CHAPTER 2

MATERIALS AND METHODS

1. Description of the study area

This study was carried out at Sichang Island (Fig.1), inner part of the Gulf of Thailand (13°09'N, 100°40'E). Average salinity recorded in the area ranged from 32.18 ppt during summer to 31.23 ppt during the rainy season (Siripong, 1984). Temperature recorded ranged from 28.89°C during the summer to 26.88°C during the winter (Siripong, 1984). After heavy precipitation, salinity may be reduced to less than 20 ppt but this condition will remain in a short period.

Three types of climatological condition annually prevail in the Gulf of Thailand.

1. Southeast monsoon or Southeast trade wind, usually occurs from February to April, which brings warm, dry air from South China Sea.

2. Southwest monsoon usually occurs between May and September, brings warm and moist air, and causes heavy precipitation.

3. Northeast monsoon, usually occurs from October to February causes heavy turbulance and strong winds (Menasveta *et al.*, 1986).

The climatological conditions of Sichang Island and some areas of the eastern coast of the Gulf of Thailand during the last 30 years are summarized and shown in Table 1. Precipitation in the Gulf of Thailand is high during the Southwest monsoon. On Sichang Island, the average maximum precipitation was 287.2 mm in September and average

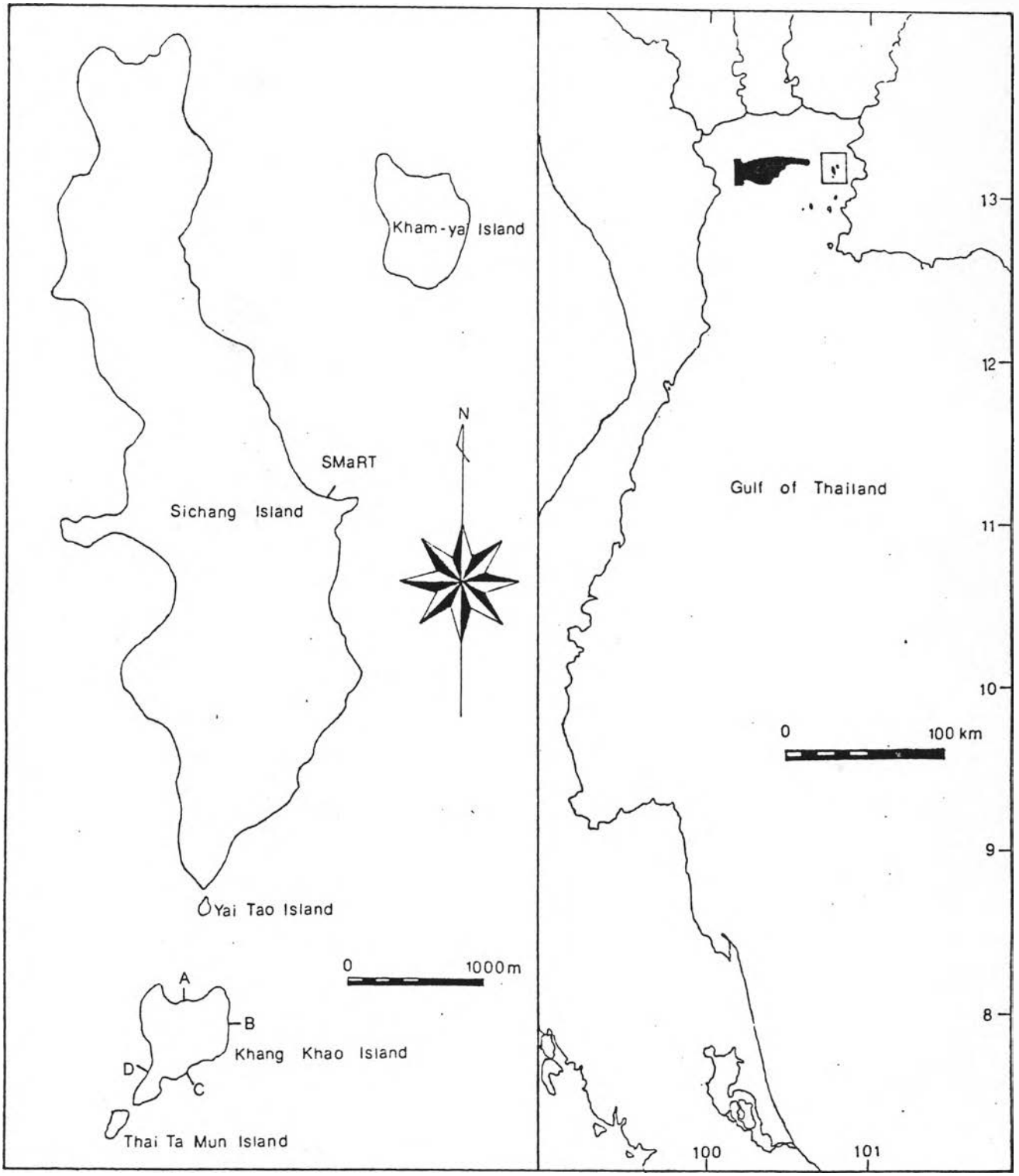


Fig. 1 Map showing the study area. General survey was done at four stations (St. A, B, C & D) on Khang Khao Island. Behavioural study was done at station B. on Khang Khao Island and station SMaRT on Sichang Island

Table 1. Climatological data of the eastern coast of the Gulf of Thailand 1951-1980 (Umnuay, 1985)

Station	Average Rainfall, mm			Average Evaporation, mm			Relative Humidity, %			Prevailing wind direction (period) Ave. in Knots	
	Max. (month)	Min. (Month)	Annual	Max. (Month)	Min. (Month)	Annual	Max. (Month)	Min. (Month)	Mean		
Chon Buri	298.3 (Sep)	8.5 (Dec)	1348.4	N.A.	N.A.	N.A.	92.6 (Oct)	48.6 (Dec)	74.0	E(Jan)	5.8
										S(Feb-Sep)	4.8-6.6
										NE(Oct-Dec)	4.6-6.1
Sattahip	283.5 (Oct)	15.0 (Dec)	1321.6	N.A.	N.A.	N.A.	93.1 (Oct)	51.9 (Jan)	77.0	N(Jan, Oct-Dec)	5.4-6.6
										S(Feb-May)	6.6-7.4
										SW(Jan-Jul)	9.0-9.2
										WSW(Aug-Sep)	7.0-8.6
Sichang Island	287.2 (Sep)	7.5 (Jan)	1223.3	N.A.	N.A.	N.A.	88.4 (Oct)	55.5 (Dec)	73.0	W(Jan-Mar, May-Sep)	5.7-7.5
										S(Apr)	6.7
Chanthaburi	534.9 (Sep)	11.8 (Dec)	2977.2	157.0 (Dec)	98.4 (Sep)	1533.3	97.0 (Sep)	50.4 (Jan)	82.0	NE(Jan, Oct-Dec)	2.7-5.3
										S(Feb-May)	2.0-2.8
										SW(Jun-Sep)	1.8-2.7
Khlong Yai	1072.1 (Aug)	21.3 (Dec)	4763.9	160.7 (Mar)	88.1 (Jul)	1514.4	96.1 (Sep)	54.5 (Dec)	79.0	NE(Jan, Mar-Apr, Nov-Dec)	4.6-5.3
										W(Feb, May-Oct)	4.2-5.0

N.A., not available

anual precipitation was 1222.3 mm. Evaporation was usually high during March and April, due to the dry and warm air. The mean relative humidity ranges from 73.0-82.0%. Sichang Island has the lowest mean relative humidity.

