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Report of a six months training period with the International Center for Living Aquatic Resources Management in Taiwan from November 1983 to May 1984.

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Contents

## Chapter 1. Introduction.

This report deals with some of my experiences during a six months training period. This field training is part of the requirements for a Masters Degree in fish culture and fisheries at the Agricultural University of Wageningen, the Netherlands. I spent these months in Taiwan, working on a research project carried out by the International Center for Living Aquatic Resources Management (ICLARM) from November 1983 to May 1984. ICLARM is a center which has been organized to conduct, stimulate and accelerate research on all aspects of fisheries and other living aquatic resources. Its operational base is in Manila, the Philippines, but projects are carried out all over South-East Asia and the Pacific region. ICLARM mostly cooperates with local institutions or universities. In Taiwan ICLARM cooperated with the Council for Agricultural Planning and Development (CAPD) in a program consisting of a Tilapia research project and a project on controlled reproduction and mass fry production of commercially important finfish.
I spent most of the time in Kaohsiung, at the Institute of Marine Biology of National Sun Yat-sen University.
ICLARM's research here concerned mainly Tilapia salinity tolerance, but I also worked on reproductive physiology of common carp. I also visited several fish farms and research institutes of the Taiwan Fisheries Research Institute.

This report consists of chapters on ICLARM's research in Kaohsiung, culture of some important species in Taiwan and my experiment on carp. Though this only concerns fish culture I learned a lot more. I think I was very lucky to have the opportunity to spend six months in a country which differs in so many aspects from my own. I really enjoyed living in the midst of Chinese people, seeing and learning about their 'way of life'. It has enriched my own life, and I still am interested in Chinese culture and religions. Off course it wasn't always easy to be there, especially since the language of the Taiwanese is not very easy to learn, but I do have very happy memories of my stay.

I believe friendship is one of the greatest gifts people can give to each other, and that is why I would like to thank some people for giving their friendship to me.
It made my stay even more rewarding !
So, thank you Dr. Ching-ming Kuo, Dr. Wade Watanabe, Mrs. Colleen Watanabe, Mrs. Mei-chan Huang, Mr. Wen-tseng Lo, Rev. Steve Hake and Faye,Jesse,Kevin,Katie,Dick and Dot.
I hope we will meet again someday.

## Chapter 2. ICLARM's research in Kaohsiung.

### 2.1. Introduction.

ICLARM's research in Kaohsiung mainly concerned salinity tolerance experiments with various Tilapias. . Tilapias are fish of the cichlid family. There are about to 70 species. Their diet is herbivorous, and they are characterized by a very strong parental care for their offspring. Allthough they originate from Africa, they have been introduced to countries all over the world. Tilapias are high valued fish, because of their strongness. Culture of these fish is not very difficult, since they are quite resistant to diseases and bad external conditions. There are, espesially in the South-East Asian region, areas in coastal zones that could be used for aquaculture. That is why there is a need for species that are tolerant to various levels of salinity. Tilapias are one possibility. In Kaohsiung experiments were done to investigate the possibility of using Tilapia for brackish water culture, or even seawater culture.
Salinity tolerance can be improved by genetic methods (like hybridization or polyploïdy) and physiological methods (acclimitization, selection). In Kaohsiung research consisted of three elements : 1. salinity tolerance determinations
2. development of acclimatization procedures
3. reproduction experiments in various salinities
In the following part of this chapter these three elements will be discussed further.

### 2.2. Salinity tolerance determinations.

Tests were done to determine salinity tolerance of a group of fishes and to compare several groups of fishes.
Parameters used were :

1. ST 50: 50% survival time. A group of fishes was transferred direct to 32 ppt salinity (seawater) and monitored. The time at which 50% of the fish had died ( and 50% still survived) is called the ST 50.
2. MST: mean survival time. This is the average of all individual survival times in a group of fishes after direct transfer to 32 ppt salinity.

Generally 25 fish from one strain were transferred directly to 32 ppt for ST 50 and MST determinations. The fish were watched continuously and whenever a fish died the time was written down on the form and the fish was taken out of the water and weighed and measured. This was done to investigate possible relationships between size,weight,condition and salinity tolerance.

3. MLS 96: median lethal salinity over 96 hours.
   Fish from one batch were placed into various salinities ( 0, 7.5, 15, 17.5, 20, 22.5, 25, 27.5, 30 and 32 ppt). During 96 hours after transfer the survival was recorded (every 24 hours) for every salinity. The longer the fish survived,the higher their score in the test. The salinity at which a score of 50% survival occurred was determined graphically and defined as MLS 96.
   Depending on availability and size 10-25 fish were used for every salinity in this experiment.

2.3. Development of acclimatization procedures.

Acclimatization of fish to saline water can be done at various stages of development. In Kaohsiung fertilized eggs and fry were used. Fertilzed eggs were taken from breeders in freshwater in the experiment and incubated in various salinities. From each batch of eggs,the eggs were distributed over different salinities, ranging from 0 to 32 ppt. Daily the number of dead eggs, dead larvae and live larvae were recorded. With these results survival rates of the eggs in the various salinities could be calculated. Data show that salinities of 20 ppt and more cause high mortality among eggs and larvae.

The same tests as described in 2.2. (ST 50, MST and MLS 96) were done with fry that were incubated at various salinities to determine whether incubation had an effect on salinity tolerance.

The fry that were used were 6-7 days old and they were transferred to 32 ppt. The results show that salinity tolerance of fry incubated in saline water appears to be somewhat higher than the tolerance of the freshwater group.

Other experiments concerning acclimatization are :

1. fixed rate transfer. Every 7, 4 or 1 day(s) the fish are transferred to water with a salinity of 5 ppt higher than they are in.Survival is monitored.
2. variable rate transfer. The fish are transferred to 5, 10, 15, 20, 25, 30 and 32 ppt saline water from freshwater and survival is monitored. The highest salinity with 100% survival after 96 hours is called the "first stage salinity". From this first stage salinity the fish are transferred to higher salinities and survival is monitored again. The highest salinity with 100% survival after 96 hours is called the "second stage salinity". This way you can determine how the fish are acclimatized quickly to high salinities.
3. gradual salinity acclimatization growth. Fish are transferred to low salinities from freshwater and fed ad libitum with Taiwanese pallet. Growth is monitored every 20 days during a 100 days period and compared to the growth of fish from the same batch living in freshwater.

2.4. Reproduction experiments in various salinities.

Tilapias of the Oreochromis genus ( O. niloticus, O. aureus and the hybrid Red Tilapia) are examined. Parameters are spawning success, fecundity, frequency of spawning etc. Fish transferred from freshwater to seawater, fish that were acclimatized as fry to seawater and a control group in freshwater are compared. The fry of these groups are checked for MLS 96, ST 50 and MST. The goals of this experiment are to find out:what is the maximum salinity for successful spawning for freshwater-reared adults, do fry acclimatized to seawater become sexually mature and also to evaluate the resultant progeny from high salinity spawns for improved salinity-tolerance and reproductive capacity in saline waters.

## Chapter 3. Aquaculture-practices in Taiwan.

In this chapter some figures will be given on aquaculture production in Taiwan and some information on important and promising species.

### 3.1. Aquaculture-production in Taiwan.

Aquaculture-production in Taiwan has been increasing steadily in the last two decades. Total production increased from 50,000 tonnes in 1963 to 216,000 tonnes in 1982, while the value increased from NT$ 576 million in 1963 to NT$ 20,384 million in 1982. The aquaculture-production is about to 20% of total annual fisheries-production.

