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Project No. T109

The continued development of a larval rearing technique for turbot.

Progress during the 1974 Season.

September, 1975.

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Research Officer

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PROJECT T109 - The continued development of a larval rearing technique for turbot.

Progress during the 1974 season.

SUMMARY

Eggs of turbot, Scophthalmus maximus, were transported from the White Fish Authority's Marine Farming Unit, Hunterston and the Ministry of Agriculture, Fisheries & Food Unit, Lowestoft at the eyed ova stage and hatched in tanks at the Authority's Marine Farming Unit, Ardtoe. The tanks, with constant illumination and temperature control, were stocked with rotifers, Brachionus plicatilis, and algae (mainly Phaeodactylum sp.) before the eggs were installed. The larvae were fed successively on rotifers, various stages of the brine shrimp, Artemia salina, and mysid shrimps, Leptomysis gracilis.

A total of 1,764 metamorphosed fish were produced, the survival of the best batch of eggs being 1.1%.

2. FACILITIES & PROCEDURE

2.1 Facilities

An 11.7m x 6.4m x 3.8m (eaves height) concrete sectional building (Kenkast Type 212) erected on a concrete raft (150mm thick) was reconstructed to house larval tankage, algae and rotifer production systems, an Artemia salina nauplia production unit and office accommodation (Fig. 1, Appendix II).

2.1.1 Tankage

Two tanks each of the following sizes, were erected on 0.6m high cast concrete plinths to allow the laying of, and access to, the drainage plumbing:-

(a) 1.5m x 3m x 0.6m deep, 2,700 l capacity. Constructed in 12mm G.R.P. reinforced with wooden battens at 61cm centres, finished with a smooth black gel coat internally. The bottom was constructed in V section having two slopes of 10° from the horizontal leading to a central groove. The end distant from the water inlet was fitted with a screened standpipe draining to waste (see Fig. 3, Appendix II for further details).

(b) 1.1m diameter, 1.1m high cylindrical tanks moulded from the proprietary polyothylene, "Valethene", with a smooth finish and translucent cream colour. No running drainage provision was made, although drain cocks were installed.

A raised walkway surrounded the tanks for ease of access, the tanks being positioned so that access could be gained on three sides. All services were provided from the fourth side.

side) adjusted to provide a stream of fine bubbles, and in the *Artemia* and algal/rotifer production units through open ended 3mm i.d. bore, glass tubes (see sections 2.1.5 and 2.1.6).

The air was not filtered and thus was not free of the carbon dust from the vanes of the compressor.

2.1.4 Illumination

High intensity light of up to 3,000 lux (at the water surface) was provided for each 2,700 l larval rearing tank by 10, 1.52m, 65 watt white fluorescent tubes (Thorn Lighting Ltd., A4Z/65) suspended from a pulley system which allowed the lights to be raised to reduce illumination and for access (Fig. 4, Appendix II).

The cylindrical 1,000 l tanks were not equipped with individual lighting but were positioned within 1m of a west facing window and within 1m of the edge of one of the above lighting batteries. Thus whilst a continuously high intensity lighting regime was not used the tanks were never dark and were sometimes very bright (in late afternoon). No measurements of light intensity were taken.

Details of illumination to the algal and rotifer production unit are given in section 2.1.6.

2.1.5 *Artemia* Unit

The previous site unit (Ref. No. 8 for further details) consisting of two 400 l incubating tanks and a 200 l concentrator/separator bath, was dismantled and transferred to a 3.0m x 3.7m fibre-board insulated room contained within the hatchery building (Fig. 1, Appendix II). Unfortunately, due to the delayed delivery

ported from the Ministry of Agriculture, Fisheries & Food Laboratory at Lowestoft as two day old fertilised eggs, the remainder resulting from strippings of the adult brood stock held at Hunterston.

By utilising the brood stocks at both Lowestoft and Hunterston an extended period of spawning was obtained. The first batch of eggs were stripped on 9:6:74 and the last on 11:9:74 giving an egg production "season" of 94 days.

Several of the then all-male potential spawning stock held in a 5.5m^3 floating sea cage in the N. Channel of Loch Moidart produced milt, the collection of adult females prior to the spawning season not having proved possible.

Where possible eggs were transported 48 hours before the predicted hatching date, however, the third and sixth batches hatched during transport - it was thought unlikely that the larvae suffered as a result.

Two types of transport container were used, 4.5 l wide-necked vacuum flasks and insulated plastic boxes (32 x 21 x 35cm i.d.). 25-30g of fertilised eggs were carried in each flask and in each of two polythene bags per insulated box.

One batch of eggs (Batch II) was transported in 4mg/l solution of the proprietary antibiotic "Furanace" in an attempt to limit any possible bacterial activity.

Discussion on the transport procedure is given in section 4.2.2. and Tables 1, 2 and 3, Appendix I give details of the dates of transfer, temperature differences and egg drop-outs.

The day after hatching had been noted to commence (Day 1) the incubation bag was split to a point about 35cm below the water level and the larvae allowed to escape. The aeration in the bag was increased to assist this. After 24 hours the larvae were out of the bags and the bottom of the bags were siphoned out and "dropped-out" eggs weighed and counted.

2.2.3 Larval Rearing

Although stocking density varied due to the availability of healthy larvae and differing tank sizes, and exchange rates also varied due to tank leakage, the basic rearing technique was as follows. (Section 4 gives detailed discussion of these variations and their consequences.)

(a) The 2,700 l larval rearing tank was filled with sea water (see 2.2.2) and an inoculum of 100 l *Phaeodactylum* culture, 20ml of 'Bio' (a proprietary plant food) and 2ml of "Crookes Multi-Vitamin injection" added. The lights, heaters and aeration were switched on.

(b) 2 to 4 days later, 5 million rotifers, *Brachionus plicatilis*, were added, after which larvae were released into the tank.

(c) Following larval release, more rotifers and algae were added at intervals as available. Observation of the tank showed a rapid build up of free-swimming rotifers to a density of approximately 10 per ml but large numbers were also clinging to the sides of the tank (up to 1 per mm^2 in places).

2.3 Food Production

2.3.1 Algae

25 x 30 l tanks were used for the production of the mixed algal culture originally derived from those species occurring naturally in the Ardtoe Pond. This was predominantly a culture of *Phaeodactylum* sp. although occasional cells of other species, e.g. *Tetraselmis* sp. were seen but not positively identified.

Each sub-culture was started with 6 l of a strong culture and 19 l of sea water. 0.2ml Crookes multivitamin compound and 3ml Bio plant food were added daily to all growing cultures.

Each day, up to 50% of all growing algal cultures were removed and added to rotifer and Artemia cultures and the on-growing tanks. Failed cultures were discarded and restarted after scrubbing the tank in fresh water.

2.3.2 Rotifers

Up to 10 x 30 l cultures of the marine rotifer, *Brachionus plicatilis*, were maintained at room temperature and under 2,000 lux illumination. The initial cultures were based on a predominantly *Phaeodactylum* culture to which approximately 6 x 10^3 rotifers had been added.

Each day about a tenth of the total culture medium was rejected after the rotifers were removed, this was replaced with the best-growing algal cultures. When all the rotifers in a culture were needed for larval feeding, the complete culture was transferred direct to the larval rearing tanks. When cultures were being built up, rotifers were separated with a 68 μ filter mesh and returned to the tank with algal culture to make up the volume. Throughout the season daily counts of rotifers were

egg-bearing adults tend to become anchored before producing young. This was generally thought not to occur with B.plicatilis but it is possible that it occurred in this instance.

2.3.3 Artemia

Newly hatched nauplii were removed from the separator bath each day and transferred to one or two 30 l glass vessels containing a predominantly Phaeodactylum culture and 4mg/l of Furanace. The culture was left illuminated for 24 hours before the Artemia, then metanauplii, were concentrated and fed to the stock. Further cultures were left for longer periods to produce larger metanauplii, these also being fed on the Phaeodactylum culture.

Adult Artemia were produced in a 100 l tank throughout the season. Algae and metanauplii were added daily and adult Artemia removed as needed with a hand net. Later in the season, Artemia were on-grown for up to 14 days in 30 l culture tanks. Normally about 10% of each stock was fed to the larvae daily and replaced with algal culture. This meant that all sizes of Artemia were being presented daily to the larvae.

Hatching rates were not regularly assessed but were very low in the region of 25-35% viability.

2.3.4 Mysids

Mysid shrimps, Leptomysis gracilis, had been used to feed wild caught turbot in 1972 and 1973. They were found in considerable quantities (up to 1kg was collected daily in good conditions) in the tidal stretches of several local burns,

(1) sea water with rotifers,

(2) boiled, filtered sea water with agar beads impregnated with haemoglobin, and

(3) boiled and filtered sea water.