The northeasterly wind previals in the Gulf of Thailand from October to January and the Southwesterly winds previals from February to September. At Sichang Island, the westerly wind previals from January to March and May to September at a speed of 5.7-7.5 knots. The Southwesterly wind previals in April at a speed of 6.7 knots. The Northeasterly wind previals from October to December with a speed of 5.8-8.4 knots.

Tides at the Sichang Islands are of mixed-cycle. Semi-diurnal were predominant but had a large difference in daily fluctuation. There were some days with diurnal cycle. From tide table published by the Hydrographic Department, Royal Thai Navy, the maximum tidal range in 1985-1986 was 0.3-4.1m, the higest tide was 4.2 m and lowest was 0.3m above the chart datum. The level of the highest and lowest tides were relatively constant throught out the year. Tidal current of this area flows from the south to the north during high tide and flows backward at low tide. The current flow speed is relatively high when the tide is begining high and low during the spring tides.

Field survey was carried out at four stations on Khang Khao Island (Fig.1), A, B, C and C, where the coral community was previously examined (Sakai *et al.*, 1986 ; Tsuchiya *et al.*, 1986 ; Kamura & Choonhabandit, 1986). Profile and composition of substrates of each

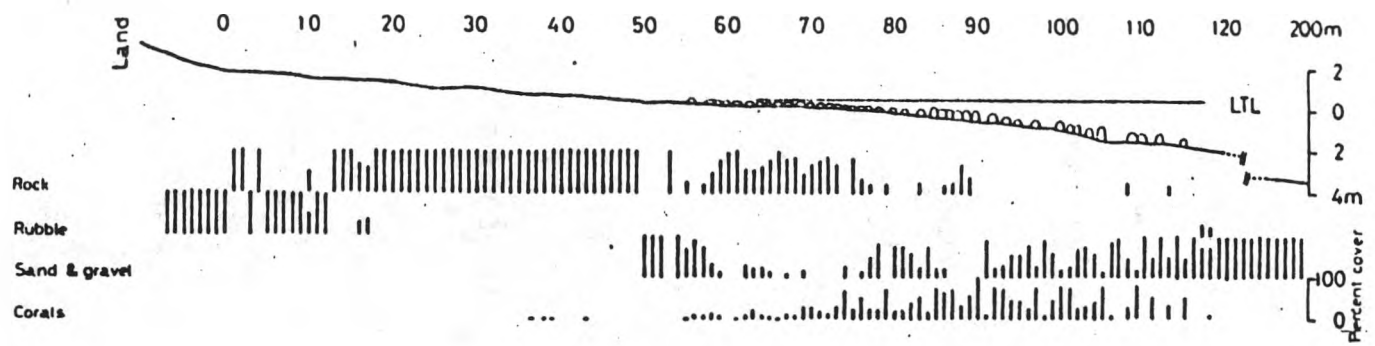
station were reported by Kamura & Choonhabandit (1986) (Fig,2). Brief description of the given stations are as follow :

Station A

Station A situated in the northern part of the Island, the effects of wind and wave on this side are lower than the other stations through out the year. Only during the Northeast monsoon that this side is affected. Coral community of Station A was relatively broader in distribution than that of the other stations. The shallower part of this station was limestone, rock and rubble. The deeper part was sand and corals with the coral zone about 60m wide. *Porites lutea* accounted for almost all coral coverage (Sakai *et al.*, 1986). The associations between the gobiid fishes and alpheid shrimps were found along the coral zone and in area surrounding the coral zone.

Station B

This station is in the eastern part of the Island. The effects of wind and wave on this station are moderate. Strong wind and waves occur during the Southeast and Northeast monsoons. The shallower part of this station was dominated by rock and living corals, *Pavona frondifera* as the dominant species. Coral zone was relatively narrow, about 20m wide. *Porites lutea* had the largest coverage and *Pavona decussata* was the next most abundant (Sakai *et al.*, 1986). The associations between the gobiid fishes and alpheid shrimps were found only in the areas surrounding the coral zone but not in the coral zone.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0
10
20
30
40
50
60
70
80
90
100
110
120
200m
LTL
2
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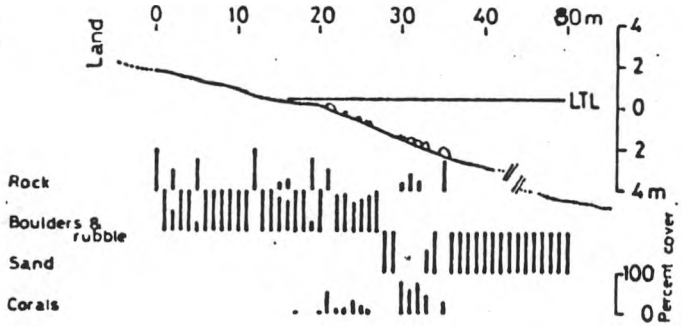
2
4m
Rock
Rubble
Sand & gravel
Corals
100
0
Percent cover

A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80m
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2
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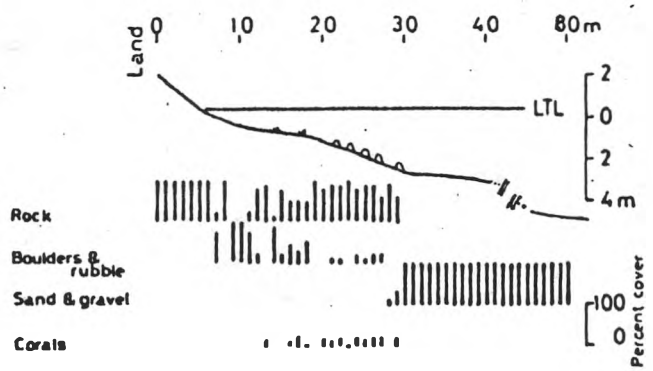
2
4m
LTL
Rock
Boulders & rubble
Sand
Corals
100
0
Percent cover