Traditionally milkfish, oysters and chinese carps were the most important species. However, Tilapia and eel became more important while milkfish-production stayed at more or less the same level. Other important or promising species in aquaculture in Taiwan are hard clam (Meretrix lusoria), grass shrimp (Penaeus monodon) sand shrimp (Metapenaeus monoceros) and the giant freshwater prawn (Macrobrachium rosenberghii).

Table 3.1. Production statistics of important aquaculture species in Taiwan.

| Year | Production (tonnes) Total | Tilapia | Milkfish | Carp | Oyster | Eel | Production ratio (%) Tilapia/milkfish |
|---|---|---|---|---|---|---|---|
| 1963 | 49,972 | 7,436 | 25,881 | 4,027 | 7,974 | 130 | 28.73 |
| 1964 | 56,291 | 7,700 | 30,686 | 4,556 | 8,495 | 182 | 25.10 |
| 1965 | 54,160 | 7,683 | 27,562 | 5,157 | 8,893 | 178 | 27.88 |
| 1966 | 58,511 | 8,334 | 29,094 | 5,671 | 10,342 | 196 | 28.64 |
| 1967 | 56,185 | 8,810 | 23,558 | 5,896 | 11,697 | 277 | 37.40 |
| 1968 | 56,595 | 9,232 | 19,709 | 6,699 | 12,573 | 620 | 46.84 |
| 1969 | 57,092 | 9,596 | 18,995 | 7,485 | 11,726 | 1,571 | 50.53 |
| 1970 | 72,724 | 11,362 | 27,857 | 8,012 | 13,072 | 1,996 | 40.77 |
| 1971 | 77,789 | 11,364 | 30,651 | 8,920 | 12,677 | 3,610 | 37.08 |
| 1972 | 81,336 | 10,923 | 24,950 | 10,925 | 13,668 | 6,926 | 43.77 |
| 1973 | 107,489 | 13,154 | 31,578 | 15,923 | 14,310 | 11,672 | 41.66 |
| 1974 | 114,472 | 15,192 | 28,907 | 17,483 | 13,371 | 11,847 | 52.55 |
| 1975 | 127,577 | 18,696 | 33,308 | 18,254 | 13,856 | 13,607 | 56.11 |
| 1976 | 135,460 | 22,222 | 25,852 | 19,984 | 13,518 | 18,771 | 85.95 |
| 1977 | 139,640 | 22,245 | 26,361 | 18,824 | 14,948 | 22,023 | 84.38 |
| 1978 | 164,405 | 28,112 | 30,153 | 23,260 | 17,966 | 21,299 | 93.24 |
| 1979 | 183,688 | 34,652 | 32,034 | 26,616 | 19,920 | 26,440 | 108.14 |
| 1980 | 175,008 | 34,781 | 19,324 | 27,379 | 20,969 | 33,079 | 179.98 |
| 1981 | 201,925 | 48,481 | 23,912 | 32,204 | 20,393 | 27,624 | 202.79 |
| 1982 | 216,436 | 51,504 | 29,524 | 28,387 | 25,202 | 28,877 | 174.49 |

## 3.2. Culture of milkfish.

Milkfish (Chanos chanos, fig.3.1) is of economie importance in the Philippines, Indonesia and Taiwan. In Taiwan milkfish is believed to be cultured since the 17th century. The farmers caught the fry in the littoral zone and stocked them in their ponds.

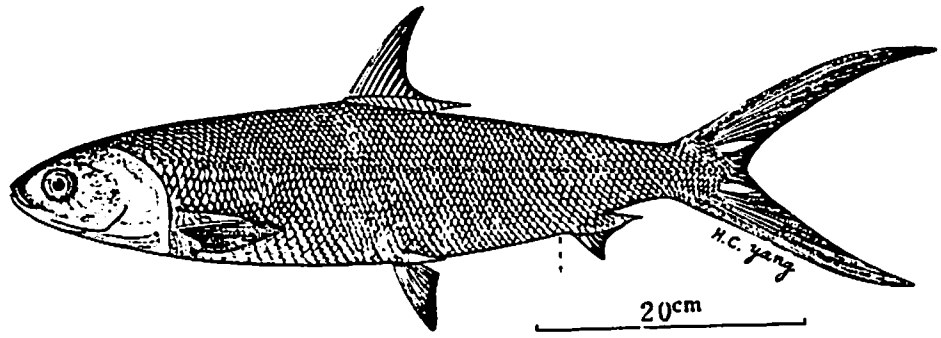



Figure 3.1. Milkfish ( Chanos chanos)

The supply of fry has always been a problem in milkfish-culture because artificial propagation of the species is difficult. Sometimes succesfull propagation is achieved, but mainly with mature fish caught from the ocean. Most of the milkfish fry are captured at full or new moon at spring tide in the months April to August with small boats or scoop nets. After their capture the fry are sold to the farmers, who stock them in their ponds at a density of 10,000 per ha. The ponds are shallow (20-40 cm) to promote the growth of bentic algae, one of the components of the milkfish diet. The fish are also fed with ricebran and soy-bean meal. In winter the milkfish are not kept in the shallow rearing-ponds. They are highly susceptible to sudden drops in watertemperature, and in the shallow ponds they do not find enough protection. Therefore there are so-called wintering ponds, long, 2 m. deep ditches, 1.5-5 m in width, protected by wind-breaks consisting of bamboo frames with a straw cover. In these ponds the fingerlings stay from November to March.
Milkfish are harvested at a weight of 200-400 grams in the period from May to November.

Recently experiments are carried out with deeper production ponds and additional feeding to find new ways in milkfish-culture. These ponds have depths of 1.5-2 m and because of this depth the growth of the benthic algae is reduced very much. The fish therefore need additional feeding. Apart from ricebran and soybeanmeal pelleted feed is given.
Mortality due to sudden changes in the weather cause great losses in the shallow ponds, and a solution for this problem (e.g. the deeper ponds) would be very wellcome to the milkfish farmer in Taiwan.

3.3. Culture of Tilapia.

Tilapia is one of the major cultured fishes in Taiwan. Its production has increased rapidly in the past ten years. In 1973 e.g. 13,154 tonnes of Tilapia were produced on 4,528 ha. or 12% of total aquaculture-production on 9% of the total area.
In 1982 production-area had increased to 10,256 ha. (16% of total), with a yield of 51,504 tonnes (24% of total).
This rapid increase of Tilapia-culture is largely due to the success of selection of good Tilapia strains and improvements in culture techniques and management.
Tilapia species were introduced in Taiwan in the period after world war II. The first species introduced was Oreochromis mossambicus (Java Tilapia) in 1946, followed by the Nile Tilapia (O. niloticus) in 1966, Tilapia zillii in 1973 and blue Tilapia (O. aureus) in 1974.
Until 1972 mainly extensive culture of O. mossambicus was practised, with a little increase in production after introduction of the red Tilapia, a hybrid of O. mossambicus ♀ and O. niloticus ♂. In the following years culture shifted to semi-intensive methods through pond fertilization, all-male culture of O. niloticus obtained through hand-sorting and promotion of integrated agriculture-aquaculture farming systems. Tilapia is the main fish crop in these systems. Of the 13,600 ha. of freshwater ponds in 1977 about to 5,000 ha. were used for integrated agri-aquaculture. Most used are ducks and pigs in combination with polyculture of chinese carps, mullet and Tilapia, mainly Nile and red Tilapia.