The results of these trials were to be judged on survival and on visual evidence in the form of sections of larvae showing artificial food in the gut.

The capsules were prepared by Dr. D. Jones of the Marine Science Laboratories, Menai Bridge and the ones used in the trial contained haemoglobin and starch. They were stored in a detergent solution and had to be scrupulously washed before presentation. The agar beads were purchased as a commercial product, Sepharose (manufactured by Pharmacia Fine Chemicals, Uppsala, Sweden and obtainable from Paramount House, 75 Uxbridge Road, London W5 S3) and impregnated with haemoglobin by Dr. P. Dean, Department of Biochemistry, University of Liverpool. The agar beads were stored in a solution of sodium azide as a bacteriostat and also required washing very thoroughly before presentation. The capsule size was approx. 40 µ - 90 µ and the agar bead size approx. 40 µ - 190 µ.

2.4 Staffing

The hatchery was purposely designed to be simple in operation and was operated successfully throughout the season with only one officer in attendance. A maximum of two unskilled assistants helped, but for most of the season the operation was undertaken by only one full time and one part time worker.

The routine work of the hatchery, i.e. readings, cleaning, feeding, operating the Artemia and algal/rotifer production

3.6 Survival Post-metamorphosis

Table 11, Appendix I gives details of the survival of post-metamorphosed fish during weaning.

Table 12 and Figure 6, Appendix I provide a record of the growth of Batch I.

4. DISCUSSION

4.1 Growth

It was thought that the stress of taking regular weight and length measurements would have had an adverse effect on final survival and for this reason only one stock was regularly sampled, Batch I (Table 12, Appendix I). Unfortunately, this batch was not typical of the hatchery-produced fish as a whole since:-

(a) Transport from the hatchery to the nursery (the first sampling) took place too early in metamorphosis and in the following 25 days 301 of the 312 fish transported had died.

(b) The 11 survivors had to be combined with a stock of wild-caught fish and have been held since October at a higher temperature (16-18°C) than the other hatchery stock (13-15°C).

Since the Batch I group of 11 fish were to be combined with wild-caught turbot stock they were marked (a piece cut off one side of the tail) as they all had good pigmentation and were otherwise not readily identifiable.

the 'polythene' bags were unsupported in the box and greater movement was likely together with a greater possibility of abrasion against the surface of the bag.

A further departure from previous egg transport practice was the use of the antibiotic 'Furanace' in egg transport (Batch II). The second batch of eggs received were transported in vacuum flasks in sea water containing 4mg/litre Furanace in an attempt to limit any possible bacterial activity. These eggs were apparently healthy on arrival and were incubated in bags containing Furanace at 10mg/litre. They hatched two days after arrival but the larvae were very unhealthy and they were all dead on the following day. Eggs transported from Hunterston to Port Erin on the same day, but without Furanace, hatched and fed with no sign of ill-health. At this time, information became available from Port Erin regarding the possibly toxic effects of Furanace on newly-hatched larvae. The effectiveness and toxicity of the drug were, however, greatly reduced by light and it was thought that the bright lights over the incubation bags would reduce the toxicity to a low level by the time the eggs were ready to hatch.

It was noted that later batches of eggs (Batch IV and V) received on 6th and 9th August showed the same bent and unhealthy appearance on hatching and poor survival as Batch II although no Furanace was used on these occasions.

4.2.3 Larval health

The technique of first-feeding in greened-up 2,700 l tanks meant that the early life of the larvae was spent in water containing a dense algal culture. It was often impossible to

to avoid the loss of larvae down the overflow. A few drops of the oil based multi-vitamin compound displaced the film for an hour or so but had no lasting effect. Eventually the film was disrupted by greatly increased aeration and shortly afterwards fish were seen to be active at the surface.

(2) In the period Day 15-20 the Batch I larvae were seen to have yellow globules on their upper surface, frequently near to the gill operculum. It was thought at first that this represented some form of parasitic infestation and specimens were sent to Dr. K. MacKenzie at the Department of Agriculture & Fisheries for Scotland's Laboratory, Aberdeen. His findings were that parasites were not involved and that the globules were composed of mucus, algae and bacteria, the mucus apparently having been produced by the gills which showed hyperplasia consistent with irritation. His recommendation was to cut down algal growth by reducing the light intensity in the belief that algae were causing the gill irritation, or reduce bacteria by treatment with Roccal (aqueous solution of alkyl-dimethyl-benzyl-ammonium chlorides) at 1:500,000. However, as there was no indication at this time that the condition was a sign of ill-health - not all mortalities showing yellow globules - and that it was considered that the addition of Roccal would be likely to kill the _Artemia_ metanauplii and the turbot larvae no action was taken. In fact it was thought that the algae were likely to be valuable in feeding _Artemia_ metanauplii in the tank and in removing metabolic waste products. It was thought that the condition (which had not been noted at Ardtoe before) could have been the result of bacteria present in the Artemia eggs

The syphon ensured that no detached weed was left in the tank. In an attempt to reduce the time spent in removing weed manually 21 full grown limpets, Patella vulgata, taken from local rock pools were added to the 2,700 l tank holding Batch III between Day 11 and Day 16. 3 were removed dead on Days 22-23 but the rest survived well and were effective in keeping localised areas of the tank absolutely clean. Unfortunately, it would have needed an estimated 100 limpets to keep the whole tank clean, which would have involved a possible risk from metabolic waste products. Apart from 2 fish seen to be trapped and killed under the shell of a limpet no ill-effects from their presence was noted. They were only used in this tank. Weed growth would have been considerably reduced if the light intensity had been cut but a high intensity was thought important to sustain the algal culture. Alternatively the water could have been filtered to remove the filamentous weed prior to stocking (see 6.2).

(5) A disease recently identified in turbot at the on-growing stage was associated with a coccidial infection. Investigations are continuing into the cause of this isolated disorder, observed on only one occasion which was attributed to poor hygiene resulting from a change in food pellet formulation. One factor which may prove significant has been the discovery of the presence of a possible parasite, termed Rhabdospora, in the walls of the intestine of affected fish. Dr. K. MacKenzie of the Department of Agriculture & Fisheries for Scotland's Laboratory, Aberdeen and Dr. R. Roberts of the Aquatic Pathobiology Unit at the University of Stirling were supplied with specimens of larvae at various ages in connection with their investigation of this disease. The

4.3 Temperature and salinity control

It was accepted that the failure to rear more than 3 turbot at Ardtoe in 1973 was due to low temperatures more than any other factor. Accordingly, the first priority in planning the 1974 trials was temperature control to keep the tanks within the range likely to be most successful. This was thought to be 16-21°C. It was intended to incubate and hatch at 16°C, raise the tank temperatures to 18-21°C for the period up to metamorphosis and reduce slowly to 16°C for the later transfer to the nursery. This, it was thought, would give the benefits of rapid growth and feeding in the larval phase whilst leaving the incubation temperature at 15°C (already known to be successful, Ref. 3), and transferring fish to the nursery at a temperature within the capacity of its water heating equipment at that time. The temperature and salinity regimes of the four batches successfully reared are given in Figs. 1 to 4. The tank temperatures stayed above 16°C with only one exception during the exercise, but two batches reared in cylindrical tanks tended to follow the ambient air temperature. The maximum daily temperature variation occurred in Batch V in late September when after the tank temperature dropped to 12.4°C (on a day when outdoor air temperatures were 3.0°C - 6.0°C), it subsequently rose to 16.3°C within 24 hours.

Each tank was equipped with 3 x 100W aquarium heaters during the summer, temperature control being obtained by switching elements as needed (2.1.2). This worked well except when extremely hot or extremely cold weather occurred (see 6.12 for future recommendations). Late in the season, when the weather

(b) to give greater depth since it had been noticed (Ref. 1) that the larval escape reaction was to swim fast downwards and were liable to be damaged on the floor of the tank.

Consequently, new tanks were constructed for the 1974 season, the 1,000 l tanks were designed to contain 0.9m and the 2,700 l tanks 0.5m of water.

Late larvae of Batch V held from Day 32 to Day 74 in a 100 l tank 1m deep showed a survival of 49%; on Day 32, 611 fish were transferred to the 1,000 l tank and on Day 74, 297 fish were transferred from it. This was the highest survival rate over an extended period in any of the hatchery tanks, and was ascribed to two main factors:-

(a) the absence of filamentous weed, and
(b) the greater depth of the tank.

The examination of larval survival in tanks of greater depth with similar control of weed growth is a high priority for future work (see 6.9).

Some small faults were discovered in the 2,700 l tanks:-

(a) the overflow standpipes were difficult to seal and having started to leak, the tank could not be sealed without draining; this meant that a constant "topping-up" flow was necessary during the rearing of Batch III.