B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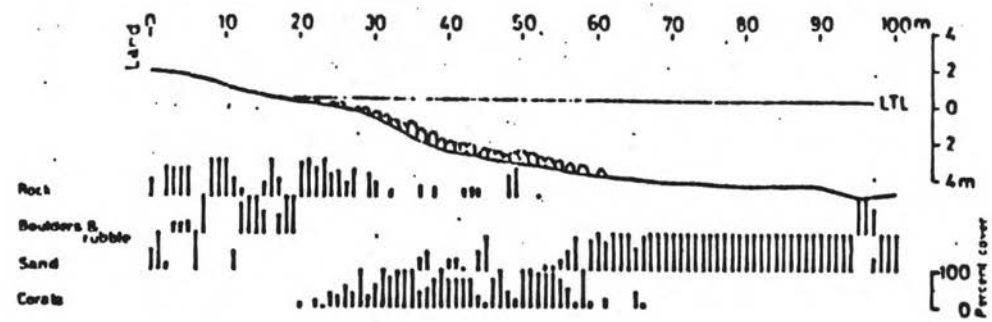
Percent cover

D



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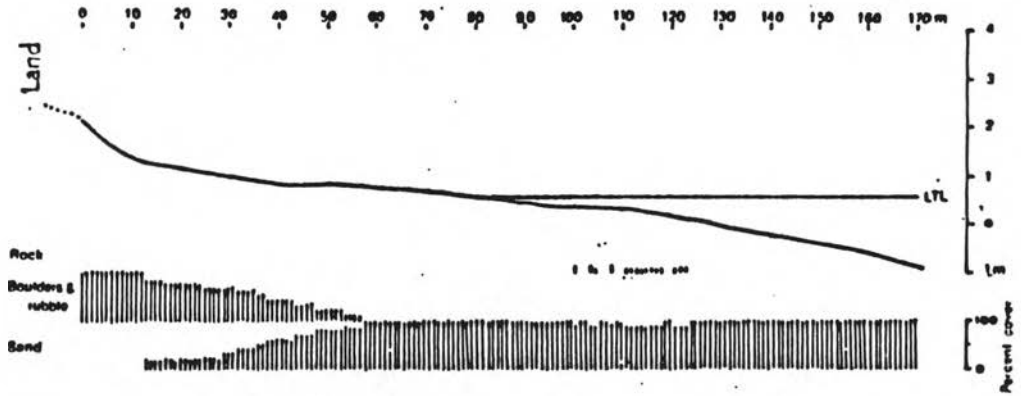


D

Fig. 2 Profies and substrate composition of each station in the study area.

A-station A B-station B

C-station C D-station D

E-station SMaRT

LTL : The lowest tide level above the lowest low water.

Station C

Station C is on the southeastern part of the Island where the effects of wind and wave are similar to Station B. The shallower part of this station was dominated by corals and rock. *Porites lutea* had the largest coverage. *Pavona frondifera* and *Platygyra daedalea* were the next most dominant species. Coral zone of this station was moderately wide, about 40m. This zone was characterized by *Porites lutea* and plate like species of, *Montipara hispida* and *Echinopora lamellosa*. (Sakai *et al.*, 1986). The associations between gobiid fishes and alpheid shrimps were found only in the areas surrounding the coral zone.

Station D

This station is on the western part of the Island. The effects of winds and waves here are relatively high. Strong wind and waves occur during the Southwest and Northeast monsoon. The shallower part of this station was dominated by hard rock and boulders. Coral zone was relatively narrow, about 15-20m wide. *Porites lutea* is the dominant species but not as abandant as in all other stations (Sakai *et al.*, 1968). The associations between gobiid fishes and alpheid shrimps were found rarely in the areas surrounding the coral zone.

The sediments characteristics of the areas surronding the coral zone of each station are the same, which is composed of coarse sand packed with pebbles, coral debris and shell fragments. At the 10m depth of each station the sediments is muddy sand.

From field survey at all of these stations found that there were many associations between gobiid fishes and alpheid shrimps at

station B, but most of them were only one association pair. Hence, only this common association pair can be observed and collected from this station, and another association pair must be studied from another areas. From general survey, the other one association pair was commonly found at the beach on the north-eastern side of Sichang Marine Science Research and Training Station. So, this area is included and designated as station SMaRT in this study. Profile and composition of substrate were studied by the same method as Sakai *et al.*, (1986), and the result is shown in Fig.2. This station is on the eastern side of Sichang Island and is affected only by Northeast monsoon. The shallower part of this station consists of rubble and gravel. The deeper part is fine sand and rubble. Few coral head, *Goniopora* sp., was found scattered in this area. Diameters of the coral heads do not exceed 60 cm. From the occurrence of young colonies, it can be noted hear that the coral community is just begining to develop in this area. The associations between the gobiid fishes and alpheid shrimps in this area were found on both sandy bottom and rubble gravel bottom.

2. Taxonomic study

2.1 Collection of specimens

Gobiid fish and alpheid shrimp specimens were collected from all stations of the study area (A, B, C, D & SMaRT). All specimens were preserved in 10% formalin solution.

Two procedures were used in collecting specimens.

First procedure, to collect gobiid fishes; underwater fishing was employed by fasten a minute fish hook (No.14-16) to nylon

fishing line and used small piece of bivalve or squid as bait. Two more piece of baits were put near the burrow entrance to attract the goby. The problem of this procedure was that the baits also attracted other fishes such as, *Pentapodus setosus* and *Scolopsis* spp. However, about half of the goby specimens in this study were collected by this procedure.

Second procedure designed to collect live, undamage specimens of gobiid fishes and alpheid shrimps lived in the same burrow for behaviovral study and also taxonomic study of alpheid shrimps. Base on the problem faced by Karplus & Vercheson (1978) in moving collecting apparatus from one place to another underwater as well as on land, the modification of shooting apparatus was made. The arrow of the regular spear gun normally used to collect specimens in this procedure was designed to sit the spear gun on when collecting specimens (Fig. 3). The shooting apparatus was set behind the burrow entrance at the time of collection, When the alpheid shrimp and its partner goby came out of the burrow, the spade-shaped arrow would be shoot to block the burrow entrance and they were then caught by hand net. Usually it would take about 5 to 15 minutes after set shooting apparatus for the goby and alpheid shrimp to come out of the burrow.

