Biotic and Abiotic Factors Influencing Initial Swimbladder Inflation of the Blue-Finned Sea Bream *Acanthopagrus cuvieri* (Sparidae), with Particular Reference to Aquaculture in Kuwait

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By

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DECLARATION

I hereby declare that this thesis has been compiled by myself and is the result of my own investigation. It has neither been accepted, or is being submitted for any other degree. All the sources of information have been duly acknowledged.

Khaled Al-Abdul-Elah

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ABSTRACT

Acanthopagrus cuvieri is a sparid fish local to Kuwait and the Arabian Gulf. It is highly valued fish with low landings from local waters and is of great inference for mariculture. Larval rearing and on-growing procedures have already been established by the Kuwait Institute for Scientific Research.

The species is prone to non development of swimbladders, and this is a common problem world wide among sparids and some other species. This abnormality can have a strong economic impact on the development of the industry for luxury species.

The swimbladder in *A. cuvieri* larvae originates as an outgrowth of the dorsal wall of the foregut on the second day after hatching at 25°C. It appears to become functional when the larvae are 4 or 5 days old. The pneumatic duct, gas gland, and rete mirabile have already been developed by that time. The pneumatic duct atrophies and becomes no longer patent on day 10-12, thus ending the outside communication via the oesophagus. The larvae clearly require access to atmospheric air to initially inflate their swimbladders. The data from the vertical migration, larval aggregation, buoyancy and length:weight studies support and give details on how the initial swimbladder inflation takes place.

The effects of selected biotic and abiotic factors on the success of swimbladder inflation were tested. Temperature, light intensity, photoperiod, aeration rate, egg batch, and rotifer:larva ratio all showed a strong effect on initial swimbladder inflation. By contrast, salinity, water-exchange and type of rotifer used did not show any clear effect on initial swimbladder inflation. The optimum rearing conditions that appear to maximize

the success of initial swimbladder inflation are; 25°C, 40 ppt, 1000 lux, 24-hr illumination, 50-70 ml/min of aeration, 8-hr water-exchange at 250 ml/min flow, 100-200 rotifer: larva, L-type rotifer and early egg batch.

Based upon the data generated in this work, the nine abiotic and biotic factors can be grouped into three major strategies, namely: reduction of water surface tension, enhancement of larval fitness, and management of the vertical aggregation of larvae. In combination, these factors contribute to successful initial swimbladder inflation and good survival of larvae.

Overall, this work summarises the development of the swimbladder in *A. cuvieri* larvae and clearly indicate how this first inflation may be maximised through careful husbandry and understanding of the biological processes involved.

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CHAPTER 1

INTRODUCTION

I. INTRODUCTION

There has been a rapid development of aquaculture world-wide during the past two decades, stimulated largely by the stagnation of landings from capture fisheries whose maximum levels of sustainable yields have been reached. Pillay (1976) predicted an increase of 5 to 10 fold in aquacultural products by the turn of the century and Robinson (1982) estimated that the increase will be from 10 million metric tonne in 1982 to about 27 million metric tonne by the year 2000. Nash (1987) gave a slightly lower estimate of about 22.2 million metric tonnes and predicted that by the year 2000 aquaculture production will account for approximately 25% of the total world fisheries harvests. This approximate doubling of aquacultural products by the year 2000 was based on the fact that an increased contribution will come mainly from finfish farming.

In 1983, finfish production was 4.4 million metric tonnes representing 43.1% of the total aquacultural production of all aquatic plants and animals (FAO, aquacultural statistics, 1983). Of this, about 0.7 million metric tonne (about 16% of the total finfish production) was marine and brackish water finfish. Milkfish *(Chanos chanos)* production alone constitutes is about 67% of the total marine and brackish water finfish farming (Jones, 1986), while the other 33% of the production is shared between Yellowtail, ***Seriola quinqueradiata***, Altantic Salmon, ***Salmo salar***, Red Sea Bream, ***Pagrus major***, Mullet, ***Mugil*** Sp., European Sea Bass, ***Dicentrarchus labrax***, Gilthead Sea Bream, ***Sparus aurata***, Turbot, ***Scophthalmus maximus***, Sea Bass, ***Lates calcarifer*** and others. These two groups (67% and 33%) differ greatly in terms of price of product, magnitude of investment, level of technology, production per unit volume, and source of fry.

There are many more marine and brackish water fish species of aquacultural potential. For example, in Japan there are 38 species which already have been subjected to research and production trials, and about 17 of which are presently under cultivation to marketable size or for restocking programs (Kuronuma and Fukusho, 1984; Fukusho, 1985). Girin (1983) listed 13 members of the family Sparidae alone for European and world potential. Similarly, Jones and Houde (1986) mentioned a number of species with mariculture potential presently being researched in the United States of America. Table (1.1) lists some of these species, their locality and their artificial propagation status.

There are a number of constraints to the further expansion of marine and brackish water finfish farming. These constraints may be market related, such as financing, insurance and marketing; or non-market related, such as site availability and licensing, feed cost, or availability and supply limitation of fry. Of these, one of the major biological problem is that of supply of larvae and fry. Most marine and brackish water finfish farming activities have been based on the collection of naturally available fry. This has been practiced for decades and much quoted examples include; Milkfish (*Chanos chanos,*) Yellowtail (*Seriola quinqueradiata*), Mullet (*Mugil Sp.*), and 30-40% of Red Sea Bream (*Pagrus major*), which are responsible for more than 90% of the total production of marine and brackish water finfish farming. There are several inherent disadvantages in the harvesting of wild fry. Supplies are restricted to natural spawning areas and seasons, and may be unpredictable due to variation in the success of natural spawning from year to year. There is a possibility of over-exploiting the wild stock to the detriment of both aquaculture and capture fisheries, and there is no opportunity for selective breeding. There may be contamination with other

Table 1.1. Artificial propagation status of some marine and brackish-water finfish of high aquacultural potential.