Mule ducks are held at a number of 2,000-4,000 per ha. of pond area, and are marketed after 2 months when they are 2.5 kg.
Some native species of ducks are kept for laying eggs.
Pigs are generally held at numbers of 150-300 per ha.
They are grown until they reach a weight of ± 90 kg.
The fish production can be upto 7 tonnes per ha. , provided good management is available.
The last few years Tilapia production increased rapidly, from 22,000 t in 1977 to 51,000 t in 1982. This increase was largely a result of culture of all-male Tilapias produced from hybridization of O. aureus O and O. niloticus O. Other factors involved in this increase were sex-inversion by steroid treatment to obtain all-male populations, and refinement of strains through genetic manipulation. Futther developments, like using pelletized feeds and improving the red Tilapia strain has led to intensification of Tilapia culture systems.
Nowadays great numbers of Tilapia are raised in ponds at high densities, fed with high-quality feeds to reach high yields.

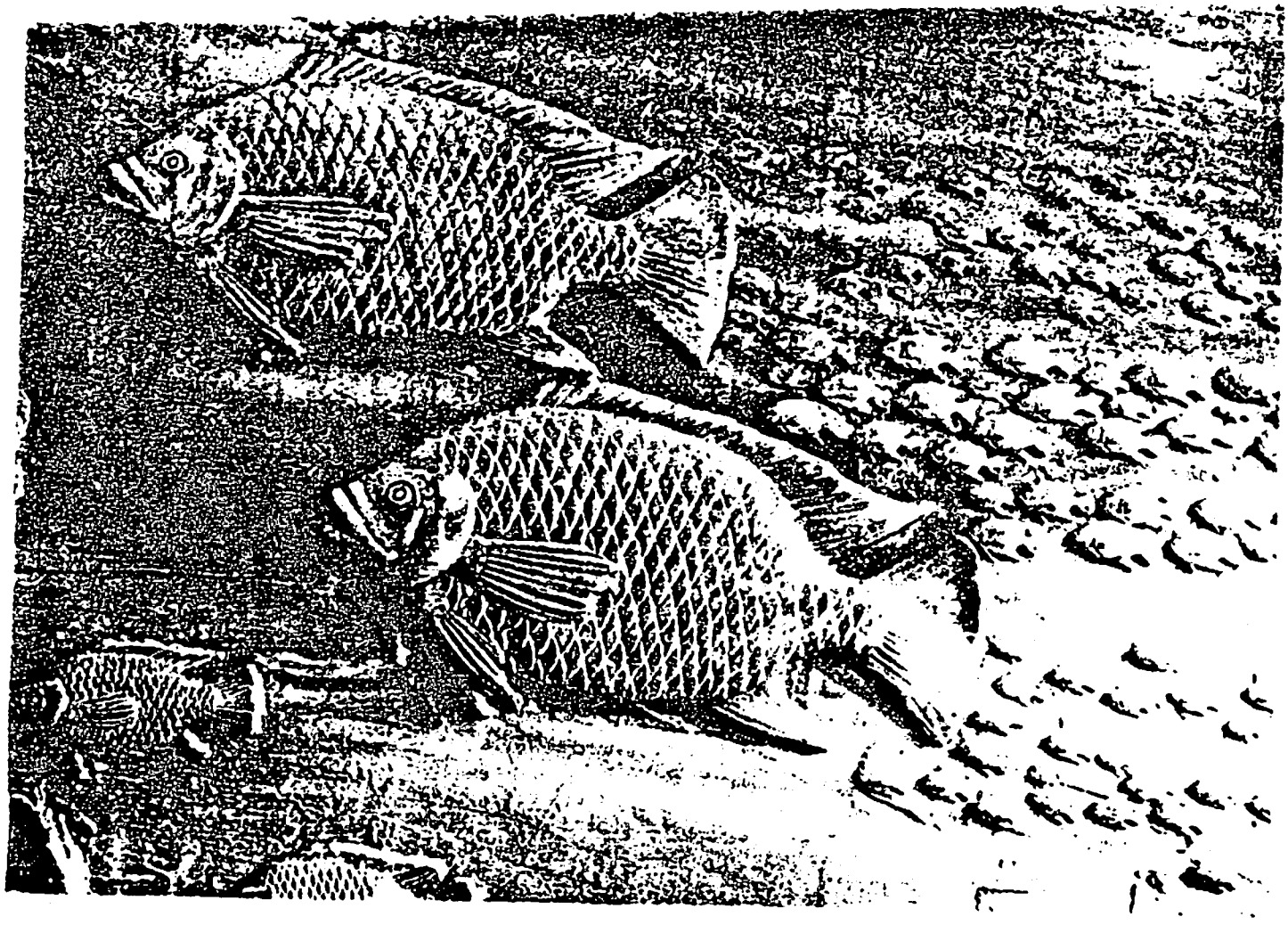


Figure 3.2. Artistic view on reproductive possibilities of Tilapia.

## 3.4. Culture of grass shrimp.

Shrimp culture is another ancient aquaculture practice in Taiwan. In the past production was limited because of the unability to produce shrimp seed. All juveniles had to be captured from the coastal waters. The main cultured species are grass shrimp (Penaeus monodon) and sand shrimp (Metapenaeus monoceros). Since the development of shrimp hatchery methods shrimp culture has developed rapidly, which can be illustrated by the growth of the number of hatcheries : 1978 150, 1981 300, 1983 600. Culture is practiced mainly in the southern part of the island where temperature is high enough for most of the year.

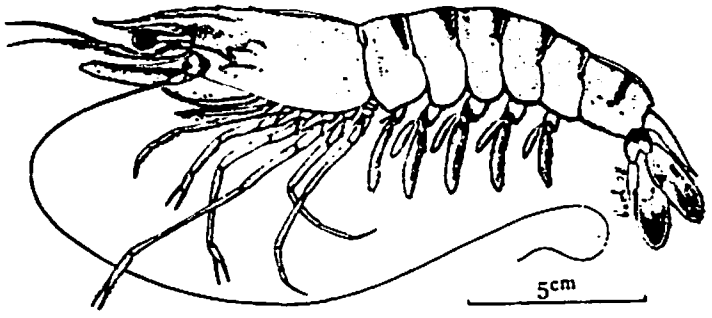



Figure 3.3. Grass shrimp ( Penaeus monodon)

Intensive monoculture is practised (stocking densities up to 300,000 per ha.) but also polyculture with milkfish or molluscs (stocking densities 5,000-20,000 per ha,).

Spawners can not easily be obtained from pond stocks, allthough some results are reported from the Taiwan Fisheries Research Institute. Most spawners are captured in shallow coastal waters by trawlers, mainly in the Malaysia and Philippines areas. They are imported to Taiwan at very high prices. A mature female can be recognized by the size of the ovaries which are visible through the dorsal part of the shell. The bigger the dark brown ovary, the more mature the animal is. To obtain copulation equal numbers of males and females are placed in tanks with aerated seawater. After moulting they copulate, which can be checked by the hard thelycum of the female.