2.2 Identification of specimens.

The specimens collected were identified. Identifications of the gobiid fishes referred to the works of Akihito *et al.*, 1984 ; Akihito & Meguro, 1978, 1983 ; Fowler, 1928, 1937, 1962 ; Gunther, 1861 ; Herre, 1927, 1933, 1934, 1936a, 1936b; Hoese & Lubbock, 1982 ; Hoese & Randall, 1982 ; Hoese & Steene, 1978 ; Koumans, 1953 ; Lubbock & Polunin,

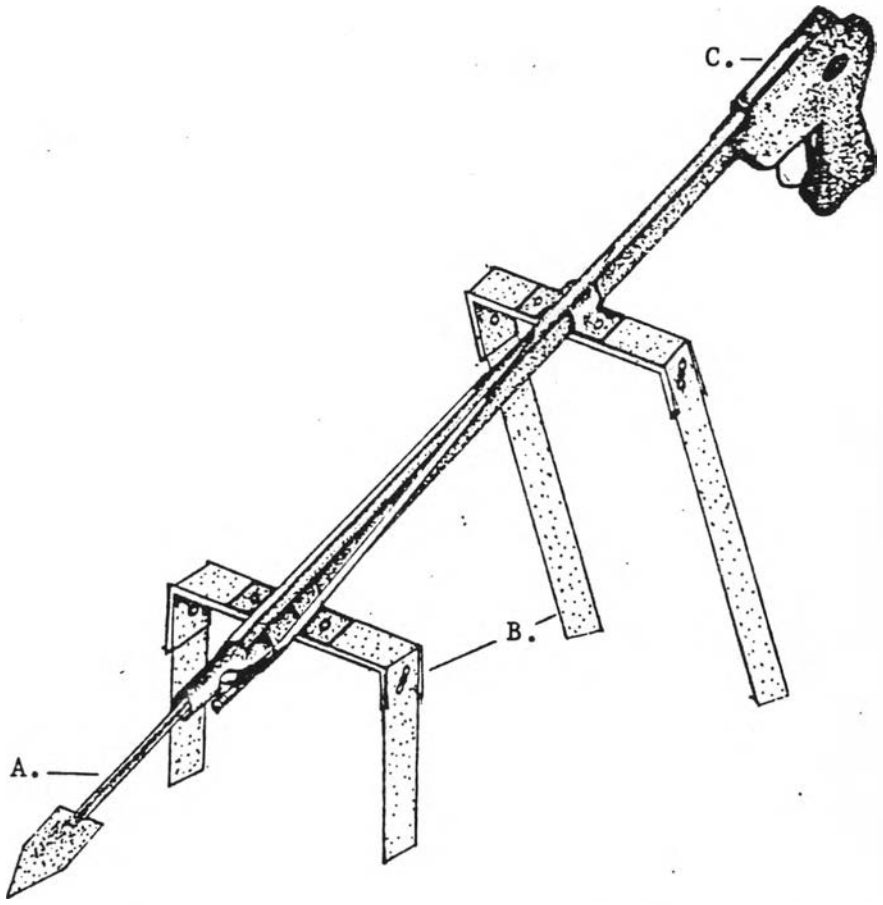


Fig. 3 Shooting apparatus used for collecting specimens of gobiid fishes and alpheid shrimps. (modified from Karpuls & Vercheson, 1978)

A. spade-shaped arrow

B. removable stand

C. spear gun

1977 ; Polunin & Lubbock, 1977, 1979 ; Smith 1945 ; Smith, 1959 ; Tomiyama 1936 ; Wongratana, 1975 ; Yanagisawa, 1978 ; Yoshino & Senou, 1983. As for the alpheid shrimps, the identifications followed the works of Banner & Banner (1966, 1982) and Miya & Miyake (1969). Some specimens were compaired with the reference collection at Kasetsart University Museum of Fisheries.

Names of canal pores on the head of goby were given as alphabetical letters designated by Prince Akihito & Meguro (1974), which is presented in Fig.4. The drawings of all species with some important characters were made.

3. Field Survey

At Khang Khao Island, some environmental parameters and the occurrence of the association pairs were studied at four stations. Salinity, temperature, dissolved oxygen and pH were measured in situ at the surface and bottom of the areas surrounding the coral zone at each station. Salinity and temperature were measured by Salinity-Conductivity-Temperature meter YSI model 33, dissolved oxygen by Oxygen meter YSI model 51B and pH by pH meter Corning M.103. The occurrence of the association pairs, species, individuals and habitat, were investigated by visual census at each station. Data were collected along the transect line, from high tide level and extended from the end of coral zone for 50m. The areas within 1m from each side of the line were observed and recorded for occurrence of gobies, alpheid shrimps and their habitat. Scuba diving was also used to collect data. All information was recorded on underwater slate board. Both gobies and alpheid shrimps were distinguished underwater and recognized in

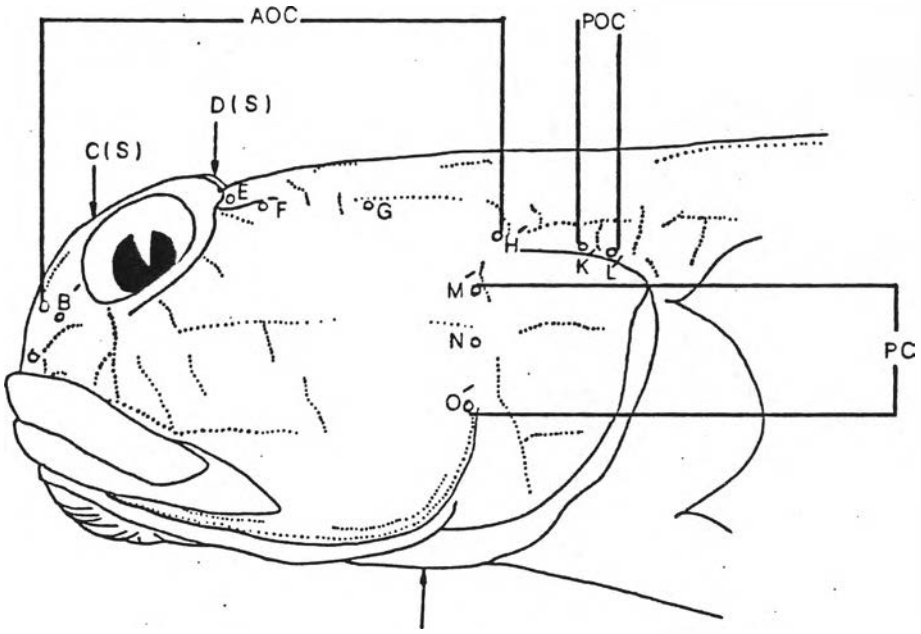


Fig. 4 Names of canal pores on the head of goby.

B'-H', pores of anterior oculoscapular canal ;

K'-L', pores of posterior oculoscapular canal ;

M'-O', pores of preopercular canal ; ', pores at tip of canal.

code before they were identified and described according to their taxonomic positions. English alphabets were used to designate the gobiid fishes and Greek alphabets for the alpheid shrimps. The important character of animals that can casily separated one from another is the colour pattern.

This field survey was carried out monthly from September 1985 to February 1986.

4. Field study on population structure effects of tidal current on direction of the burrow entrance and daily activities of the association pairs.