(b) the overflow screens were not capable of preventing the escape of larvae even up to the 15mm stage, it being impossible to improve the screens once the larvae were in the tank.

The removal of Batch I to the nursery on Day 45 and the transfer of Batch IV to a 1,000 l tank on Day 2 were the result

to aeration. Larvae were often seen carried by the current into a vigorous stream of bubbles but continued to feed after leaving it.

4.6 <u>Illumination</u>

In many previous hatching trials (References 1, 3 and 4) a dark period was left so that a natural diurnal rhythm of lighting was maintained, however, it was thought possible that a dark period would merely reduce feeding time and lights were left on continually. This also avoided the possibility of low dissolved oxygen levels (as noted in Reference 1) caused by oxygen uptake of algae during the dark period.

The main disadvantage of constant, high intensity lighting was the excessive growth of filamentous weed on the sides of the tanks. Two adjustments were made to control this:-

(a) the lighting units were raised,

(b) alternate fluorescent tubes were removed.

Both reduced light intensity but did not significantly affect the growth of weed at this stage (see 4.2.3).

4.7 <u>Food Production</u>

Given a regular supply of rotifers, they can be presented to the stock in two ways:-

(a) the turbot larvae can be kept to a registered area in which a high food density is maintained, e.g. the chiffon rearing tanks used in 1973, or

temperatures and the strong light depressing the growth rate. Since insufficient adult *Artemia* were available much greater reliance was placed on the feeding of Mysid shrimps. Live shrimps were accepted, frozen ones only after a few days. Although it was not possible to conduct a trial to compare mysid feeding and *Artemia* feeding, it is thought possible that faster growth rates and better survivals might have been obtained, if sufficient adult *Artemia* had been available to produce metamorphosed turbot on *Artemia* alone.

It is considered that this phase of feeding will present most difficulties in the establishment of a production technique, turbot between 1.5 and 3.0cm failed to accept extruded pellets and live mysids produced a far poorer feeding response than adult *Artemia*.

4.7.1 Artificial foods

During the larval rearing programme, three trials were undertaken using artificial foods for newly-hatched larvae. The intention was to demonstrate that larvae would take inert particles at an early stage and thus commence the development of an artificial diet.

The tanks available for these trials were small (2.5 l) glass containers within the algal culture unit. These tanks could have been totally unsuitable for early turbot larvae since no larvae survived longer than a few days, even when fed rotifers which were known to be an acceptable diet. The success of the trial was judged purely on the evidence of stained sections of larvae showing gut contents, (see Appendix III).

Clearly, the tank stocked with larvae at over 40/1 produced the most metamorphosed turbot. Larval density was not thought to be limiting at this level. However, it must be stressed that overall survival was not considered to be good at any density.

4.9 Larval movement

During the course of the hatching trial there were frequent occasions when larvae had to be moved. Great care was taken to limit mechanical and thermal stress at this time. Several observations were made, some of which could be more fully investigated in the future:-

1. Eggs which hatched during transport appeared to suffer no setback as a result.

2. The use of mild aeration to move newly hatched larvae out of incubation bags into the main tank caused no detectable damage to the larvae.

3. Larvae held in water could be handled very roughly in the first few days after hatching without showing immediate total mortality (i.e. in the transfer of Batch IV from the 2,700 l to the 1,000 l tank by beakering and pumping), however, final survival was low.

4. Early larvae which had been subjected to great handling stress were noticeably darker than larvae kept in stress-free conditions. The development of partial albinism in later larvae did not appear to be affected by this darkening in the earlier stages.

5. During a period of high mortality when larvae were nearing metamorphosis, there was an immediate reduction of

for example the air distribution system was not connected to the site compressor and was 'jury rigged' from small bore polythene tubes instead of the planned P.V.C. ring main and distribution outlets. Nevertheless the unit was able, successfully, to rear larvae throughout the summer, from the first eggs arriving on June, 12th to the last metamorphosed fish being transported to the nursery on October, 22nd. There were no major interruptions to the routine during this time.

5.3 Two things in particular were found to be limiting to larval rearing:

(a) the poor insulation of the building making larval rearing, Artemia hatching and live food production too vulnerable to changes in ambient temperature.

(b) the continuously rapid growth of weed in the large rearing tanks which trapped larvae and necessitated continuous cleaning.

5.4 It was seen that larvae grew well and were not harmed when tank temperatures were greatly in excess of 20°C and it is probable that larval rearing would be more rapid if tank temperatures higher than 14°-18°C were used.

5.5 Trials with artificial food were promising in that there were definite indications that first-feeding larvae would accept an inert particle.

5.6 Although it was comparatively simple to supply larvae with sufficient rotifers, considerable difficulty was encountered in producing a sufficient supply of on-grown Artemia.

6. RECOMMENDATIONS

As a result of recommendations on the basis of the 1974 results the following works were put in hand for the 1975 season:-

6.1 The hatchery building was insulated and heated so that a minimum internal temperature of approx. 17°C could be maintained throughout the season.

6.2 The sea water supply to the hatchery was filtered and irradiated with UV light before use in larval rearing tanks, and rotifer-algal cultures and filtered before use in the *Artemia* unit to remove filamentous weed and possible harmful organisms.

6.3 The *Artemia* unit was fitted with a heated header tank.

6.4 Space was made available for the installation of tanks specifically for ongrowing *Artemia* nauplii.

6.5 The rotifer tanks were to be held at a constant temperature of 20°C.

6.6 An algal unit was installed to supply several species of algae (instead of just *Phaeodactylum* which may cause gill damage) for greening-up tanks.

6.7 The 2,700 l ongrowing tanks were modified to give:-

(a) a watertight seal at the overflow,

(b) a larva-proof screened overflow system, and

(c) the facility for a recirculation system using a calcereous sand filter to reduce short term temperature and salinity fluctuations.

APPENDIX I

3. Results

3.1 Transport and Incubation

Table 1 Batches of eggs received.

Table 2 Hatching rates.

Table 3 Temperatures during incubation.

3.2 Larval Survival

Table 4 Survival of larvae related to stocking density.

Table 5 Assessments of larval numbers during the season.

3.3 Daily Records

Table 6 Mortalities related to batches and age in days after hatching.

Fig. 1 Temperature and salinity record of Batch I

Fig. 2 " " " " " " III

Fig. 3 " " " " " " IV

Fig. 4 " " " " " " V

3.4 Rotifers

Table 7 Rotifers added to larval rearing tanks ($x10^5$)

Table 8 Rotifer counts in larval rearing tanks.

Table 9 Rotifer production - daily counts.

Fig. 5 Rotifer production related to air temperature.

3.5 Water Exchange

Table 10 Tank water exchange records.

3.6 Survival of Fish

Table 11 Survival of fish in the nursery unit.

Table 12 Growth data of Batch I.

Fig. 6 " " " " " weight.

Fig. 7 " " " " " length.

3.7 Artemia Production

Fig. 8 Artemia eggs hatched daily in 1974.

TABLE 3

Temperatures during incubation.

Batch No.	Date received	Temperature range during incubation at Hunterston °C	Temperature on reception at Ardtoe °C	Temperature at hatching °C
I	12:6:74	11.8 - 12.7	13.9 - 14.5	17.0
II	15:7:74	10.4 - 10.4	13.0 - 13.3	16.3
III	29:7:74	17.3 - 17.5	16.9 - 18.6	hatched on arrival
IV	6:8:74	14.9 - 15.5	15.7 - 16.0	19.0
V	9:8:74	15.0 - 15.5	15.5 - 15.7	18.1
VI	16:9:74	11.0 - 15.6	15.0	hatched on arrival

TABLE 4

Survival of larvae related to stocking density.

Batch No.	Calculated No. of larvae stocked	Tank volume l	Larvae per l	Survival No.	%age survival
I	28,400	2,700	10.5	312	1.1
II	55,000	2,700	20.4	Nil	-
III	120,000 *	2,700	44.5	1,070	0.9
IV	150,000	1,000	+	85	0.06
V	87,000	2,700	32.2	297+++	0.34
VI	1,600	200	++	Nil	-

* larvae hatched on arrival, number calculated from figures at start of incubation at Hunterston.

\+ very poor hatch, few surviving transferred to 1,000 l tank on day 2.

++ larvae hatched on arrival, most larvae committed to artificial food trial, estimated 1,000 larvae transferred to 200 l container but died when temperatures fell.

+++ included sample of 10 fish sent to DAFS Marine Laboratory, Aberdeen within 10 days of transport to nursery unit.