Three days later one of the eyestalks is cut to induce spawning. Light is reduced and spawning occurs about to 7 days later. Each female produces ± 300,000 eggs, which measure 0.3 mm and hatch in 15-20 hours at temperatures of 27-29°C.
After hatching the nauplius stage 1 occurs. In all there are 6 nauplius stages. It takes 2 days to develop into the first zoea stage, which occurs after metamorphosis. During the 3 zoea stages size increases from 1.1 mm to 3.2 mm. Development of the eyes takes place, and the body becomes segmented. After 3 to 5 days the second metamorphosis occurs, and the third zoea stage becomes the first mysis stage. The 3 mysis stages can be distinguished by the developing pleopods. After 3-4 days, in which the mysis grows from 3.8 to 4.6 mm, the third and final metamorphosis takes place. The result is the postlarva stage, the final stage of larval development. The postlarva are named according to their age in days : P1, P2 etc. In general P10 is moved outdoor to growout tanks, and the P17-20 stage is sold to shrimp farmers for growout in ponds. It takes 3 weeks from spawning to moving outdoors (P10) and after the postlarvae are sold to the farmer it takes 4-5 months to reach a marketable size (20-40 gr.).

Feed.

The nauplius stage grows by absorbing egg-yolk and doesn't need to be fed. Starting with the first zoea stage the larvae have to be fed. Usually they are fed with diatoms (planktonic) like Skeletonema costatum at this stage. This alga is cultured in tanks by the hatcheries. To obtain a flourishing culture of Skeletonema fertilizer is added. Each farmer has his own prescription. The one I spent some time with added 100 g $KNO_3$, 10 g $Na_2HPO_4$, 1 g $Na_2SiO_3$ and 5 g FeCl per ton of seawater. The stock of algae was held in a small tank with some straw and manure, and for every culture some of the algae were removed from this tank with a phytoplankton net, and put into the culture tank (5 or 10 t) At normal temperatures (25°C) it takes 1½ day to produce a high density of Skeletonema. Harvesting is done with a phytoplankton net to concentrate the algae, and they are put into the tanks in which the zoea of the grass shrimp are. Since the shrimp larvae are fed twice a day several tanks with algae have to be prepared in advance in a continuous operation.

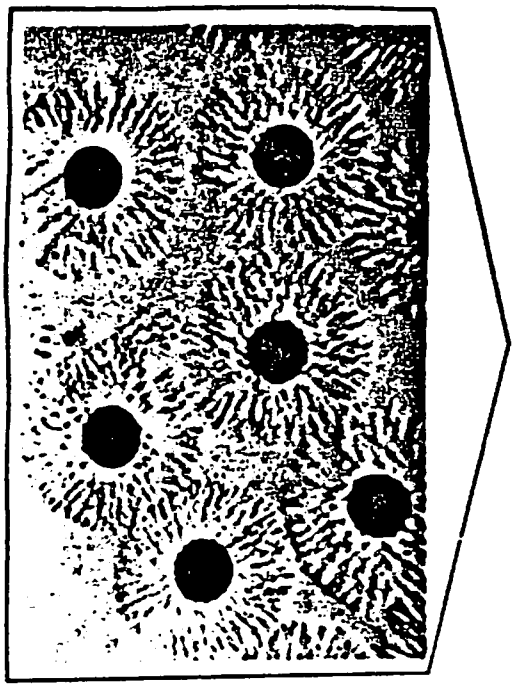
Starting in the mysis stage the larvae are also fed with Artemia salina nauplii. In postlarva stage eggyolk sometimes is fed with the Artemia nauplii. After the P5 stage minced meat,or more common nowadays, pelleted feed is used. I visited a food enterprise producing a specialized shrimp feed in 5 grades, for postlarvae to large shrimps. In Taiwan there are about to 20 enterprises producing shrimp feed, which illustrates the importance of shrimp culture. Feeding rate declines gradually from 20% of the live weight per day at the start to 3% of the body weight per day at 50 g per piece.

Some other remarks should be made on shrimp farming.

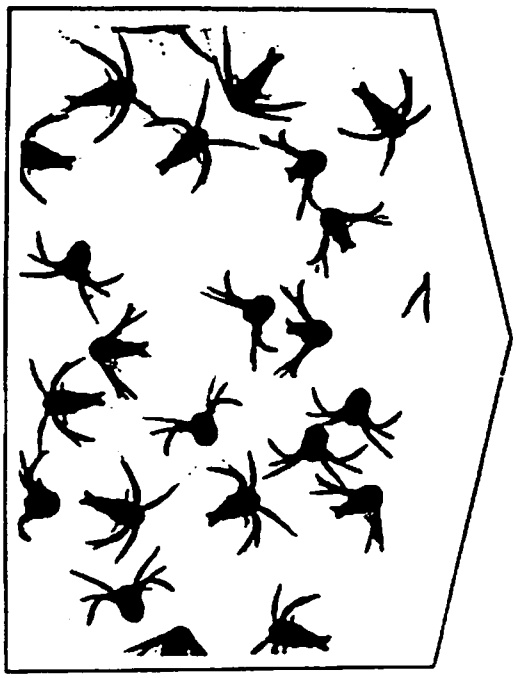
It does bear some risks in it. The farmer and hatcher never can be assured of a reasonable high price for his products. We visited some farms and hatcheries which were not in operation due to very low prices for both postlarvae and marketable shrimps.

Still, with a little research on demand shrimp farming can be a worthwhile business.

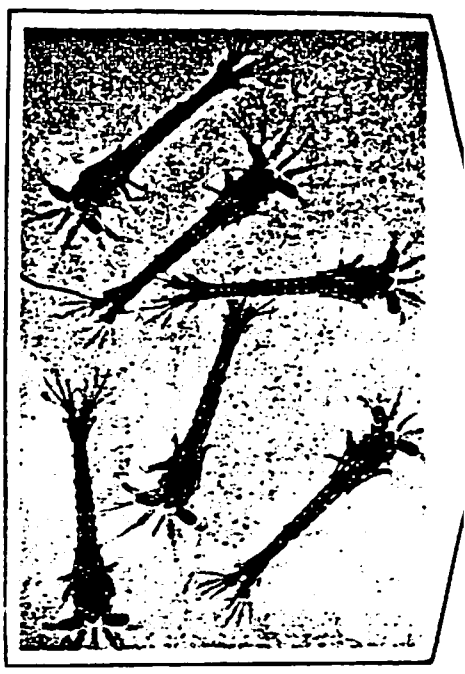
Figure 3.4. Marketable grass shrimp.



剛產下的蝦卵
Fertilized eggs.

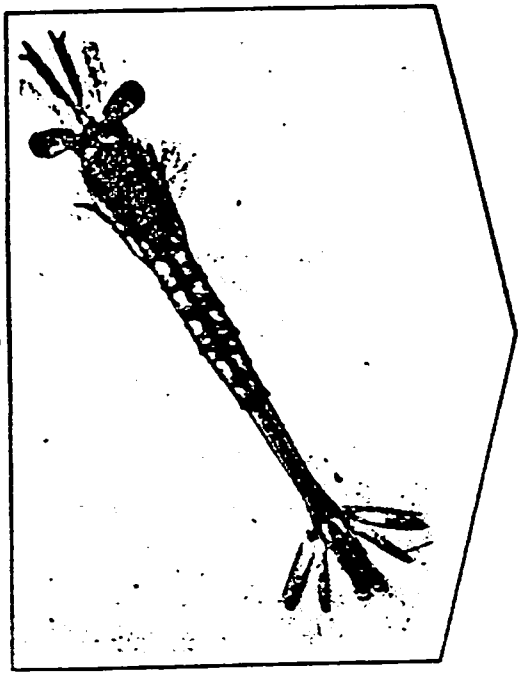


剛孵化的蝦苗——無節幼蟲
Newly hatched larvae–Nauplius.