At station B, a 25m x 25m quadrat was set up on the sandy bottom of the area surrounding the coral zone. The depth ranged from 5m to 8m (relative depth when compared with MSL). This quadrat was divided into twenty five 5m x 5m sub-quadrats. The quadrat was set up to observe population structure and the effects of the tidal current on direction of the burrow entrance. Position and direction of the opening of the burrow entrance, species and all individual fishes and shrimps in the same burrow were recorded. Direction of the tidal current from underwater observations were also recorded. The total underwater observation time added up to 12 hours.

Also at station SMART, a 8m x 8m quadrat was set up on the sandy bottom of the intertidal zone. The depth ranged from 1m to 1.5m. This quardrat was devided into sixty four 1m x 1m sub-quadrats. Daily activities of the association pairs in this quadrat were observed and recorded. The position and direction of the opening of burrow entrances, species and all individuals of the fishes and the shrimps in each burrow were recorded everyday, for 5 days. Activities of the association pairs

were observed both day and night. These studies were carried out during March to May 1986. The underwater observation time added up to 60 hours.

5. Behavioural study

Behavioural studies were carried out during the period of March to July, 1986.

5.1 Field observations

For comparison, two association pairs were selected for behavioural study. From field studies, *Cryptocentrus* sp.1 and *Alpheus bellulus* were selected to be studied at station B on Khang Khao Island, while *Cryptocentrus singapurensis* and *Alpheus djiboutensis* were stelected to be studied at station SMaRT on Sichang Island.

Slate board and stop watch were used in underwater recording. Behaviour of both fish and shrimp were observed and recorded at the burrow having only one fish and one shrimp, but in case there were two or more shrimps in a burrow only Fixed Action Patterns (FAP_s) of the first shrimp were recorded. Behaviours of fish and shrimp were recorded after the diver reached the selected burrow for 5 to 10 minutes and continued watching by keeping motionless at the distance of 1.5-2 m away from the burrow entrance. This method seems not to distrub their normal behaviours. Behaviours of fish and shrimp were recorded 20-25 minutes for each observation, but only 20 minutes of the records were used to calculate cummulative frequency of two-FAP sequences. Forty observations were made for each selected pair without repeating observation on the same pair. Total observation times

for each selected association pair were at least 800 minutes. The FAP_s of gobiid fishes and alpheid shrimps were analysed and described.

General Fixed Action Patterns of gobiid fishes are listed in alphabetical order. (some FAP_s are shown in Fig. 5)

A = Attack : The goby charge at the intruder fish who swims into its territory.

D = Dash : The goby rapidly moves above bottom surface and takes a quick gulp of planktonic organisms.

E = Emerge : The goby emerging its head from its burrow.

F = Flee : The goby rapidly enters the burrow, head first.

G = Guard : The goby sits still at the burrow entrance with its caudal fin generally extending into the burrow. Sub-FAP (signalling) are released when there is intruder animal who swims into its territory.

Ga = Guard away : The goby sits still at any position on a shallow depression formed by the shrimp. Its caudal fin generally extending into the burrow. Signals are given only through the antennal contact maintained by the shrimp.

I = In burrow : The goby residing inside the burrow and cannot be seen.

Ld = Lateral display : The goby spreads its fins fully, lifts its head with the mouth and the operculum widely open.

Ld↑ = Soaring Lateral display : The goby soars above the bottom surface while fully spreading its fins and pauses for a moment.

M = Move : The goby changes its position within the distance of 15cm from the former position.

Ma = Move away : The goby changes its position beyond the distance of 15cm from the former position.

N = Nip sand : The goby takes a quick bite of sand. The sand is filtered through the goby's gills and falls beneath each operculum.

S = Sit : The goby sits at any position away from the burrow entrance.

W = Withdraw : The goby stops its former act and moves directly back to the burrow.

General Fixed Action Pattern of the alpheid shrimps are listed in alphabetical order. (some FAP_s are shown in Fig,5).

D' = Decorate the burrow : The shrimp uses its large chela, small chela or walking legs removing objects at the burrow entrance.

E' = Emerge : The shrimp emerges out of the burrow. Only the antenna can be seen. (Other FAP_s can be seen after the shrimp moves out of the burrow.)

F' = Flee : The shrimp rapidly enters its burrow, usually backwards.

I' = In burrow : The shrimp hides in its burrow and cannot be seen.

M' = Manipulated objects : The shrimp picks up various objects with its large chela.

P' = Plough : The shrimp holding its large chelae forward to push sand or pebbles in front of it.

S' = Stand : The shrimp stands still, appearing to be doing nothing.

W' = walk : The shrimp moves on the bottom surface by its walking legs.