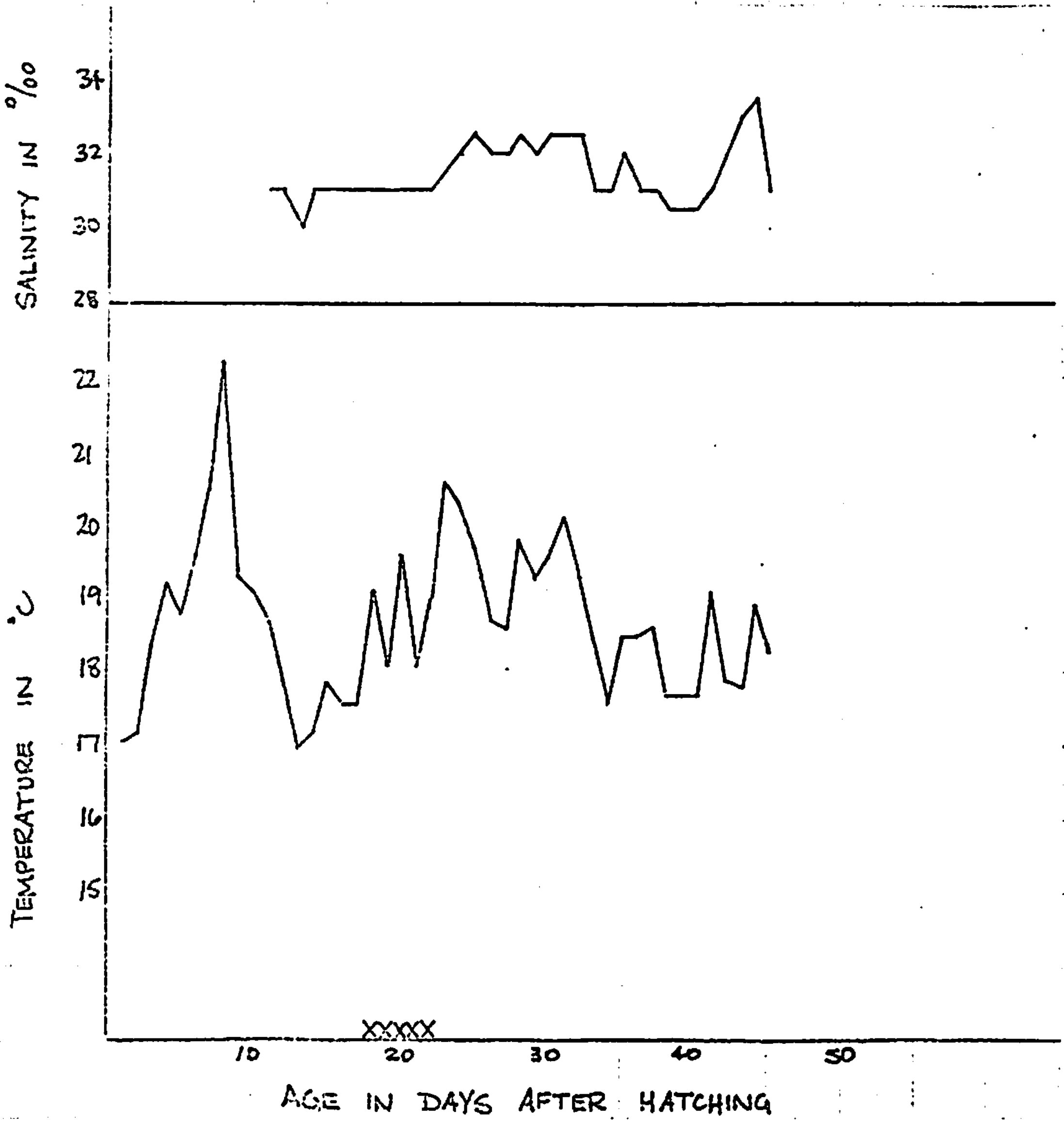
TABLE 6

Mortalities related to batches and age in days after hatching

Day	Batch Number I	II	III	IV	V	VI
1						
2						
3						
4	None recorded due to opacity of water					
5					None recorded due to opacity of water	
6						
7						
8				Deep tank with poor visibility - no mortalities recorded		
9						
10						
11						
12						
13						
14						
15						
16			65			
17	265		75			
18			78			
19	319	Total mortality on Day 1	123			Total mortality on Day 21
20	518		83			
21	178		53		16	
22	219		0		93	
23	74		33		19	
24	14		15		7	
25	7		12		16	
26	10		7		13	
27	7		18		18	
28	0		0		0	
29	0		22		0	
30	5		8		10	
31	0		19			
32	0		4			
33	1		12			
34	3		17			
35	0		14			
36	5		37			
37	9		20			
38	1		24			
39	5		61			
40	3		90			
41	9		83			
42	11		72			
43	0		70			
44	7		57			
45	9		70			
	Transfer					
	Total morts. 1,679					

Table continued on p43.

FIGURE 1. TANK RECORDS BATCH I



x Daily mortality over 100

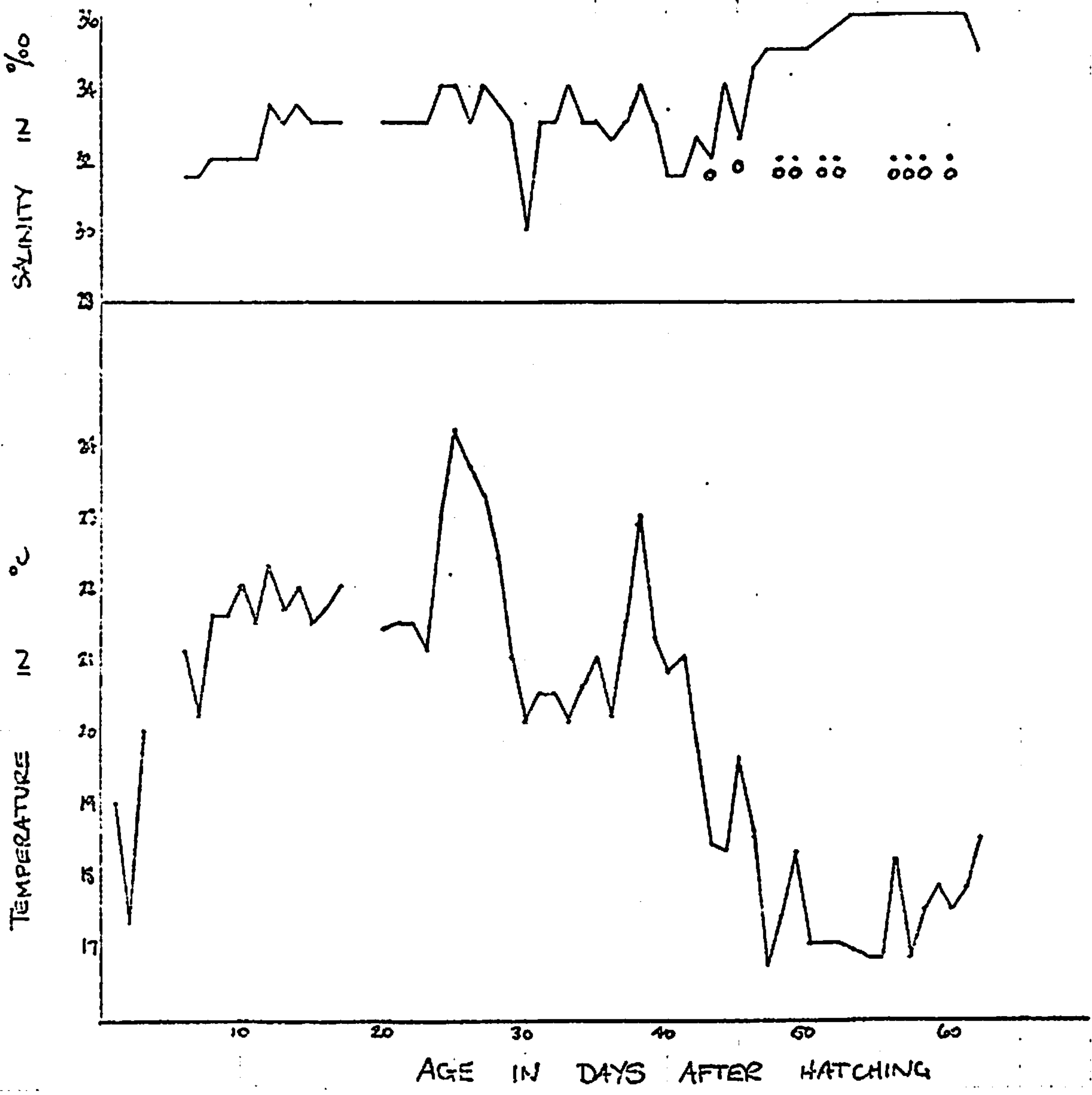


FIGURE 3.
TANK RECORDS — BATCH IV
SALINITY IN ‰
TEMPERATURE IN °C
AGE IN DAYS AFTER HATCHING
Suspect reading
Small batch held in deep tank
- mortalities not recorded for most of trial

TABLE 7

Rotifers added to larval rearing tanks ($x10^5$).

