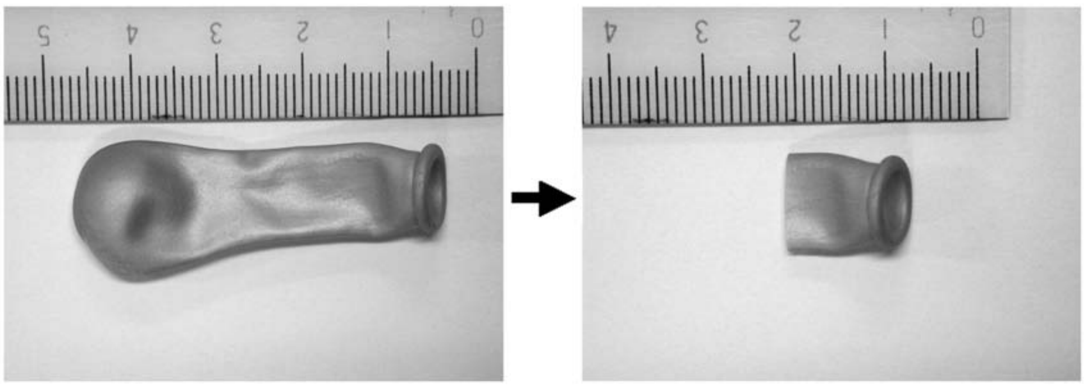

Fig.4

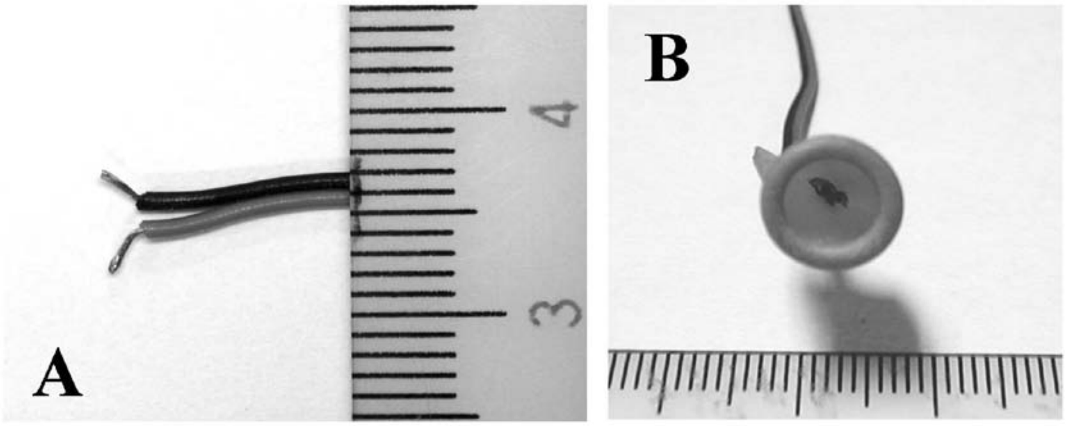

Fig.5

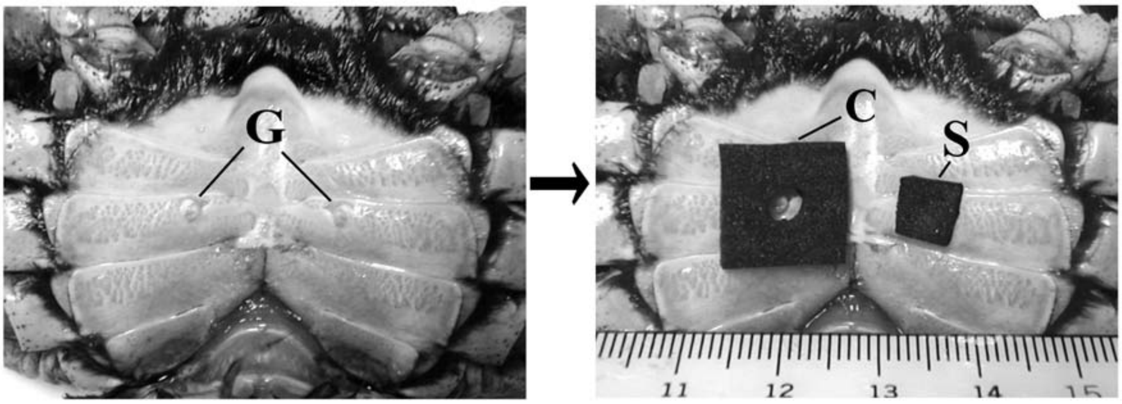

Fig.6