第二生長階段的蝦苗——眼幼蟲
The second stage of larvae–Zoea.

第三生長階段的蝦苗——糠蝦期幼蟲
The third stage of larva–Mysis.



第四生長階段的蝦苗——後期幼蟲
The fourth stage of larva–Post larva.

孵出後一個半月的蝦苗
One and half month old larvae.

Figure 3.5. Development of grass shrimp.

## 3.5. Culture of giant freshwater prawn.

Another aquaculture practice gaining influence in Taiwan is the culture of the giant freshwater prawn (Macrobrachium rosenberghi) This species is not indigenous to the Taiwan area, but was introduced from Thailand in 1970. In 1976 the first prawns were produced for marketing purposes. Since that time production has increased rapidly. A number of private hatcheries is involved in supplying the juvenile prawns to the farmers.

Reproduction is obtained by placing mature, hard-shelled males and ripe, soft-shelled females, which have just completed their pre-mating moult together in a pond or tank. Copulation results in deposition of semen in a gelatinous mass on the underside of the thorax of the female. A few hours after copulation "egg-laying" occurs : the eggs are released from the ovary and fertilized by the semen attached to the female's body, and transferred to a broodchamber at the underside of the abdomen. Movement of appendages supplies fresh water to aerate the eggs. Within 3 weeks egg-laying takes place. Normally 10,000-30,000 eggs are produced ( $\pm$ 1000 eggs per gram live weight). It is important to use females in the same stage of ripeness, when you want good results. This can be checked by the colour of the eggs. Females with orange-coloured eggs should not be used. Eggs that are grey or black will hatch within 2 to 3 days. These females are called berried. After hatching (= egg-laying) the spent females are removed from the tank. Hatchability is best in brackish water. (salinity $5^{o}/oo$). The larval life is completed in $\pm$ 16 days, and consists of 8-11 stages. Larvae grow from 2mm in the first stage to 7 mm in the last. Then metamorphosis into postlarval stage occurs. This stage resembles the adult prawn, but it is much smaller. It is translucent, with a light orange-pink head. Older juveniles are blue or brown coloured. The second pair of walking legs is much larger than the others and ends in a claw. Male prawns are larger than females, and the second walking leg is much larger and thicker.

The larvae feed constantly. Their main food is brine shrimps, Artemia nauplii, but also fish eggs and specially prepared feeds are used.

In Taiwan Macrobrachium rosenberghii is cultured mainly in the central and southern area. In the southern part stocking time is early due to higher temperatures (March), and therefore two crops per year can be obtained. In the other areas stocking is not practised until late April, so only one crop is possible. Both monoculture and polyculture, mainly with milkfish and grey mullet is practised. The finfish help to reduce filamentous algae which might hinder feeding and moulting of the giant freshwater prawn. Feeding rates in growout ponds are 1-8% dry weight of live body weight, depending on feed matter and age of the prawn. Trash fish, barley and artificial feed are the most important components of the diet.
Harvesting is mainly complete harvesting, prawns weighing 50-100 grams. Yields can reach 3,000 kg per ha.

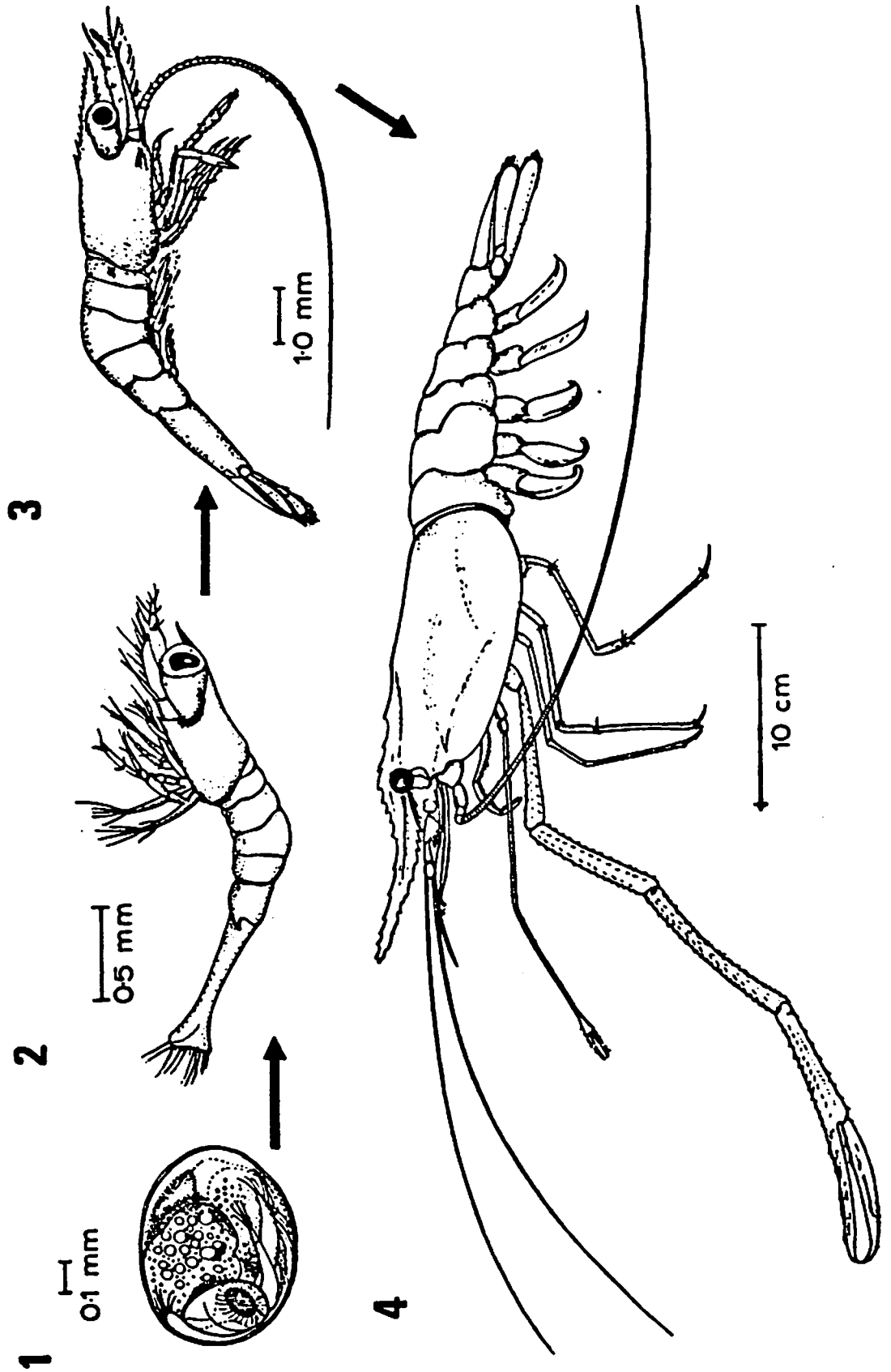



Figure 3.6. The life cycle of a caridean prawn: 1. egg; 2. larva; 3. postlarva; 4. adult. (Forster and Wickins, 1972)