Scientific Name (Common English Name)	Egg Diameter (mm)	Total length at hatching (mm)	Achieved production[a]	Approximate Survival (%) (0.2-0.4 g)	Country/ies	References
Acanthopagrus cuvieri (Blue-finned sea bream)	0.78-0.87	1.72-1.96	250,000 P**	10-12	Kuwait	Hussain *et al.* (1981) El-Zahr *et al.* (1988)
Acanthopagrus latus (Yellow-finned sea bream)	0.77	1.80-2.20	100,000 E**	10-15	Kuwait, Japan	Al-Abdul-Elah *et al.* (1985) AkazaKi and Tokito (1979)
Acanthopagrus schegeli (Black sea bream)	0.75-0.93	1.7 -2.18	3,805,000 C**	-	Japan	Kuronuma and Fukusho (1984)
Chanos chanos (Milkfish)	1.1 -1.25	3.2 -3.46	2,859 E	-	Taiwan, Philippines, S. E. Asia	Liao *et al.* (1979); Bagarinao (1986).
Dentex dentex (Common dentex)	0.96	2.17	- E	-	Southern Europe	Glamuzina *et al.* (1989)
Dicentrarchus labrax (European sea bass)	1.2	4.0	6,000,000 C	20-25	Southern Europe	Jones and Houde (1986); Jones (1986); New *et al.* (1987); Girin (1979a).
Epinephelus akaara (Red grouper)	0.7 -0.77	1.45-1.56	-	-	Japan	Mito *et al.* (1967); Ukawa *et al.* (1966)
Epinephelus tauvina (Brown-spotted grouper)	0.7	2.0 -2.4	50,000 P	05-10	Kuwait S.E. Asia	Hussain *et al.* (1975, Chen, *et al.* (1977)
Gadus morhua (Cod)	1.4 - 1.75	4.2 -4.4	- E	-	North Europe	Makhotin *et al.* (1984)
Hippoglossus hippoglossus (Halibut)	3.08	6.4	- E	-	North Europe	Blaxter *et al.* (1983)
Lates calcarifer (Sea bass)	0.74-0.8	1.5 -1.72	20,000,000 C	10-15	Thailand Southeast Asia	SiriKul (1982); Maneewongsa and Tattanon (1982); Tattanon and Maneewongsa (1982).

Table 1.1. (cont'd)

Scientific Name (Common English Name)	Egg Diameter (mm)	Total length at hatching (mm)	Achieved production*	Approximate Survival (%) (0.2-0.4 g)	Country/ies	References
Morone saxatilis (Striped bass)	-	-	- P	30	USA	Kerby, *et al.* (1983).
Mugil cephalus (Grey mullet)	0.93	2.65	58,000 P	05-25	USA; Hawaii S. E. Asia	Kraul (1983); Pullin and Kuo (1981), Kuo *et al.* (1973).
Pagrus major (Red sea bream)	0.8 -1.2	2.0	22,623,000 C	25	Japan	Kafuku and Ikenoue (1983); Kuronuma and Fukusho (1984).
Sciaenops ocellata (Red drum)	0.95	1.71-1.79	- E	-	South and East of USA	Robert *et al.* (1978).
Scophthalmus maximus (turbot)	0.95	2.7	270,000 C	05-10	European countries	Jones *et al.* (1981) Jones (1986).
Seriola quinqueradiata (yellowtail)		3.5	230,000 E	-	Japan	Kuronuma and Fukusho (1984)
Siganus oramin (Rabbit fish)	0.65	2.6	300,000 P	05-10	UAE, Kuwait S.E. Asia	Akatsu *et al.* (1984) (Personal communication with Akatsu)
Solea solea (Dover sole)	1.0 -1.4	3.2-3.7	1,000,000 C	35-50	North Europe	Girin (1979a)
Sparus aurata (Gilthead sea bream)	0.9 -0.96	2.7	1,635,000 C	5	Southern Europe	Girin (1983); Jones (1986); Pullin and Kuo (1981).

* Production of 0.2 - 0.4 g fry

** Production scale as; E = Experimental, P = Pilot and C = Commercial.

species some of which may be competitors or predators, and wild fish may be vectors of pathogens.

The shortage of juveniles is one of the major bottlenecks in the future development of marine and brackish water finfish farming (New *et al.*, 1987; Jones, 1986 and 1981). European examples where these shortages occur are the Sea Bass *Dicentrarchus labrax* and the Gilthead Sea Bream *Sparus aurata* (New *et al.*, 1987). Other examples, are the Sea Bass *Lates calcarifer* in Thailand and neighbouring countries and the Red Sea Bream *Pagrus major* in Japan.

There is wide acceptance of the fact that expansion of the aquaculture industry for many high value species cannot continue to be based on the availability of natural fry. As a consequence there has been an increase in efforts to develop larval rearing techniques for many important species of marine and brackish water fish over the last two decades. The development of larval rearing techniques will permit fish culture operations to become independent of natural supplies of fry. Advantages will also accrue through the ability to program hatchery output to meet the production requirement of individual grow-out units. In the longer term this will enable the selection of desired inherited traits in farmed species and makes possible other aspects of stock manipulation, such as control of maturation by gynogenetic techniques or hormonal treatment.