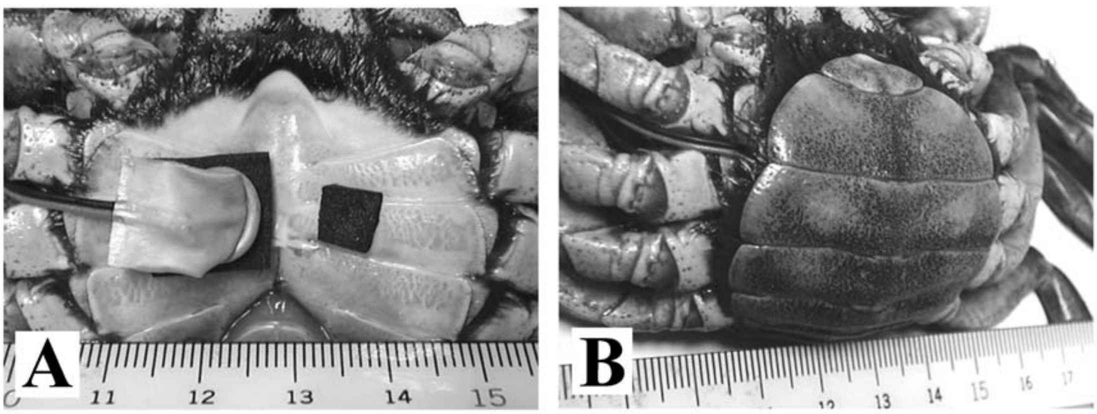

Fig.7

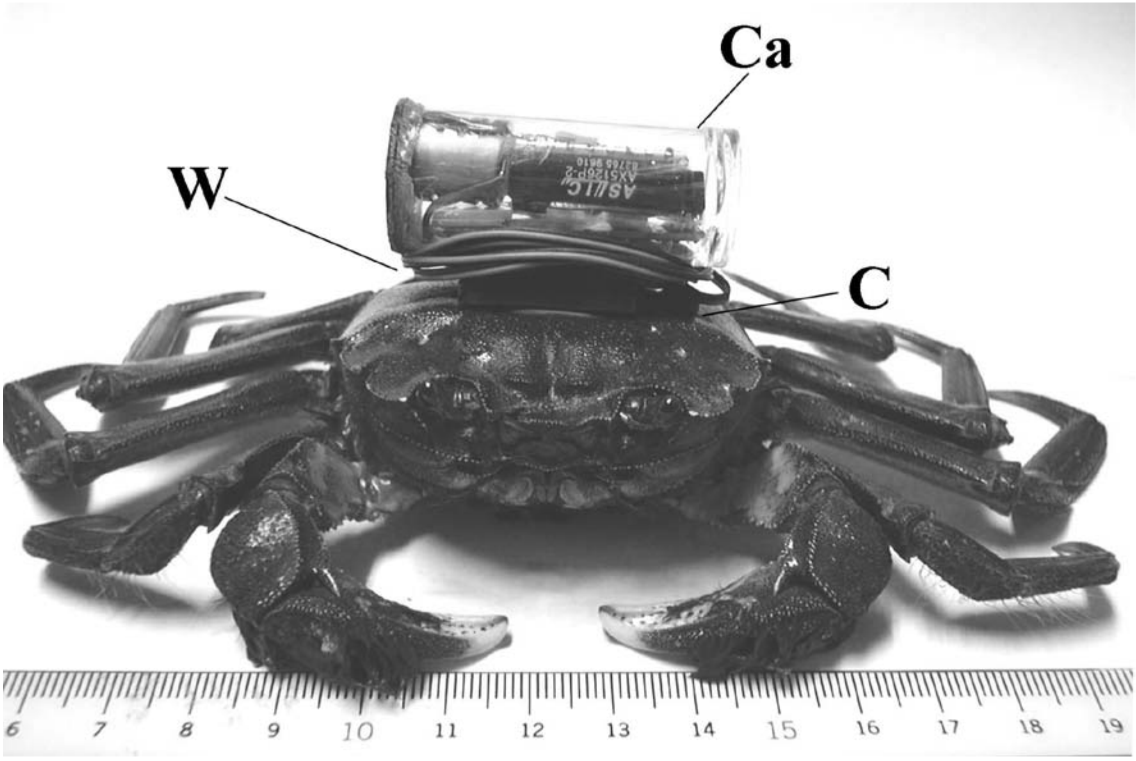

Fig.8

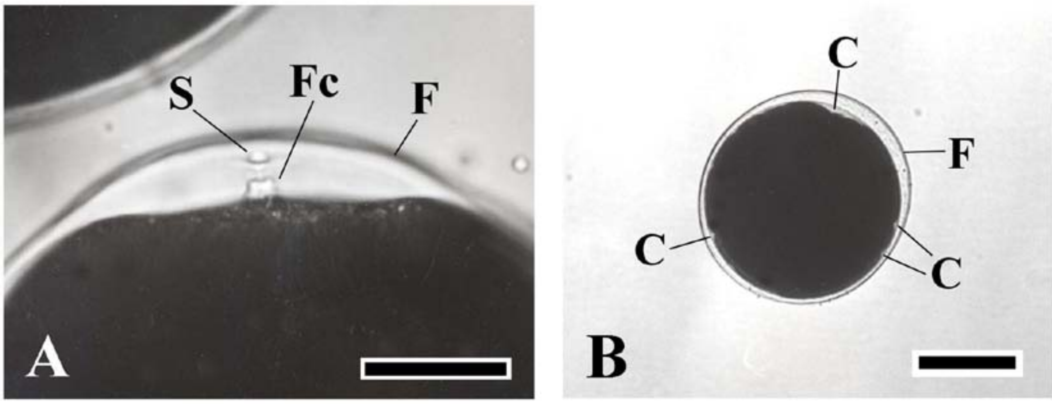

Fig.9