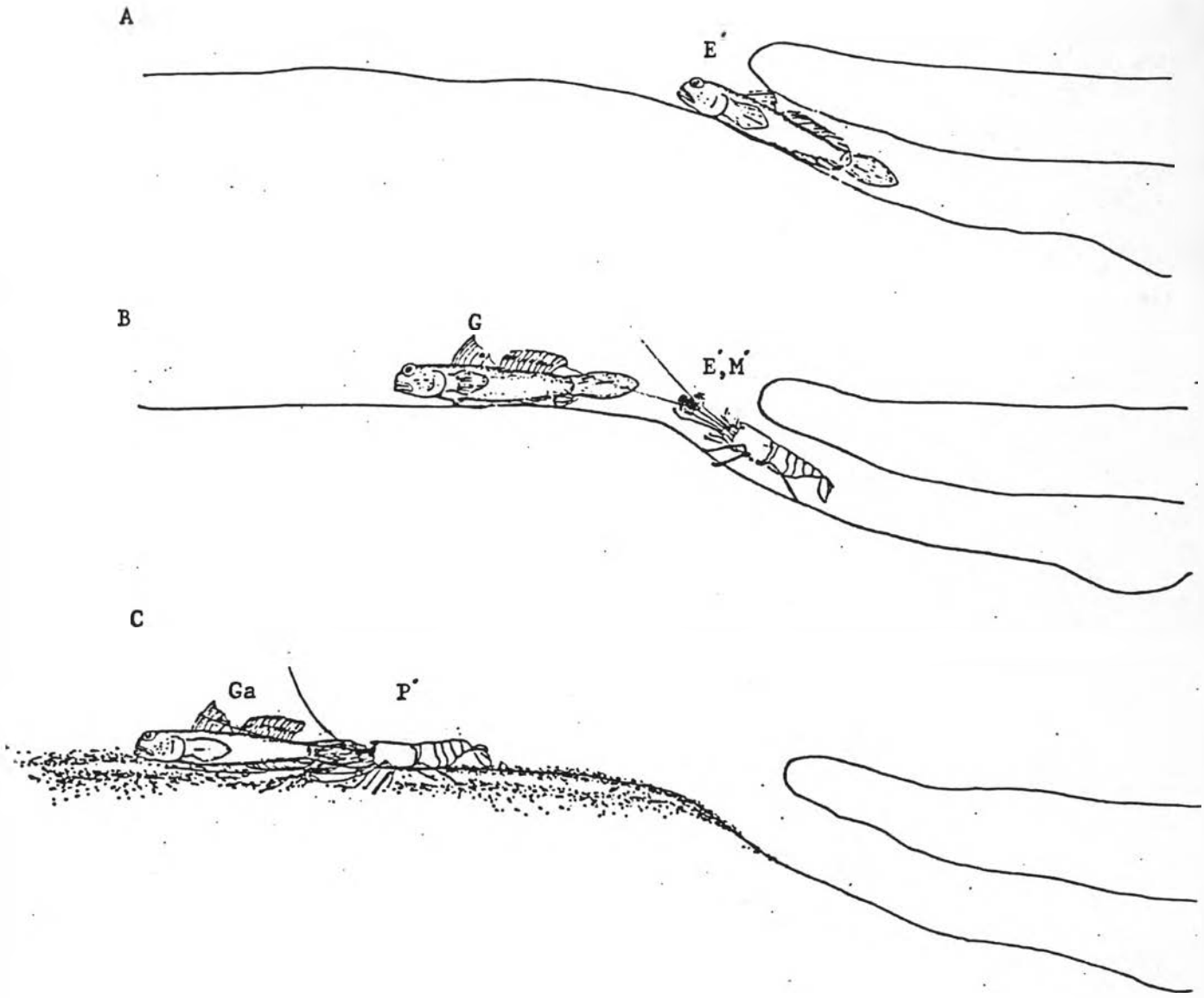


Fig. 5 Fixed Action Pattern of gobiid fish and alpheid shrimp.

A. The goby emerged (E) at the burrow entrance.

B. The goby guarding (G) at the burrow entrance while the shrimp emerged (E) with manipulated objects (M').

C. The goby guarding away (Ga) from burrow entrance by moving (M) together side by side with alpheid shrimp which is ploughing sand (P) to construct a depression.

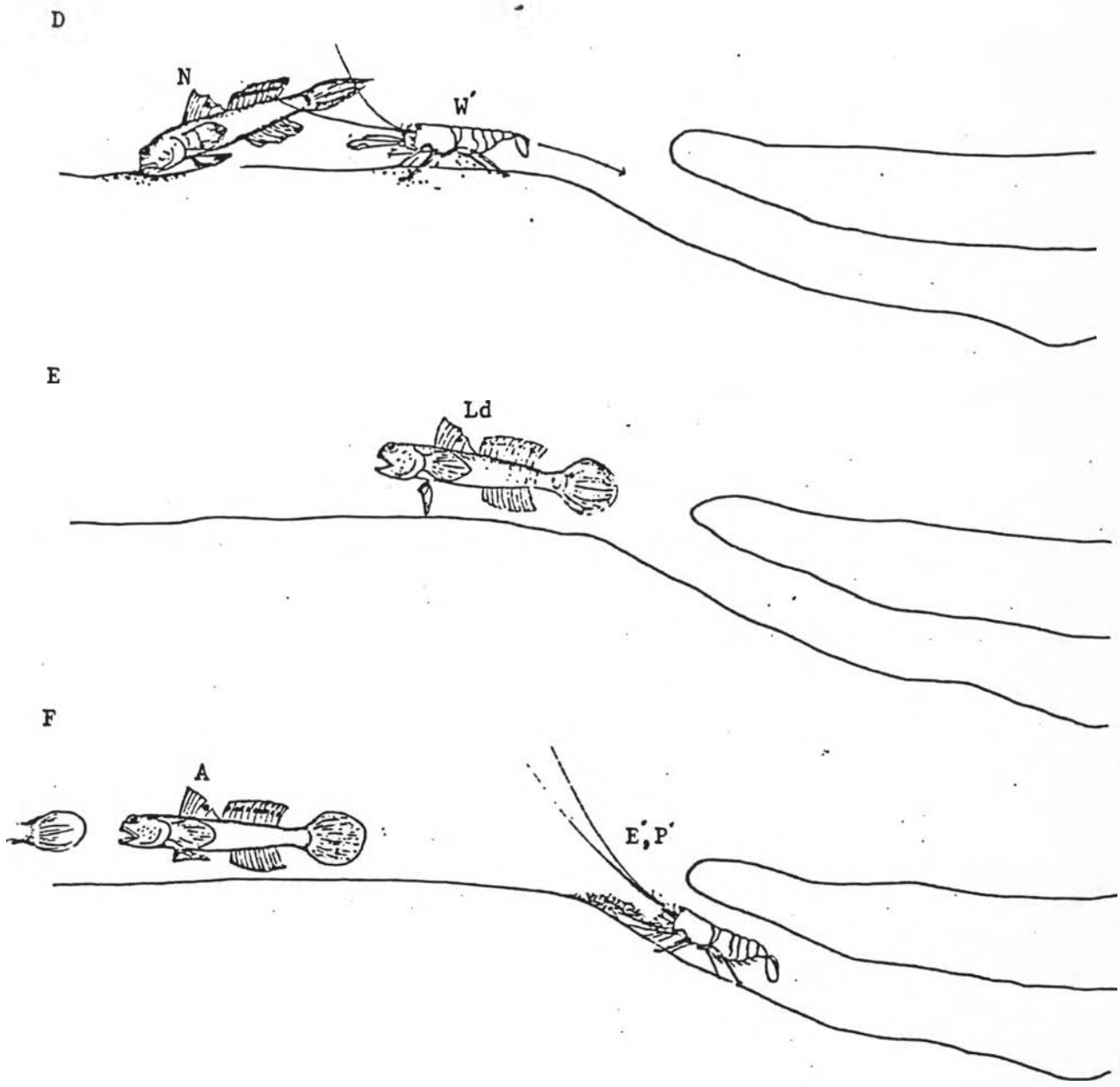


Fig. 5 (continued)