II. MAJOR PROBLEMS IN LARVAL REARING OF MARINE AND BRACKISH-WATER FINFISHES

The mass rearing of marine fish larvae remains the most difficult area of fry production. Its problems have been the subject of a number of reviews (May, 1970; Houde, 1973; Nash and Kuo, 1975; Kinne, 1977; Chaudhuri

and Tripath, 1979; Girin, 1979a; Houde and Taniguchi, 1979; Nash and Shehadeh, 1980; Pullin and Kuo, 1981; Hunter, 1983; Kuronuma and Fukusho, 1984; Al-Abdul-Elah, 1984; Jones and Houde, 1986). It is now generally agreed that the smaller the egg diameter the greater are the problems in mass larval rearing, especially in those species which have pelagic eggs smaller than 1 mm in diameter. The major problems in mass larval rearing stressed in these reviews are survival, physical abnormalities and consistency and predictability.

A. Survival

Mortalities during larval rearing can occur for different reasons and at different times throughout the hatchery phase. Survival of planktonic marine fish larvae is determined by the interplay of various environmental and nutritional factors. Good survival is a major measure of larval rearing success, but is not necessarily the most important criterion for selecting marine species for culture. Marine and brackish water finfish species usually have higher fecundities and larval survival rates are naturally lower than could be accepted in freshwater species such as Trout, *Oncorhynchus mykiss* (*Salmo gairdneri*) Gall and Groot (1990) . Survival rates from newly hatched larvae to fully weaned fry range from 10% to 25% (Table 1.1) and are considered acceptable for mass rearing and could lead to industrial scale operation.

Major mortalities usually occur during the first 10-15 days after hatching (Jones, *et al.*, 1981; Jones and Houde, 1986; and Bagarino, 1986). Small peaks in mortality are also experienced during addition of the second live-food (Artemia) particularly if there is any nutritional deficiency or other problems such as contaminants. The process of weaning, usually result

in a wide size range of metamorphosed larvae which can also contribute to mortalities, because of cannibalism. Sudden and unexplained mortalities are always attributed to diseases.

Some of the critical parameters which may have a major role in the occurrence of these early mass mortalities of larvae are; egg and larvae size, yolk and oil quantities and qualities, egg handling and incubation, quality and quantity of the initial live-food, feeding schedule and proper rearing environment. The parameters that can influence the early larval survival fall broadly into two groups, the biotic and abiotic factors.

A.1. Effect of biotic and abiotic factors on broodstock and subsequent egg quality.

Both biotic and abiotic factors have a direct or indirect influence on gamete quality through broodstock husbandry, eventually affecting early larval survival. They can also be considered as the parental effect on the gametes and could result from various broodstock husbandry practices. These can be further categorized into nutritional, biological and environmental factors. A thorough understanding of the interplay of these factors will result in a better gamete quality which can give a substantial boost to survival in the subsequent larval rearing phase.

A.1.a Gamete quality

Most of the knowledge on gamete quality is of a descriptive nature. The identification and quantification of the chemical constituents of a "good egg" is still in the research phase, although some ideas have already been verified. Most cultured marine and brackish water species produce small, pelagic, buoyant eggs ranging from 0.65 mm diameter, as in *Siganus oramin* (Lam, 1974), to 3.08 mm as in *Hippoglossus hippoglossus*

(Blaxter *et al.*, 1983). There are some exceptions among the Siganidae where they have very small, adhesive, demersal eggs (0.45mm - 0.68mm; Lam, 1974). Commonly, in marine or brackish water finfish hatcheries, good pelagic egg quality is judged by the following decriptive criteria; percent of buoyant eggs, egg diameter, average number of oil globules (if present), position of oil globule, hatching percent, percent of normal hatchlings obtained and their swimming activities.

The oil globule does not exist in the eggs of some cultivated species, such as the Milkfish *Chanos chanos*, while some of the Siganids have more than one oil globule and this is considered as normal. The normal position of the oil globule differs from species to species. For example, the oil globule is anterior in relation to the yolksac in Sea Bass (*Lates calcarifer*), while in Red Sea Bream (*Pagrus major*) it is in the posterior position. Kuo, *et al.*, (1973), and Fujiya (1979) have both drawn attention to the importance of the species-specific configuration of ovarian oil globules in relation to quality. Nash and Kuo (1975) stated that premature inducement of grey Mullet (*Mugil cephalus*) will result in eggs containing multiple oil globules which will not produce viable larvae.

Poor quality eggs sink rapidly while good ones remain buoyant until hatching in most of the cultivated species (Kuo *et al.*, 1973; Kjorsvik and Lonning, 1983). Buoyant eggs, in general, have a high hatching rate and normal larval development; whereas sunken ones mainly consist of unfertilized or dead eggs (Watanabe *et al.*, 1984a). Other criteria for good egg quality in Cod (*Godus morhua*) were given by Kjorsvik and Lonning (1983) as follows; short cortical reaction (10-15 min.), the synchrony of cleavage within the same egg batch and the larger perivitelline space. Watanabe *et*

al. (1985a, b) attempted to set some criteria for good egg quality through chemical analysis of buoyant and sunken eggs.