## Chapter 4. Carp reproduction experiment.

### 4.1. Introduction.

Part of my stay was devoted to a small experiment on controlled reproduction of common carp, Cyprinus carpio.
Like most teleost fishes carp-oocytes have several stages of development during maturation. Within the cyprinid-family numbers vary from 6 stages for Catla mrigala ( Lal, 1962 and 1963) 7 for Ctenopharyngodon idella ( Kurt and Chow, cited by Chen et al., 1969) to 8 for Carassius auratus ( Yamamoto and Yamazaki, 1961). Chen et al.(1969) describe the stages of development as follows :

1. Chromatin-nucleolus stage : small cells embedded in the ovigerous lamellae, found throughout the year
2. Perinucleolus stage : larger cells with numerous nucleoli on the periphery of the nucleus, these cells always remain unabsorbed (recruitment stock)
3. Yolk vesicle stage : yolk vesicles are formed in the ooplasm which accumulate at the periphery of the ooplasm
4. Primary yolk stage : yolk globules are formed between the yolk vesicles, nucleus becomes polyhedral, nucleoli become randomly distributed in the nucleoplasm
5. Secondary yolk stage : yolk globules are formed rapidly, yolk vesicles pushed outwards form rows between yolk and egg membrane, nucleus has irregular shape
6. Tertiary yolk stage : yolk globules increase in number and size, yolk vesicles reduced to one or two rows, nucleus becomes spherical again
7. Migratory nucleus stage : nucleus leaves its central position and moves towards the periphery (animal pore, micropyle)
8. Prematuration stage : nucleus at micropyle, germinal vesicle breaks down, no boundary between nucleoplasm and cytoplasm

Prematuration stage is followed by hydration, ovulation and oviposition (spawning). The process from tertiary yolk stage to spawning can be induced artificially by injecting a hormone. This process takes 8 to 24 hours for common carp, depending on water temperature ( Woynarovich, 1975).

The regulation of ovarian development is shown in figure ( Kuo and Nash, 1975). In this figure hormones often used for artificial inducing of spawning are indicated.

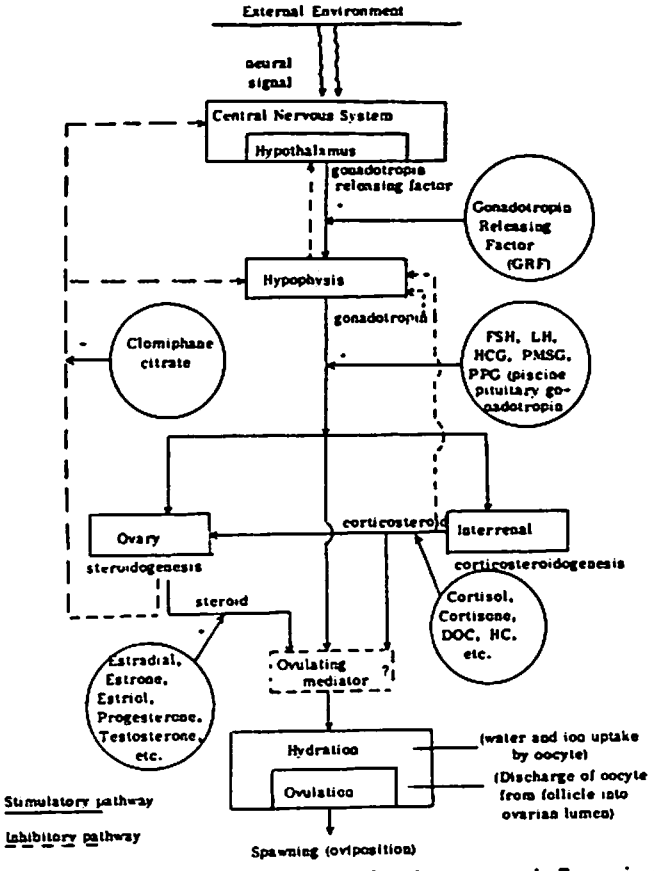



Figure 4.1. The interrelationship of endocrine organs influencing ovarian development and ovulation. The effective hormones are circled; the injection location is marked by an arrow and a reference to stimulatory (+) or inhibitory (—) reactions.

In this particular experiment main object of interest was the process of hydration. During this process the oocyt becomes much larger. Question was what causes this swelling. Which mechanisms are involved in the take up of water and ions to maintain osmotic balance with the environment.
In the gills the so-called chloride-cells play an important role in the osmoregulatory mechanism. Mitochondria and agranular endoplasmic reticulum point to an active way of transport ( Philpott and Copeland, 1963). The enzyme sodium-potassium-adenosinetriphosphatase ( Na/K-ATP-ase) plays a role in this active part of ion transport. Juvenile spring chinook salmon (Oncorhynchus tschawytscha) e.g. showed maximum ATP-ase activity at the time they would enter the sea ( Buckman and Ewing, 1982), and so did coho salmon ( O. kisutch) ( Giles and Vanstone, 1976).

But not only enzymes play a role in osmoregulation. Hormones can have an effect on mineral balance in fishes as well. Pituitary gland, adrenal cortex, hypothalamus, thyroid gland, suprarenal bodies and gonads are tissues that may be involved in maintaining mineral balance ( Lagler et al., 1977). In this experiment we tried to investigate in what way the osmoregulation in the oocytes takes place.

4.2. Material and methods.

Mature carps were bought from a local market and treated against parasites with Neguvon ( 0,5 ppm). Males and females were seperated. To distinguish the females they were fin-clipped.
To check the stage of development of the ovaries egg samples were taken with a polyethylene canula. The eggs were fixed in a solution containing acetic acid, formalin and ethanol ( 6 : 3 : 1). In this solution the nucleus became visible ( dark brown colour, while the rest of the oocyt became translucent), so the stage of maturity of the females could be distinguished. Females with eggs in the tertiary yolk stage were selected for the experiment. They were injected with a combination of carp pituitary gland ( CPG) and human chorionic gonadotropin ( HCG) to induce final maturation. The carp pituitaries were homogenized in 0,6 % NaCl-solution, the HCG was obtained from a company in powder-form. The doses were $\pm$ 10 mg CPG and 3000 i.u. HCG per kg liveweight, given in two injections. The first injection at the start of the experiment ( time 0), 1/3 of the total dose, and the second injection 4 hours later ( time 4) 2/3 of the total dose. The female was placed in an aquarium ( 200 l) immediately after the first injection together with two "running" males (milt flowing out upon gentle pressure). The males were injected with $\pm$ 3 mg CPG and 1000 i.u. HCG per kg liveweight. In this way 7 females were tested during 5 weeks. The temperature in the aquarium varied from 23°C to 26°C. Every three hours, starting immediately after the first injection and ending at the moment spawning occurred egg samples were taken with polyethylene canula.
These samples were processed in the following way :