Day No.	Batch I	Batch II	Batch III	Batch IV	Batch V	Batch VI
-2	54.9		0		13.7	
-1	0		0		0	
0	0	62.7	0		0	
1	7.6	49.8	42.0		15.4	
2	7.2	Trial terminated due to mortality of larvae	21.7	Not counted - larvae moved after high mortality	22.4	Not counted - larvae used in artificial feeding trials
3	4.0		26.9		14.1	
4	7.7		17.8		0	
5	7.7		22.7		8.0	
6	5.4		19.3		9.7	
7	5.7		16.0		12.8	
8	8.9		0		23.8	
9	9.9		0		0	
10	8.1		7.4		20.7	
Total added	127.1	112.5	173.8	-	140.6	-

TABLE 8

No. of rotifers per ml in larval rearing tanks.

Day	Batch I	Batch III
0	1.7	
1	1.5	
2	1.6	
3	5.2	3.0
4	5.5	2.8
5	4.8	3.6
6	6.3	4.9
7	9.8	2.8
8	12.0	13.4
9	-	7.5
10	6.0	
11	3.4	

TABLE 9 (Contd.)

Date	Total No.	No. with eggs	No. removed
8:7:74	4,250,000	1,325,000	
9:7	7,600,000	1,750,000	
10:7	12,105,000	2,357,000	
11:7	11,000,000	2,225,000	
12:7	12,225,000	2,750,000	
15:7	11,625,000	4,900,000	
17:7	21,950,000	5,900,000	6,270,000
18:7	17,425,000	3,525,000	4,980,000
19:7	16,825,000	3,625,000	
22:7	16,250,000	2,750,000	783,000
25:7	16,770,000	3,800,000	
26:7	14,620,000	4,580,000	
29:7	14,680,000	4,540,000	4,200,000
30:7	7,000,000	2,250,000	2,170,000
31:7	11,075,000	3,675,000	2,690,000
1:8:74	6,250,000	1,475,000	1,780,000
2:8	7,900,000	2,375,000	2,275,000
3:8	6,775,000	1,300,000	1,930,000
4:8	5,600,000	975,000	1,600,000
5:8	5,025,000	825,000	
6:8	5,375,000	1,300,000	
7:8	5,000,000	1,000,000	740,000
8:8	5,325,000	1,125,000	
9:8	4,800,000	1,450,000	1,370,000
10:8	3,575,000	650,000	
12:8	5,375,000	1,375,000	1,540,000
13:8	5,225,000	1,250,000	2,238,000
14:8	3,300,000	1,075,000	1,413,000
15:8	3,125,000	1,275,000	
16:8	3,425,000	932,500	803,000
17:8			974,000 approx.
18:8			1,284,000 approx.
19:8	7,200,000	2,100,000	2,384,000
21:8	4,825,000		2,070,000
26:8	4,275,000	725,000	1,682,000
28:8	5,950,000	1,625,000	
29:8	4,167,500	757,000	1,597,500
30:8	2,900,000	600,000	230,800
31:8	6,825,000	775,000	1,974,000
2:9:74	8,525,000	475,000	
3:9	6,800,000	200,000	1,945,000
4:9	3,250,000	150,000	713,000
5:9	2,075,000	175,000	
6:9	1,800,000	440,000	

Total rotifers removed to larval rearing tanks equalled 68,500,000

TABLE 10

Tank water exchange records.

Batch I - Reared in 2,700 l tank T2.

Day	Water Exchange l.	Notes
-4	Tank filled	Four days before hatching
5		Foam in overflow chamber
6	+50	First time the bottom could be seen
9		Surface film of algae
11	+200	200 l changed (collar on standpipe during filling)
14		Skimmed off surface film
21	+50	Compensation for leakage
24	+250	Compensation for leakage
25	+50	Compensation for leakage
26	+250	Compensation for leakage
28		Fish seen to be trapped in weed
32	+1280	Water exchange overnight
33	+920	" " "
39	+1370	" " "
41	+1800	" " "
45		Fish removed - tank cleaned

Batch III - reared in 2,700 l tank T2 (later also T1).

Day	Water Exchange l.	Notes
1	Tank filled	Compensation for leakage
3	+500	Compensation for leakage
4	+500	" " "
5	+390 per day	Continuous flow until Day 13
12		Surface film of algae noted
13	+1224	Continuous flow until Day 15
15	+3456	" " " " 19
19	+2880	" and thereafter 1 change/day
47		Transfer of smallest stock to T2 (2700 l)
		Thereafter no change in T2 until transfer on Day 83. 1 change daily in T1 until transfer

FIGURE 6. GROWTH OF BATCH I WEIGHT

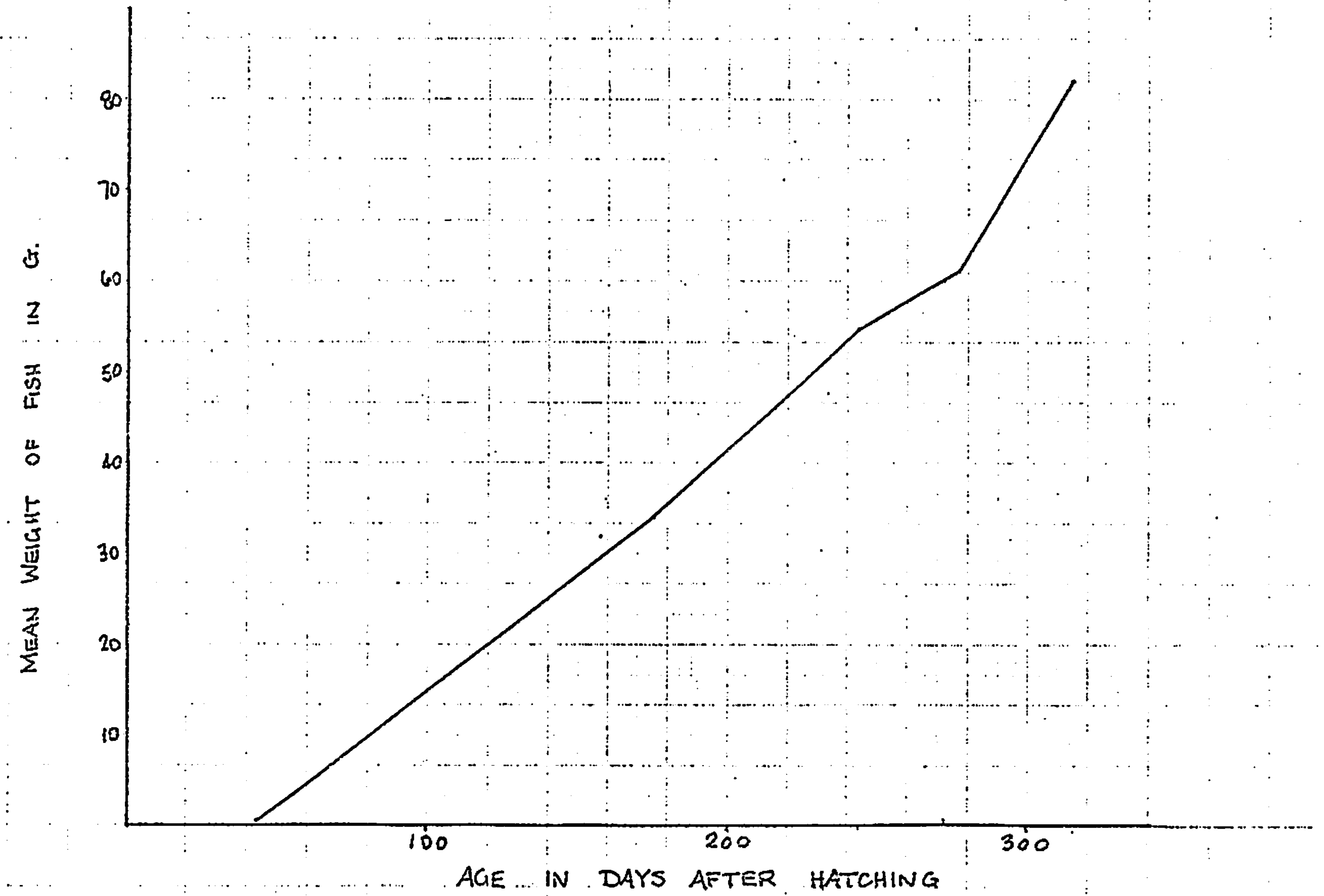


FIGURE 5.