The quality of spermatozoa is equally as important as the eggs, but little work on their quality has been published, since it appears to be less problematic than the eggs although this may be a sweeping assumption. Billard *et al.* (1977) reported that the spermatozoa of *Dicentrarchus labrax* age as the spawning season progresses and their quality, as measured by duration and intensity of motility on dilution, becomes lowered.

A.1.b Nutritional effect

Although nutrition is known to have a profound effect upon gonadal development, maturation and spawning parameters (Watanabe, 1986b), precise information on the nutritional requirements for gonadal development and maturation in marine and brackish water species is lacking. It has been generally agreed that in addition to quality and quantity of feed, the feeding regime also has an important influence in successful spawning, in promoting good egg quality and viability of hatched larvae (Watanabe *et al.*, 1984a, b, c, 1985b; Kanazawa, 1985; Jones and Houde, 1986). However, research on broodstock management to date has placed more emphasis on other factors which affect gonad maturation and spawning such as hormone injection, selective breeding, hybridization and control of environmental factors such as temperature, photoperiod and stocking density.

Much nutritional information is available on freshwater broodstock. Lovell (1979) working on the effect of diet on the reproductive performance of Channel Catfish (*Ictalurus punctatus*) found that denial of a vitamin supplement could effect the quality of spermatozoa. Shimma *et al.* (1977) found that the hatchability of carp ova was greatly reduced when

dietary C22:6W3 fatty acids were reduced below 10%. Broodstock feeding regime and ration size has a significant effect on ova diameter, number of eggs produced and successful spawning in Brown Trout (*Salmo trutta*; Bagenal, 1969) and Rainbow Trout *Oncorhynchus mykiss*; Springate and Bromage, 1984). Takeuchi *et al.* (1981) showed the significant role of manganese concentration in broodstock diets for Rainbow Trout *Oncorhynchus mykiss* in producing better egg quality. The nutritional requirements for marine and brackish water broodstock have generally been little researched, and Watanabe *et al.* (1984a, b, c, d, and 1985a, b) are from the few papers published on this subject on the Red Sea Bream, *Pagrus major*.

The natural food of a wild fish is usually very varied and it is not practical to collect and store it for feeding to captive broodstock, or to present it in a natural way. Therefore,the broodstock nutritional requirements must be identified and quantified for the major cultivated species. These nutritional requirements may be variable with time, such as before spawning, during spawning and after spawning. Some hatcheries use commercial pellets made for on-growing fish as a broodstock diet although these diets are not specifically designed for broodstock and the formulation of a specific broodstock diet based on their nutritional requirements would be the practical solution. The use of fresh natural feed as a supplement may be helpful in these cases.

The source of protein, lipid, vitamins, minerals, fat-soluble pigments, HUFA and phospolipids and their constituents is another area of research important in developing a broodstock diet comparable to that in the natural environment. These research areas were stressed in a series of publications by Watanabe *et al.* (1984a, b, c, and 1985b) and many important findings were established. Table (1.2) summarizes some of these findings.

Table 1.2. Collected data on the effect of different broodstock diets on spawned eggs, hatched and fed larvae of Red sea bream (*Pagrus major*)

Test diet	Control *	Cuttlefish meal *	Frozen Krill **	HUFA Deficiency **	Control plus Krill oil extract ***	Control plus 250 mg vit. E/ 100 g diet ***
Protein source	White fish meal	Cuttlefish meal	Frozen Krill	White fish meal	White fish meal	White fish meal
Crude protein	43.1	43.0	14.5	45.7	45.5	44.6
Lipid source	Cuttlefish	Cuttlefish	-	Corn oil	Krill oil extract	Cuttlefish oil
Crude lipid (%)	9.4	9.1	2.3	16.6	12.0	10.4
E ω3 HUFA (area %	25.8	30.0	34.3	5.2	24.1	25.3
Feeding period before spawning (months)	5	5	During spawning (high protein diet used before spawning)	During spawning high HUFA diet used before spawning	26 days before spawning and during spawning	26 days before spawning and during spawning
Spawned eggs						
Average eggs produced/fish (10^4)	205.1	197.3	202.1	58.3	153.8	132.7
Floating eggs (%)	61.8	83.5	82.7	18.2	84.1	77.9
Average number of oil globule	2.0	1.0	1.09	3.43	1.81	1.64
Hatched Larvae						
Hatching rate (%)	70.0	97.5	90.3	27.3	85.4	84.1
Normal Larvae (%)	24.2	79.5	68.1	1.2	65.0	58.4
Feeding Larve (25 days)						
Survival (%)	17.5	66.5	15.4 (High Cannibalism)	-	-	-
Total length (mm)	9.36	10.0	11.2	-	-	-
Inflated (swim-bladder (%)	96	96	93.3	-	-	-

Source:
* Watanabe *et al.* (1984c)
** Watanabe *et al.* (1984d)
*** Watanabe *et al.* (1985b)

They showed that in fishes which accept diet actively during spawning, like the Red Sea Bream, *P. major*, the quality of diet given to broodstock even shortly before or during spawning, can have a great effect on their reproduction and on the egg quality produced (Watanabe *et al.*,1985b). Another finding was the superiority of Cuttlefish meal over white fish meal as a protein source in broodstock diet of Red Sea Bream, which could not be explained through intensive chemical analysis of broodstock and eggs produced (Watanabe *et al.*,1984b, d; 1985a). Despite this, a remarkable piece of information was observed, which was the decrease of concentration of W3 HUFA in spawned eggs from each of the five groups of broodstock as the spawning progressed. The concentration of W3 HUFA in the eggs from the broodstock fed on Cuttlefish meal which is high in W3 HUFA (29.6%) showed a slight decrease, but a relatively high steady level throughout the spawning period, while the eggs from the broodstock fed on white fish meal (control) which is low in W3 HUFA (18.5%) showed a constantly decreasing level throughout the spawning period. The survival of larvae 14 days after hatching from eggs obtained from the broodstock fed on Cuttlefish meal and white fish meal was 24.3% and 7.3% respectively (Watanabe *et al.*,1984a).