1. Approximately 100 eggs were taken randomly for measurement of the mean egg diameter. The eggs were classified in classes of 0,13 mm and the number of eggs in each class was multiplied by the mean diameter of the class. The total of these multiplications was divided by the number of eggs to obtain the mean egg diameter.
2. Approximately 50 eggs were used for determining the stage of development of the eggs. The eggs were fixed in beforementioned solution and qualified in 6 stages : central nucleus, nucleus halfway center and periphery, nucleus at periphery, germinal vesicle breakdown, hydration and ovulation.
3. A sample of ± 200 mg of eggs was placed in a test tube and dried to constant weight ( 100°C for 48 hours). % of dry matter was calculated. The samples then were ashed at 500°C for 36 hours. % of ash was calculated. The samples were treated with conc. $HNO_3$, dried and dissolved in 70 % $HClO_4$ and dried again to prepare them for cation analysis via Atomic Absorption Spectrophotometry. Due to lack of time I wasn't able to do this cation analysis.
4. Another sample of ± 200 mg was homogenized with ether on ice. The homogenate was centrifuged at 2500 rpm and the ether layer was decanted into another tube. This was repeated once with ether and once with dichloromethane; the pooled supernatants were dried under $N_2$, the remainder was to be analyzed for steroïds using Radio Immuno Assay, which couldn't be done either.
5. A sample of eggs was taken to test Na/K ATP-ase activity. This sample was placed in a pre-incubation medium ( ice-cold) containing test-tube and homogenized for 45 seconds. This homogenate (2 ml was poured through 4 layers of gauze. The remaining filtered homogenate was used for the ATP-ase assay. The principle of the assay is that two different incubation media are used, one of them containing ouabain, a glycoside which inhibits the Na/K ATP-ase enzyme. By substracting the activity of the Na/K ATP-ase inhibited fraction from the total ATP-ase activity (the other incubation medium) we find Na/K ATP-ase activity of the homogenate. This ATP-ase activity is expressed as the amount of inorganic phosphate released per amount of protein.

The composition of the pre-incubation and incubation media are listed in table 4.1.

Table 4.1 Pre-incubation medium.

| | | |
|---|---|---|
| tris-buffer | 50 | mM |
| sucrose | 250 | mM |
| EDTA | 5 | mM |
| sodiumazide | 2.5 | mM |

Incubation media.

| | medium A | | medium B | |
|---|---|---|---|---|
| NaCl | 150 | mM | 180 | mM |
| KCl | 30 | mM | 0 | mM |
| $MgCl_2.6H_2O$ | 7.5 | mM | 7.5 | mM |
| tris-buffer | 37.5 | mM | 37.5 | mM |
| ouabain | 0 | mM | 1.5 | mM |

all three media were pH adjusted to 7.6 with HCl

For the experiment 0.2 ml of homogenate was added to 0.8 ml of incubation medium (5 tubes with medium A, 5 with medium B) at a temperature of 28°C. Then 0.2 ml of ATP (disodium salt, 30 mM) was added to start the reaction. After 15 minutes of incubation the reaction was stopped by adding 0.7 ml of 17% trichloro-acetic acid ( TCA). After 10 minutes in an ice-bath, to facilitate precipitation, the tubes were centrifuged at 3000 rpm. The supernatant was analysed for phosphate content according to Subbarov ( Bartlett, 1959), the precipitate was analyzed for protein content according to Lowry ( 1951).
Before the first injection and after spawning a sample of blood was taken with a 20 gauge $1\frac{1}{2}$" needle. After clotting for 45 min on ice the blood was centrifuged at 3500 g under refrigeration.
0.2 ml of the serum was removed, 2 ml of ether was added and then mixed. After separation of the two phases the ether layer was removed. This was repeated and the pooled ether extracts were treated as in 4.

## 4.3. Results.

The results of the egg diameter measurements are listed in table 4.2 The results of the stage of development-determinations can be found in table 4.3. Combining these results shows that eggs do not become much larger until hydration takes place. Egg diameter remains at the same level until a few hours before spawning.
In figure 4.2 the changes in dry matter % during final maturation is shown.

Table 4.2 . Egg-diameter of carps in their final maturation, measured every three hours, in mm, and % of time 0.

| Fish nr. \ Time (hr) after injection | 0 | 3 | 6 | 9 | spawning | |
|---|---|---|---|---|---|---|
| 1 | 1,30 | 1,41 | 1,32 | 1,67 | 1,74 (10 hr) | mm |
| | 100 | 108 | 101 | 128 | 133 | % |
| 2 | 1,39 | 1,42 | 1,49 | 1,41 | 1,47 (12 hr) | mm |
| | 100 | 102 | 107 | 102 | 105 | % |
| 3 | 1,16 | 1,16 | 1,14 | 1,18 | 1,30 (12 hr) | mm |
| | 100 | 100 | 98 | 101 | 112 | % |
| 4 | 1,30 | 1,33 | 1,33 | 1,50 | 1,61 (12 hr) | mm |
| | 100 | 102 | 102 | 115 | 124 | % |
| 5 | 1,12 | 1,11 | 1,14 | | 1,34 ( 8 hr) | mm |
| | 100 | 99 | 102 | | 119 | % |
| 6 | 1,05 | 1,07 | 1,07 | | 1,15 ( 9 hr) | mm |
| | 100 | 102 | 102 | | 110 | % |
| 7 | 1,31 | 1,33 | 1,34 | | 1,58 ( 9 hr) | mm |
| | 100 | 102 | 102 | | 121 | % |

Table 4.3 . Stages of development of the eggs, in % of total.

C = central position of nucleus
H = nucleus halfway between center and periphery
P = peripheral position of nucleus
G = germinal vesicle breakdown
Hy= hydration
O = ovulation

| Fish nr. | 0 | | | | 3 | | | | 6 | | | | 9 | | | spawn | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | H | P | G | C | H | P | G | C | H | P | G | G | Hy | O | G | Hy | O |
| 1 | 14 | 50 | 36 | - | 6 | 28 | 66 | - | 4 | - | - | 96 | - | 76 | 24 | - | 72 | 28 |
| 2 | 50 | 40 | 10 | - | 26 | 64 | 10 | - | 16 | 34 | 50 | - | 100 | - | - | - | - | 100 |
| 3 | 88 | 12 | - | - | 62 | 36 | 2 | - | - | - | 100 | - | 100 | - | - | - | - | 100 |
| 4 | 66 | 34 | - | - | 40 | 60 | - | - | - | - | 100 | - | 8 | 92 | - | - | 52 | 48 |
| 5 | 66 | 34 | - | - | 16 | 84 | - | - | - | - | - | 100 | | | | - | - | 100 |
| 6 | 68 | 32 | - | - | 62 | 38 | - | - | - | - | - | 100 | | | | - | 94 | 6 |
| 7 | 50 | 46 | 4 | - | - | 40 | 60 | - | - | - | - | 100 | | | | - | - | 100 |