D. The goby nipping sand (N), some species of alpheid shrimps responded by flee (F) into the burrow.

E. The goby showed lateral display (Ld) when other small fishes passes into it territory.

F. The goby move directly to attack (A) other small fishes which come close to its territory.

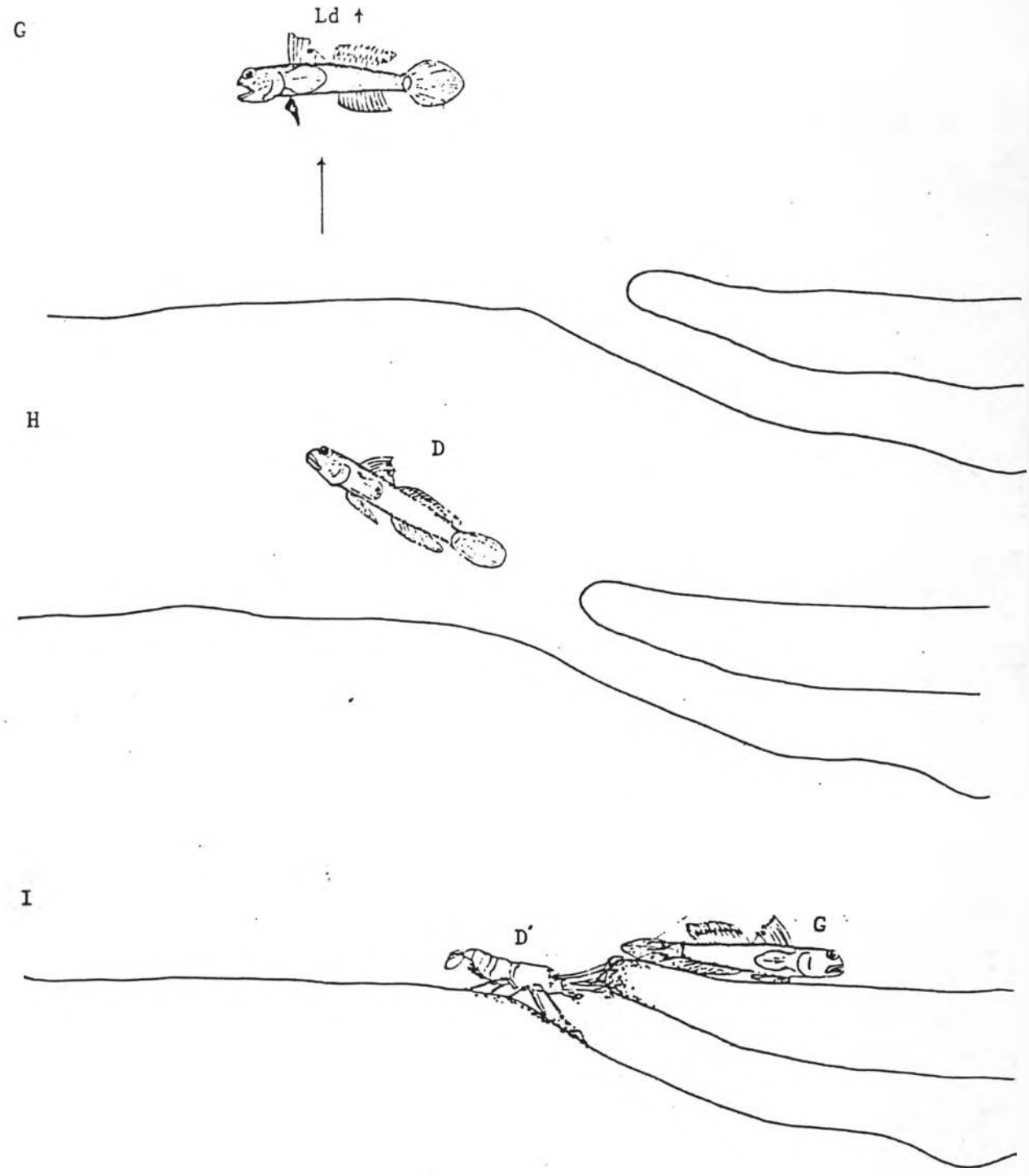


Fig. 5 (continued)

G. The goby soaring 15-20 cm above bottom surtace and showed Lateral display (LD↑).

H. The goby dash (D) above the bottom surface to take a gulp of planktonic organisms.

I. The goby move (M) to guard at the other side while the alpheid shrimp decorate (D') the burrow entrance.

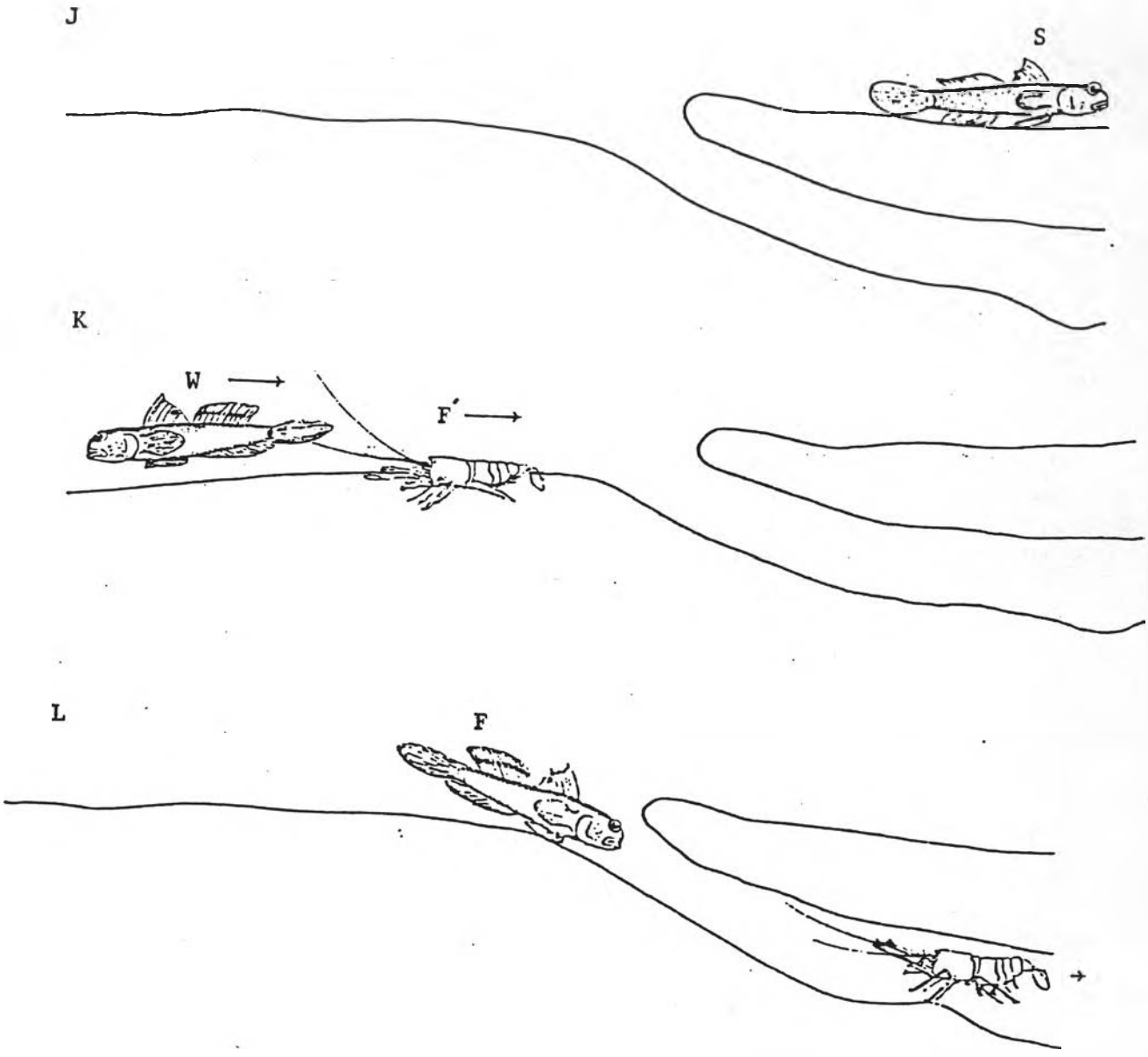


Fig. 5 (continued)

J. The goby sitting (S) at any position from the burrow entrance.

K. The goby withdraw (W) tail first after guarding away or sitting far from the burrow entrances. The alpheid shrimp always responses by fleeing.