A.1.c <u>Biological effect</u>

Many biological features affect the performance of broodstock including the type of spawning, relative fecundity, spawning age or size, sex ratio, whether the broodstock are wild or cultured, and strain or race.

Spawning of broodstock by hormonal inducement is more common than natural spawning. Blaxter (1981) stated that results from induced spawning are not always as good as with natural spawning. Nash and Kuo (1975) described oocytes which have been accelerated by the use of hormones as

inferior to those allowed to develop naturally. Hatcheries require a large amount of eggs of a similar age (spawned within 24 hours) to simultaneously stock a large number of rearing tanks, and this generally precludes the adoption of a natural spawning scheme, especially if the broodstock are of a small size and have relatively low fecundity. Natural spawning of large broodstock with high relative fecundity will, however, meet the demand of a large amount of eggs spawned the same day. The trend now-a-days in European marine hatcheries is to use hormonal inducement and natural release of gametes to synchronize ovulation and fertilization and to produce the maximum amount of similar-aged eggs with the minimum number of broodstock possible.

The selection of a specific size or age of brooders can either depend on their relative fecundity, egg size, percentage of floating and hatching eggs, deformed hatchling percentage and larval survival, or to factors concerning broodstock handling and transfer, and can be of both. Young adults as first-time spawners are of questionable value as broodstock. An evidence of this, Gall (1974) showed that for Rainbow Trout *Oncorhynchus mykiss* progeny from second spawning females (age of 3 years) grew more rapidly than those from first spawners (age of 2 years). Springate and Bromage (1984) noted that older brooders of rainbow trout will produce bigger eggs. Bagenal (1971) quoted Simpson (1959) stating that older female Plaice (*Pleuronectes platessa*) produce larger eggs than young adult females. On the other hand, Kittaka (1977) stated that the average rate of fertilization of Red Sea Bream (*P. major*) eggs in 80% from younger breeders and 34% from the older ones. The average hatching rate of floating eggs is also higher at about 85% for the younger group and 55% for the older one. Bagenal (1971) concluded that some marine species with pelagic eggs have a difference in egg volume of over 100% and that there is a decrease in size as the spawning

season progresses. He attributed this to biological factors rather than environmental ones, such as salinity or temperature, quoting Simpson (1959) who stated that these biological factors could be due to progressive change in age composition of spawners. Thus, the older fish spawn first and younger ones will dominate towards the end of the spawning season. Watanabe *et al.* (1985b) found that the egg diameter of six groups of Red Sea Bream (*P. major*) breeders of similar size and age, fed on different diets of various protein source, decreased from 0.90 - 0.92mm to 0.83 - 0.88mm as the spawning season progressed, and the smallest eggs were observed with diet No. 5 (Cuttlefish meal) giving a reduction in egg diameter from 0.90 to 0.83mm. It could be a function of increased fecundity and not necessarily a detrimental effect, since larval performance was good.

Another biological aspect of broodstock is the sex ratio, where a reduction of spawned eggs or even complete failure of spawning could result from an excess of males in the tank which tend to spend most of the time in fighting and chasing each other. On the other hand, very few males may also lead to reduction of spawned eggs.

Inbreeding is another problem associated with using only cultured breeders usually from the same hatchery and this should be avoided to obtain genetically normal progeny. Annual addition of wild fish to the broodstock will help to maintain a large genetic pool.

The failure to understand these biological aspects of a broodstock can lead to inferior gametes reflected by over-ripening, premature release, smaller egg diameter, low egg production, low fertilization and hatching rates and low percent to buoyant eggs. This will be reflected in larval rearing and early survival.

A.1.d Environmental factors

The total environment is affected by many factors, including broodstock holding tank size and design, water quality and photoperiod. Shehadeh (1975) reviewed previous work and concluded that there is a lack of knowledge of the effect of environmental stimuli on reproduction. Holding the broodstock in net-cages for 8-10 months prior to spawning and then transferring them indoors (or outdoors) for spawning and egg collection is practiced in many European and Japanese hatcheries (Fujita, 1979; Kafuku and Ikenoue, 1983; Lisac, 1986). This procedure has proved useful in minimizing stress, diseases and water quality degradation before spawning which are very important in assuming normal gonadal development and spawning. It is well known that photoperiod triggers gonadal development and that water temperature is the fine tuner for the speed of gonadal maturation. The procedures for influencing spawning date through photoperiod and temperature manipulation are well documented (Kuo *et al.*, 1974; Kuo and Nash, 1975; Girin and Devauchelle, 1978; Gillet *et al.*, 1978; Bromage, 1986; Davies *et al.*, 1986a, b; Bye, 1987 and many others) and are now becoming more widely used.