ARTEMIA EGGS HATCHED DAILY IN 1974.

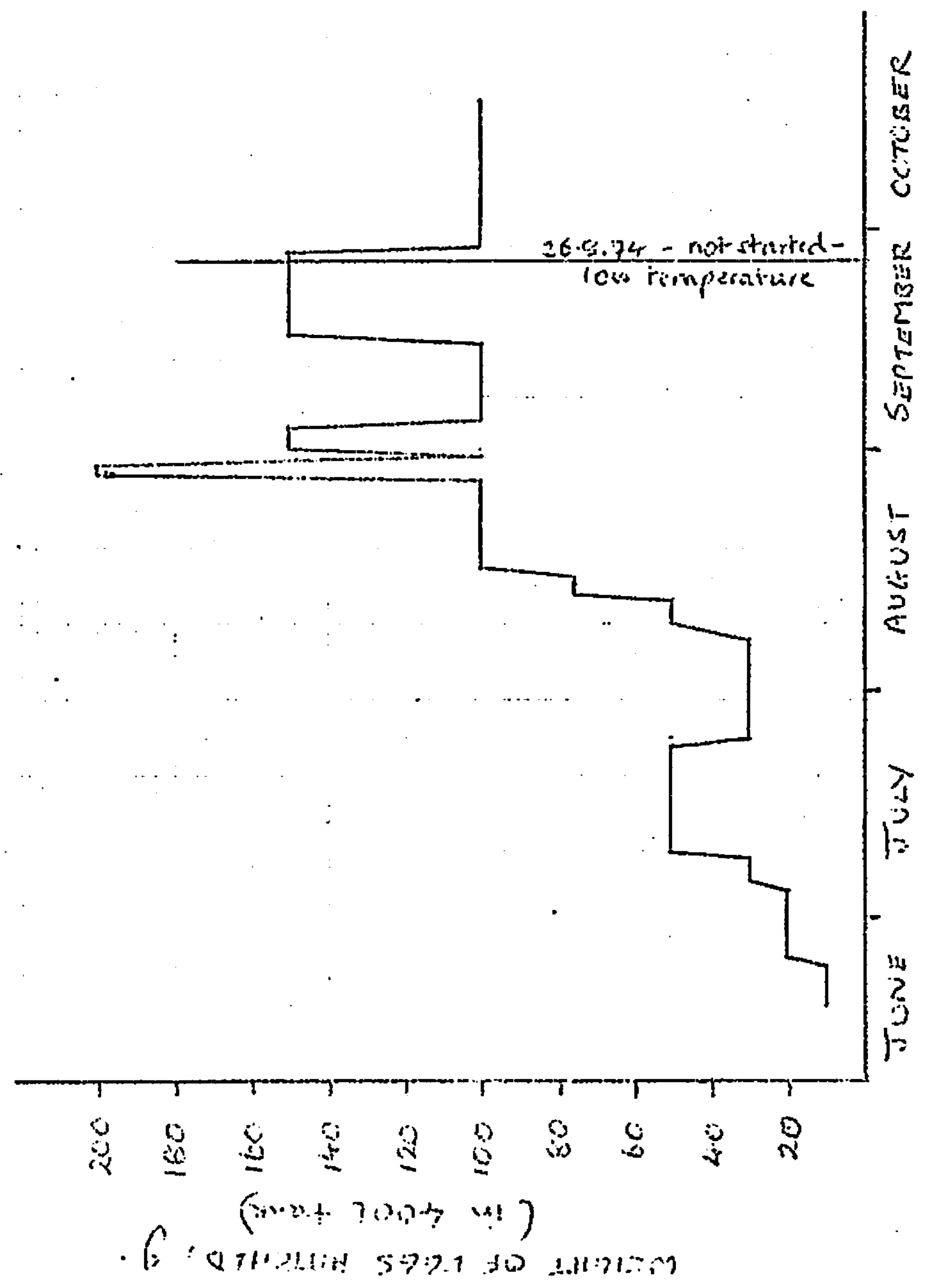


FIGURE 1. ARDTOE HATCHERY ARRANGEMENT 1974

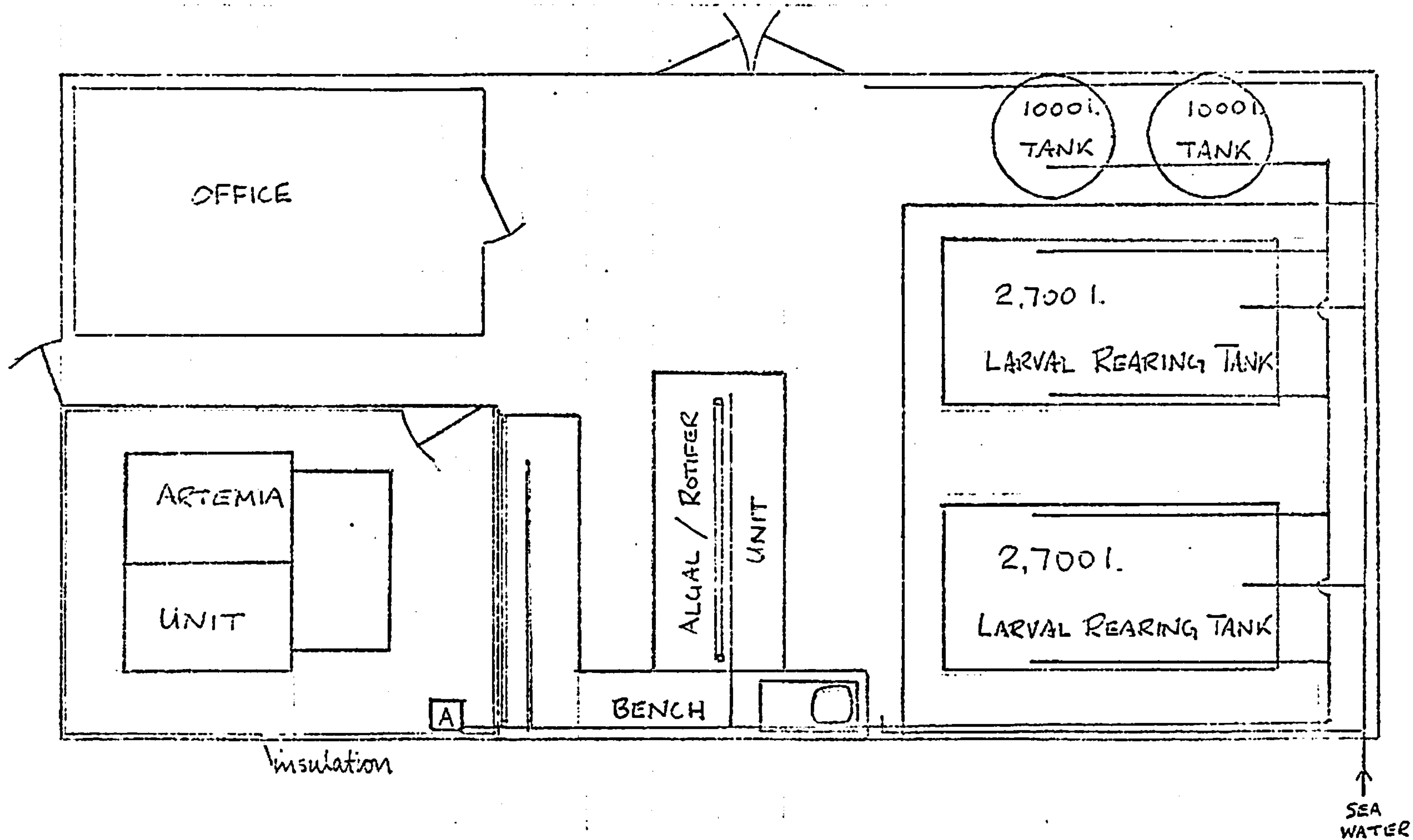
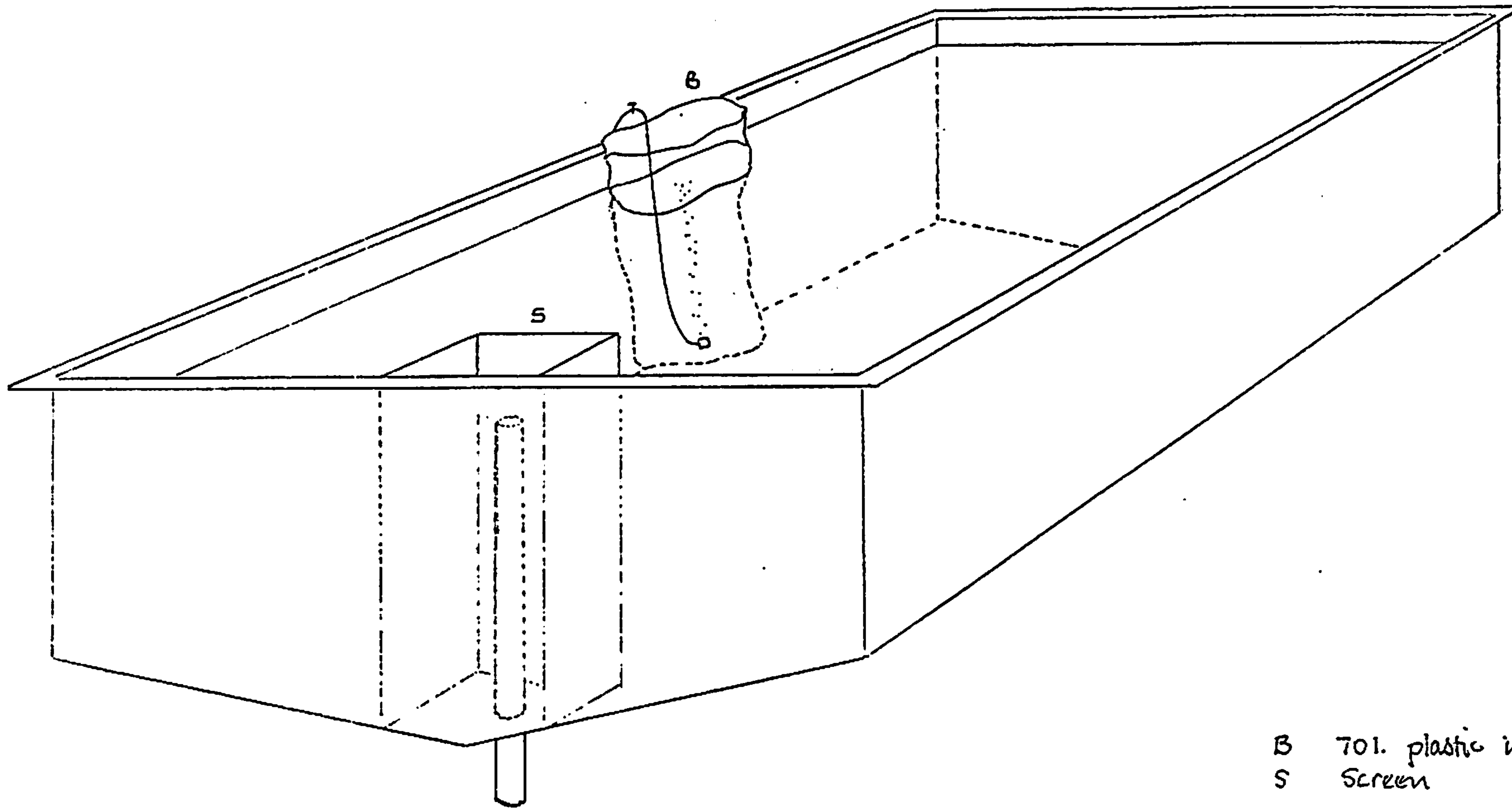