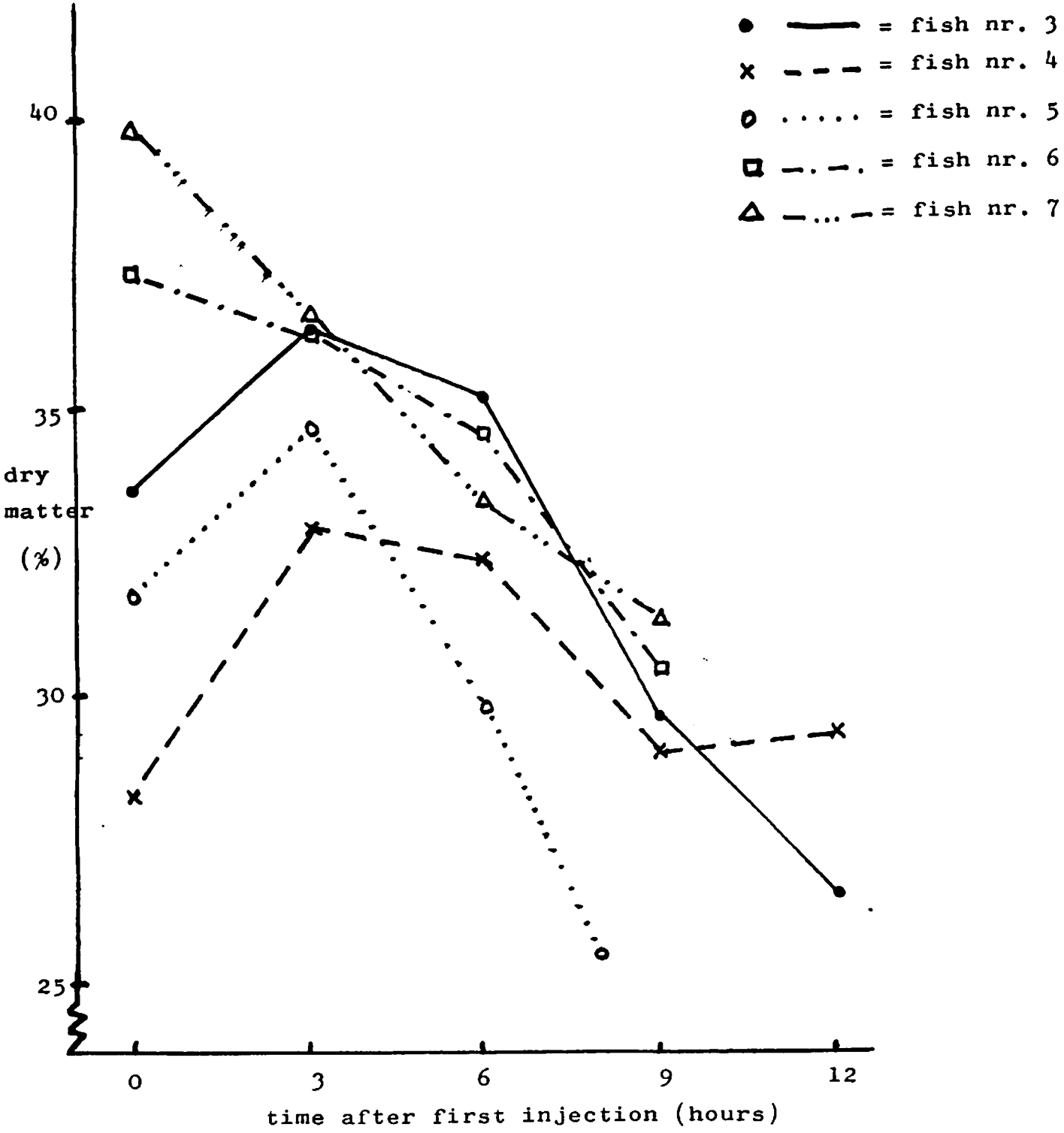



Figure 4.2. Dry matter as % of fresh weight of eggs taken at three hours intervals during final maturation.

Allthough the results are not very consistent a decline in dry matter % towards the end of the maturation can be noticed. Therefore we might conclude that the swelling of the eggs is mainly due to the uptake of water and ions, not to heavier molecules.

Table 4.4 . Results of the ATP-ase assay.

| Fish nr. | time after injection (hr) | total ATP-ase activity * | Na/K-inhibited ATP-ase activ. * | Na/K ATP-ase activity * |
|---|---|---|---|---|
| 3 | 0 | 0,14 ± 0,01 | 0,06 ± 0,01 | 0,08 |
| | 3 | 0,12 ± 0,07 | 0,19 ± 0,04 | -0,07 |
| | 6 | 0,18 ± 0,01 | 0,16 ± 0,03 | 0,02 |
| | 9 | 0,18 ± 0,02 | 0,16 ± 0,04 | 0,02 |
| | 12 | 0,10 ± 0,04 | 0,11 ± 0,05 | -0,01 |
| 4 | 0 | 0,08 ± 0,03 | 0,09 ± 0,02 | -0,01 |
| | 3 | 0,07 ± 0,02 | 0,10 ± 0,05 | -0,03 |
| | 6 | 0,16 ± 0,01 | 0,15 ± 0,03 | 0,01 |
| | 9 | 0,17 ± 0,02 | 0,20 ± 0,03 | -0,03 |
| | 12 | 0,14 ± 0,02 | 0,18 ± 0,02 | -0,04 |
| 5 | 0 | 0,11 ± 0,03 | 0,16 ± 0,03 | -0,05 |
| | 3 | 0,13 ± 0,05 | 0,18 ± 0,03 | -0,05 |
| | 6 | 0,23 ± 0,03 | 0,26 ± 0,03 | -0,03 |
| | 8 | 0,18 ± 0,04 | 0,22 ± 0,02 | -0,04 |
| 6 | 0 | 0,17 ± 0,02 | 0,19 ± 0,04 | -0,02 |
| | 3 | 0,13 ± 0,03 | 0,21 ± 0,02 | -0,08 |
| | 6 | 0,20 ± 0,01 | 0,17 ± 0,02 | 0,03 |
| | 9 | 0,23 ± 0,01 | 0,26 ± 0,03 | -0,03 |
| 7 | 0 | 0,17 ± 0,01 | 0,15 ± 0,02 | 0,02 |
| | 3 | 0,14 ± 0,01 | 0,19 ± 0,01 | -0,05 |
| | 6 | 0,18 ± 0,02 | 0,24 ± 0,02 | -0,06 |
| | 9 | 0,19 ± 0,01 | 0,24 ± 0,03 | -0,05 |

* expressed in M $P_i$/ mg protein / 15 minutes ± standard deviation

In table 4.4 the results of the ATP-ase assay are shown. The levels of ATP-ase activity are very low, with high standard deviations, both for total ATP-ase and Na/K ATP-ase activity. There are several Na/K ATP-ase values that are negative, a result which is contrary to the theory of the ATP-ase assay. In the discussion some remarks will be made on this.

## 4.4. Discussion.

The results of egg diameter measurement, egg stage determination and dry matter % point to the uptake of water,and probably ions to maintain osmotic balance, towards the end of the maturation process. Unfortunately we were not able to determine what processes are involved in the swelling of the eggs. Steroïd involvement could not be proved due to lack of time, while the ATP-ase assay showed no satisfactory results. As mentioned before the results gave very low total ATP-ase levels and sometimes even negative Na/K ATP-ase activity. Some remarks can be made on the assay :

1. We used media for incubation containing sodium and potassium both for medium A and B first. In a previous experiment on ATP-ase activity ( Van Dam, 1984) this gave unsatisfactory results too, so we decided to use an incubation medium B (with ouabain) without potassium , like Rivera (1974). In some trials with Tilapia gills this gave better results. In this experiment however the results were not very good.
2. In this experiment we homogenized the complete oocytes. This homogenate was used for the ATP-ase assay. There might be better methods testing ATP-ase activity in eggs. The ATP-ase activity is probably limited to the membrane of the egg, being the part in contact with the environment. If this part could be seperated from the rest of the egg (e.g. with the use of a microsome, like McCarthy and Houston (1974) did for gills of rainbow trout, Salmo gairdneri) maybe better results can be obtained.
3. Maybe ATP-ase activity does not play an important role in this process, that would explain the low ATP-ase activity too.

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Illustrations.

Table 3.1 and figure 3.2 are from :

Kuo, C.M., 1984. The development of Tilapia culture in Taiwan. In : ICLARM newsletter, vol. 7, no. 1.

Figures 3.1 and 3.3 are from :

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Figures 3.4 and 3.5 are from :

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Figure 3.6 is from :

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Figure 4.1. is from :

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Other figures and tables are by the author of this report.