Tank design should satisfy the swimming, courtship and spawning behaviour requirements of the broodstock. For example, fast surface swimmers with very active courtship and spawning behaviour might require more surface area and less depth. Further examples are given by Al-Abdul-Elah (1984). Broodstock stocking density is another factor which is important in determining the size of the tank and which is also affected by the breeder size and activities. Natural spawning of sea breams (gonads development, maturation and release of egg) has been achieved in Japan and Kuwait at about 1 Kg/m^3 (Fujita, 1979; Teng *et al.*,1984).

Low dissolved oxygen could affect feeding rate and possibly affect body conditioning of the breeders. Carlson and Herman (1978) working on *Pomoxis nigromaculatus* found that low dissolved oxygen reduced spawning. Accumulation of faecal matter, uneaten food and dead algae provide an excellent substrate for harbouring pathogens. Multiple infection of the breeders prior to spawning can markedly affect spawning and gamete quality produced. The direct effect of bad environment on the breeders' gamete quality, and later on larval survival, is not clear, but there could also be an indirect effect. A clear direct effect on spawning rather than gamete quality can be easily inferred.

A.2. Effect of biotic and abiotic factors on egg incubation, hatching and larval rearing.

The main biotic and abiotic factors that govern larval survival and performance can be grouped under two headings; environmental and nutritional. These biotic and abiotic factors influence the ability of larvae to establish themselves as successful feeders, and have a major effect on larval survival. Collectively they constitute sound procedures for larval rearing practice.

A.2.a Environmental factors

A knowledge of the environmental requirements for egg incubation and larval rearing is essential for successful fry production. Fletcher (1976) who was quoted by Pullin and Kuo (1981) made a practical assessment of the environmental requirements of larvae and some ingenious proposals for simulating the natural environment as far as possible. However, high density incubation and rearing in hatcheries will usually, of necessity, present eggs and larvae with a vastly different environment from their own in natural

waters with respect to all aspects of water quality, including dissolved oxygen, accumulation of metabolites, temperature, salinity and microbial populations.

A.2.a.1. Egg-incubation and hatching

Even when good eggs are obtained through sound broodstock husbandry, unsatisfactory practice in egg collection, handling or incubation might result in poor hatching or weak hatchlings. Mechanical or thermal stress during egg handling and transfer should be avoided. During subsequent incubation of marine pelagic eggs, many factors can have a strong influence on quality of hatchling, including, oxygen-uptake, osmoregulation and buoyancy, temperature tolerance, illumination, mechanical damage due to excessive turbulence or rough handling. The best combination of temperature and salinity for total resorption and best utilization of yolk should be adopted. In general, however, the incubation of ova present few problems in fish culture, far fewer than those associated with larval rearing.

In egg incubation oxygen-uptake increases rapidly during the course of embryo development. A herring egg at fertilization has an O_2-uptake of 0.01 ul/h, but at hatching it is 0.07 ul/h (Braun, 1973). Oxygen-uptake in temperature-dependent and herring eggs had a standard O_2-consumption of 1.5 ul/mg/h at 8°C and 4 ul/mg/h at 12°C (Hempel, 1979). Generally, in marine fishes with pelagic eggs, O_2-uptake does not pose a serious problem if the water is kept near saturation. This is due to the fact that the chorion is very highly permeable to O_2 and oxygen transfer occurs readily (Hempel, 1979). Buoyant pelagic eggs tend to collect at the surface around the sides of tanks or hatching nets. The use of gentle aeration will keep them in suspension and break their dense mat-forming at

the water surface layer which could otherwise cause localized oxygen depletion.

Alderdice and Forrester (1968) showed that for *Pharophrys vetulus*, there is a considerable range of temperature-salinity combinations from which the majority of eggs give normal larvae. The same authors (1971) working on Petrale Sole (*Eopsetta jordani*) noted that optimum combinations of temperature and salinity (6-7°C and 27.5 - 29.5 ppt) during egg incubation yield greatest number of viable larvae of largest size at yolk exhaustion. Their work showed how each species has its own requirements. Santerre and May (1977) studied the effect of salinity on the thermal tolerance of eggs and larvae of *Polydactylus sexfilis* (Threadfin) and suggest that salinity can influence the thermal tolerance range of both eggs and larvae. Blaxter (1981) noted that the osmotic regulation by the egg is not fully functional until the blastopore closes; before this the developing egg cannot withstand wide ranges of salinity for long periods.

Hatching time is dependent on incubation temperature. For example, Cod (*Gadus morhua*) eggs hatch within 7-8 days at 13°C, but require 30-32 days at 2°C, (Blaxter, 1981). Blaxter suggested that, as a guide it would seem acceptable to use rearing temperatures within ±2°C of the natural incubation temperature. An optimum incubation temperature is judged by hatching percent and the properties of normal larvae which are properly pigmented and active in movements. Camus and Koutsikopoulos (1984) working on *Sparus aurata* eggs, tested a range of incubation temperatures (7.7 to 26.3°C). They found that the highest hatching rate and the lowest rate of larval abnormalities were both observed at 14.5°C which is also the spawning temperature. Gulidov and Popova (1979) examined the influence of incubation temperature on larvae hatching and abnormalities in Roach (*Rutilus rutilus*).

They found that 10-12°C is the best temperature range for incubation. Irvin (1974) working on *Solea solea*, established that the embryonic stages are stenothermal, whereas the larval stages become increasingly eurythermal with age.