FIGURE 3. 2,700 l. LARVAL REARING TANK



B 70 l. plastic incubation bag
S Screen

FIGURE 5. ALGAL / ROTIFER UNIT - ARRANGEMENT OF TANKS

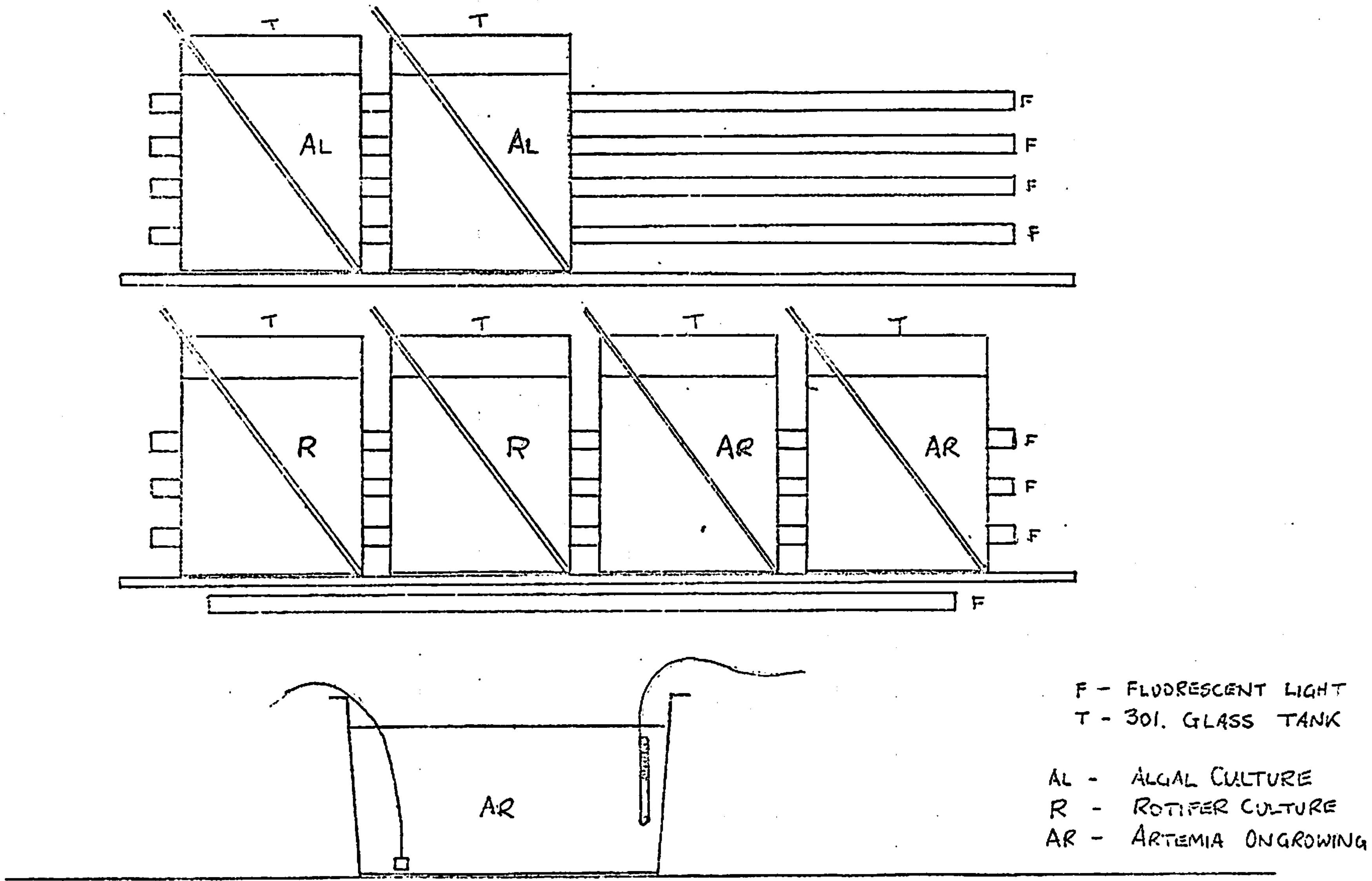
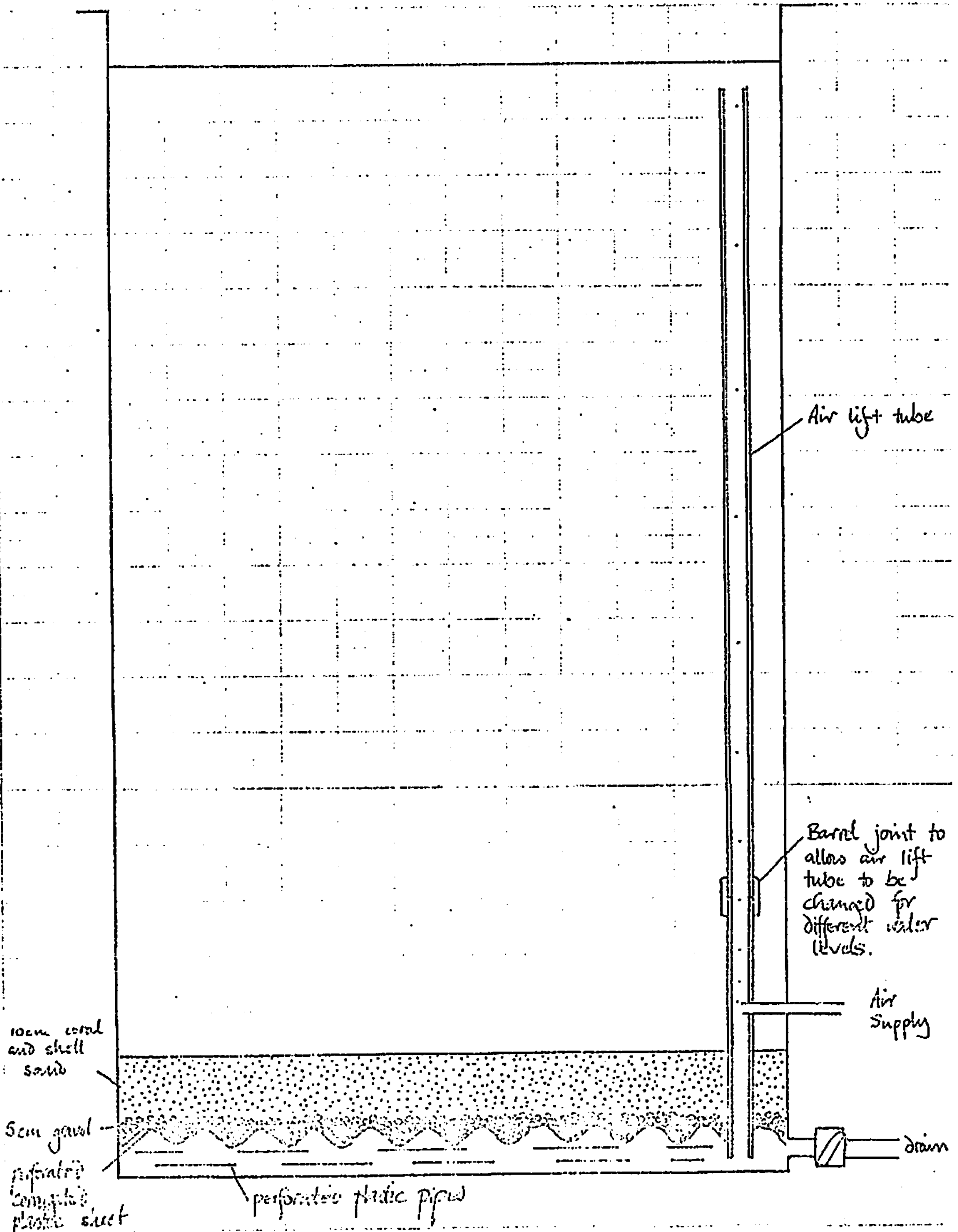