L. The goby flee (F) into the burrow, head first, when there is an intruder fish.

To analyse the behaviour pattern of each animal, the fixed action patterns were drawn up from the observed records into sequence pattern. The frequencies of each step of the FAP or between any two-FAP sequence of each animal were added up as the commulative frequency (Y_i) of each two-FAP sequence of that animal. The summation of all cummulative frequencies is the total commulative frequencies (ΣY_i). Percentage frequency (X_i) of each two-FAP sequence can be calculated by the formula :

$$\% \text{ frequency of two-FAP sequence } (X_i) = \frac{\text{cummulative frequencey } (Y_i) \times 100}{\text{total cummulative frequencies } (\Sigma Y_i)}$$

when $i = 1, 2, 3, \ldots\ldots, n$

The results are shown in "Ethogram" with percentage frequency of two-FAP sequence unless the frequency is less than 1%.

5.2 Laboratory observations.

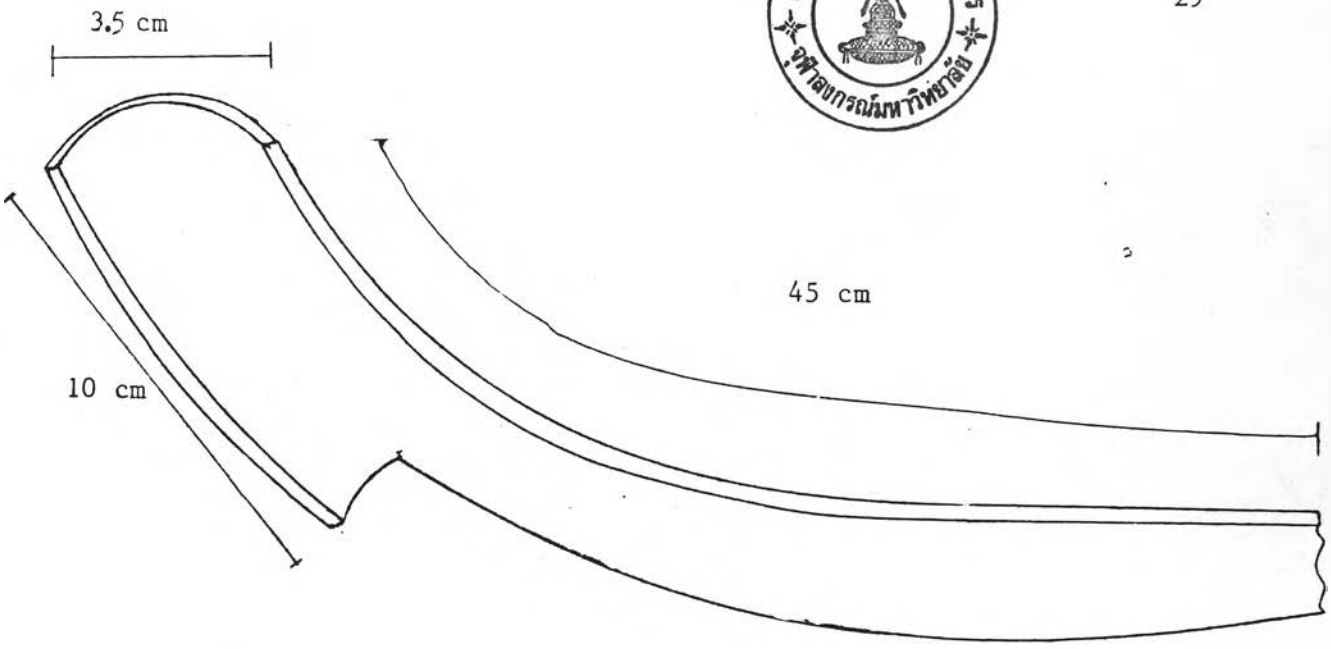
Few data are available on the behaviour of the partners inside the burrow. During the night, the partners live in the burrow together. In the day time, the gobies spent most of their time on activities outside the burrow, while the alpheid shrimps spent their time performing activities both inside and outside the burrow. Outside burrow observation alone is not enough to understand their relationship and roles in nature clearly. In order to elucidate the full relationship, observations on the partners within the burrow are required and the laboratory observations were carried out for this need.

Two replications in the behavioural observations of two association pairs : *Cryptocentrus singapurensis-Alpheus djiboutensis*

and *Cryptocentrus* sp.1-*Alpheus bellulus* were carried out in the laboratory at Sichang Marine Science Research and Training Station. The aquarium (45cm x 120cm x 60cm) with semi-closed water circulation system (sub-sand filter) which half the volume of sea water was changed every 2 weeks was used. The aquarium was kept in open room condition at the temperature 28-29°C, salinity 30 ppt and maximum light intensity 1000 lux.

Each artificial burrow was created by using plastic tube of 3.5 cm in diameter and of 45 cm long. First, the tube was cut along its length into half. Then, except the first 10cm which was left to be used as the burrow entrance, the half tube was cut away along its length again for another half. Hence, the one fourth of the tube left was used as the top part of the burrow (Fig. 6A) The cut surface of plastic tube was faced toward the front of the glass aqurium. Coral fragments, rubble and sand were used to decorate this artificial burrow (Fig.6B). At the position of the artificial burrow, a sheet of aluminum foil was used to cover on the outside of the qurium to make the burrow dark. This sheet will be removed during the observation periods.

Pairs of gobiid fish and alpheid shrimp were collected together from station B (*Cryptocentrus* sp.1-*A. bellulus)* and station SMaRT *(C. singapurensis-A. djiboutensis)*. The observation pair were placed to acclimate in prepared aqurium for approximately one week prior to the observation period. They were fed twice daily 8.00 hr and 18.00 hr , with live adult brine shrimp, *Artemia salina,* and bivalve flesh. During the observation period, gobiid fish and alpheid shrimp were observed four times a day; 7.00, 12.00, 18.00 and 21.00 hrs. Night observations were carried out under a dark red light. Each observation period lasted one hour. A total of 28 hours was observed for each replicate.



A

B

Fig. 6 Laboratory observation equiipments

A.) Sketch of the cut-off plastic tube to be used as artificial burrow.

B.) Setting of the aqurium with artificial burrow (Arrow)