Pelagic eggs are often highly resistant to visible light, although they are normally not protected by pigmentation. Breder (1962) discussed the importance of transparency of eggs and larvae for their survival. Transparency is typical of marine pelagic eggs occurring in brightly illuminated places and is considered as a protection, particularly against heat radiation which will pass through with little absorption. However, eggs exposed to ultraviolet radiation have a reduced hatching rate in various marine species (Marinaro and Bernard, 1966). Pommeranz (1974) studying the risk for Plaice eggs (*Pleuronectes platessa*) existing near the sea surface, found that mortality was not directly dependent upon the applied dose of total daylight but upon its ultraviolet component. The threshold for mortality was between 250 and 460 ly/day (ly = 1 Langley = 1 cal/cm^2).

There are two kinds of mechanical damage possible while handling eggs. Abnormalities and mortality which can be caused by vibration and shaking during gastrulation prior to closure of the blastopore (Blaxter, 1981) and secondly, destruction of the chorion. Pommeranz (1974) and Kjorsvik and Lonning (1983) studied Plaice (*P. platessa*) and Cod (*Gadus morhua*) eggs and found that maximum resistance of the eggs to crushing was 700g and 120g respectively. In Plaice (*P. platessa*) eggs, deformation had to exceed 70% before the thin chorion burst and it was only the bursting of the chorion which led to mortality. Hardening of the chorion is a very quick process. During the first 10 hours after fertilization, the required crushing force for the egg chorion rose from 1.5g to about 500g and reached

700g at the end of gastrulation. A steady decrease in chorion strength was then noted, reaching its lowest value at hatching. It is thus recommended that eggs are handled after the end of gastrulation to avoid any possible damage (Pommeranz, 1974).

A.2.a.2. Larval rearing

Rearing marine species from newly-hatched larvae to fully weaned fry is a difficult task. Many larvae tend to refuse to feed and exhibit narrow, specific environmental and nutritional requirements. These must be met immediately because the tolerance of larvae to prolonged environmental stress or to starvation is lower than that of any other life-cycle stage and they are also generally much less adaptive(Kinne,1977). The interaction between the environmental and nutritional factors is very strong. The environmental factors that may affect larval survival are many, some of the major ones being tank size, colour and shape, light intensity and photoperiod, water temperature, and aeration level (mechanical and chemical effects). More complex ones are the addition of unicellular algae as a water conditioner, microbial and macrobial populations in rearing water and water flow changes to suit the progressive development of the larvae.

Kinne (1977) stated that the spatial requirements of captive fish larvae depend to a large extent on their locomotory activities. Kuo *et al.* (1973) attributed high mortalities of *Mugil cephalus* larvae to their diel vertical migration behaviour and hence to mechanical damage brought about by prolonged contact with a solid surface (tank bottom). They support this observation by the fact that lower mortalities were noted when deeper (1.5m) rearing tanks were used. Hunter (1983) suggested the use of 100 to 400 liter cylindrical black containers made of fiberglass as a minimum for better

growth and survival. Houde (1973) recommended using large rearing systems for better survival and growth and he observed that some larvae were trapped in the corners when rectangular glass tanks were used. Many other authors generally support this idea that large and circular rearing systems are associated with higher survival and growth rates.

Internal matt black-walled rearing tanks have been recommended by Shelbourne (1964) and Blaxter (1962, 1970, 1981). The matt black background appears to make food organisms more visible to the larvae (Nash and Kuo, 1975). Shelbourne (1964) noted the dangers from crevices and internal reflective surfaces in the rearing tanks. Typical marine larval rearing tanks used by European and American mariculturists tend to range from 2 to 20 m^3 in volume. The Japanese, by contrast, use large concrete tanks of 20-200 m^3 in volume.

Light intensities reported to be appropriate for larval rearing range from 250-10,000 Lux (Houde, 1973) although a 500-3000 Lux range has been used most often (Houde and Taniguchi, 1979). Literature on the effects of light intensity on survival of marine larvae is contradictory. Barahona-Fernandes (1979) working on *Dicentrarchus labrax*, using artificial light (fluorescent day light), found that high light intensities (1400-3500 Lux) gave poor survival. Kiyono and Hirano (1981), by contrast, working on Black Sea Bream (*Mylio macrocephalus*) using fluorescent day light found that best survival was obtained at 3000 Lux. Tandler and Mason (1983) working on *Sparus aurata* and using fluorescent daylight, found that increasing light intensity from 205 to 1370 Lux gave better survival. The evidence, reviewed by Blaxter (1970), demonstrates that the preference of fish for light of specific intensities varies among species and also among individuals. Kraul (1983) working on *Mugil cephalus*, tested various light

sources and found that direct sunlight (shading the tanks during the first week) and diffused through fiberglass roughing gave the best survival. Most of the literature on the effects of photoperiod on larval survival agree that constant illumination results in the highest survival (Barahona-Fernandes, 1979; Tandler and Mason, 1983; Tandler and Helps, 1985; and El-Zahr *et al.*, 1986).

Hayes and Pelleut's (1945) work on salmonids as quoted by Pullin and Kuo (1981) showed that the efficiency of yolk utilization was 42% at 5°C or below, but reached about 60% when temperature was raised to 12°C. Ryland and Nichols (1967) working on Plaice (*P. platessa*) larvae found that they have a sharply defined thermal optima of utilization efficiency between 6.5-8°C resulting in 10% larger larvae at the time of first feeding. Sea Bream (*Archosargus rhomboidalis*) larvae were found to retain more yolk at the onset of feeding at 26°C that at other temperatures tested. They thus have more yolk to sustain themselves if food is not present and would have a longer learning period for food recognition and capture (Houde, 1974). All the above examples clearly indicate that an optimum temperature during egg incubation and early larval rearing enhance larval performance and hence survival.