FIGURE 7: CONSTRUCTION OF FILTER IN PROPOSED DEEP LARVAL REARING TANKS



APPENDIX III

AR.6.4
24:4:75

A trial to prove the acceptability of nylon-coated microcapsules to early larvae of the turbot, *Scophthalmus maximus*

1. Introduction

The rearing of turbot from the egg has only been accomplished during the last four years, using a first feed of the rotifer, *Brachionus plicatilis*. Subsequently, the larvae were presented with *Artemia salina*, firstly as nauplii, later as metanauplii and adults.

The culture of rotifers and the on-growing of Artemia have proved to be difficult and considerable interest has developed in the possibility of using artificial foods throughout the larval phase.

This trial was designed to investigate (a) whether first feeding turbot would accept a microencapsulated food, (b) whether It would be an advantage to present rotifers with the encapsulated food and (c) whether the digestive system of the larval turbot could break down the nylon and cross-linked protein walls of the capsules.

2. Method

Microcapsules of two size ranges: small (20 μ - 70 μ) and large (40 μ - 90 μ) were prepared by Dr. D. Jones of Marine Science Laboratories, Menai Bridge. The capsule walls were of nylon and cross-linked protein and the active contents of the capsules were as follows:-

Protein	10% (Haemoglobin)
Carbohydrate	5-10% (Starch)
Fat	4% (Cholesterol)

Two glass jars of 2.5 l capacity were used, each filled with filtered sea water and stocked with 100 turbot larvae two days after hatching, i.e. at the first-feeding stage. In the first

Photomicrographs clearly showed that the capsule membrane had been broken down in the gut. It remains to be established if turbot larvae could be reared for extended periods on microcapsules but it might be reasonable to assume that this trial has shown the principle of larval rearing on artificial diets to be feasible.

4. Recommendations

(a) More trials to be set up in which rotifers are not presented before microcapsules and using larger containers, e.g. 50 l tanks.

(b) A range of microcapsule diets to be prepared, incorporating the same capsule structure, e.g.

	% Diet A	% Diet B	% Diet C
Protein	50	60	40
Fat	35	35	50
Carbohydrate	14	4	9
Vitamins	1	1	1

Suggested constituents:-

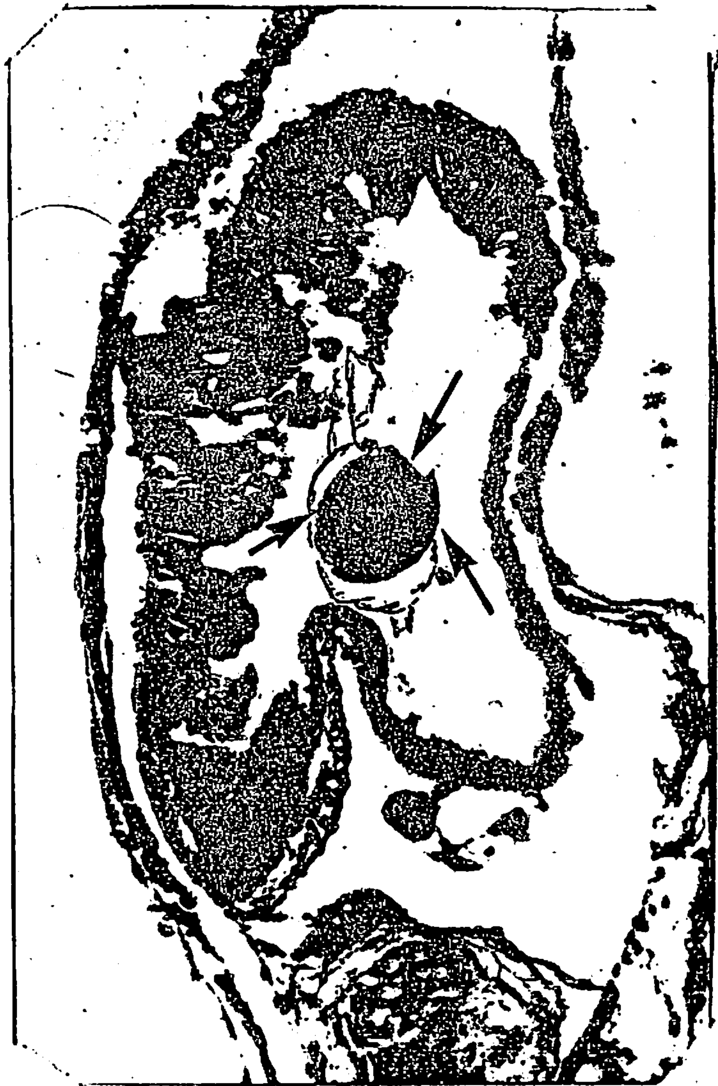
Proteins:	Egg Albumin Plasma Albumin Plasma Globulin Haemoglobin Casein Gelatin
Fats:	Lecithin Cholesterol
Carbohydrates:	Glucose

Vitamin additive (added as 1% of the diet).

Vitamin/

Fig. 1: Photomicrograph showing ruptured microcapsules (3), approx. 50µ, in the gut of a 4 day old larval turbot.

Fig. 2: Photomicrograph showing a ruptured microcapsule, approx. 50µ, in the gut of a 4 day old larval turbot.



APPENDIX IV

Glossary of terms used in the text

Artificial foods	Foods prepared entirely from refined substances.
Azide	An enzyme-inhibitor used to control bacterial activity.
Batch	Group of eggs produced on one occasion (although not necessarily from one female).
Coccidial infection	An infestation by protozoan parasites known as Coccidia.
Drop-out	Dead eggs which have increased in density.
Eyed ova	The last stage of egg development before hatching.
Greening-up	Promotion of a dense algal bloom by the use of high intensity lighting, nutrients etc.
Hatchery	Unit producing metamorphosed fish from eggs.
Hyperplasia	Excessive multiplication of cells in a specific tissue.
Larvae	Juvenile form which is markedly different to the adult.
Metamorphosis	Phase during which the eyes of flatfish migrate to the 'upper' surface.
Metanauplius	The stage of development of crustacean larvae after the nauplius.
Nauplius	The earliest stage of development of crustacean larvae.

Nursery	Unit holding metamorphosed fish until the ongrowing stage.
Pigmentation-normal	In metamorphosed flatfish - having a fully pigmented upper surface and an unpigmented lower surface.
Stripping	The manual removal of eggs from adult fish.
Waterbelly	Oedema of the abdominal region of marine fish larvae.

APPENDIX V

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