The provision of aeration to maintain adequate dissolved oxygen, even mixing of live-food and creating desirable gentle turbulence is now a standard hatchery procedure. Barahona-Fernandes (1978), using 150 liter cylindriconical tanks, indicated that very low aeration rates (about 0.5 - 1.0 ml/min) gave poor survival rates of *D. labrax* larvae, where larvae remained near the surface and tank walls in dense patches. Medium or strong aeration rates (40 to 100 ml/min) gave better survival and the larvae showed rheotactic behaviour and were more evenly distributed; it also reduced the

bacterial population in the larval rearing tank. Chatain (1982) working with *P. major* larvae, found that 50 ml/min gave better survival than 1000 ml/min in 1000 liter rearing tanks.

The use of "Green water" (unicellular phyto-plankton) as a "water conditioner" in the larval rearing systems is widespread but not always necessary. It is mainly used with the cultivated sparids and other species. Green water is not used in European Sea Bass (*D. labrax*) larval rearing procedures. It is well documented that the larvae benefit from the addition of unicellular algae to their rearing tanks during the rotifer feeding period which varies with species from 14 to 30 days after mouth opening (Jones, 1970; Anonymous, 1970; Houde and Palko, 1970; Houde, 1973; Howell, 1973; 1979; Spectorova and Doroshev, 1976; Scott and Baynes, 1979; Moffat, 1981; Jones and Houde, 1986). The unicellular algae is a source of HUFA and the nutritional benefit comes either directly from larvae drinking rearing water or indirectly through the rotifer feeding on the algae. There is some disagreement on the role of the unicellular algae in larval rearing tanks. The debate is persisting as to whether the beneficial effects of algae derive mainly from their direct or indirect food value or from improvements in water quality such as increased dissolved oxygen and decreased nitrogenous compounds, particularly ammonia (Alderson and Howell, 1973; Alderson, 1979; Howell, 1979). Others suggest that unicellular algae have a general stabilizing effect on the larval rearing environment through a competitive reduction of the bacterial population, or that they provide a supplement of some necessary vitamins or amino acids in solution (Nash and Kuo, 1975).

Algal blooms in the larval rearing tank can cause massive mortality. This phenomenon is accompanied by oxygen super-saturation and affected larvae tend to float forming a mat of dead larvae. These larvae

usually have an air bubble in their stomach or an over-expanded swim bladder (Kafuku and Ikenoue, 1983; Cornacchia and Colt, 1984). Light intensity, amount of unicellular algae added and grazing rate of the remaining rotifers appear to determine the occurrence of this phenomenon. The worst combination is high light intensity (>10,000 Lux), unicellular algal count of more than 400 x 10^3 cell/ml of rearing water and low grazing rate by the rotifers. This phenomenon usually occurs during the first 2-3 weeks of larval rearing and it should not be confused with swim bladder stress syndrome. It is common in hatcheries using diffused sun light as their illumination source. Another problem with unicellular algae is the species selected, which should be easy to scale-up, digestible by the rotifers and have an adequate concentration of HUFA.

One of the main sources of increasing microbial populations in larval rearing tank is the daily addition of rotifers. Washing and filtering the rotifers before adding to the larval tanks only reduces the load added. The complete harvest of rotifers tends to introduce less microbial populations than the continuous (batch harvest) method. Antibiotics and antifungal agents can be used as routine prophylactic measures in the larval rearing tank to reduce microbial contamination and help in attaining better survival. Blaxter (1981) found that bacterial counts of 1000/ml showed no enhanced mortality on Herring (*Clupea harengus*) larvae, although normal levels were 200-400/ml. Routine prophylaxis with antibiotics and antifungal agents does, however, encourage the development of resistant strains of pathogens, as in any other branch of animal husbandry. Microbial populations can be stabilized by filtration and by sterilization using ultraviolet light (Spanier, 1978), ozone (Sander and Rosenthal, 1975) or a variety of antibacterial substances such as nitrofurans, (e.g. Furnance)

(Barahona-Fernandes, 1977). It should also be noted that high populations of other organisms, especially ciliate protozoa, are implicated in high larval mortalities (Nash *et al.*,1977). They recommended the use of a combination of reduced salinity and formalin baths to control such organisms.

A.2.b Nutritional factors

Before considering the nutritional factors affecting early larval survival it must be borne in mind that the condition of the larvae is crucial. The initial well-being of larvae will determine whether or not they will establish themselves after mouth-opening as successful feeders. Some larvae hatch at very early stage of development with no digestive system and their mouth closed as in *A. cuvieri* (Hussain *et al.*, 1981) while others hatch with well developed digestive system as in haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) (Lawrence, 1978). Larval fitness is determined by egg quality, proper incubation and the environmental conditions of rearing during the few days before mouth-opening. Weak larvae usually do not survive beyond the first 10 days (depending on species and rearing temperature). Having produced strong larvae, then knowledge of their nutritional behaviour and requirements becomes important. Associated with this, the necessity to understand the live-food organisms, including their size, nutritive value, mass production and the larval ability in recognition, capture and ingestion.

A.2.b.1. Larval nutrition

Larval nutrition is probably the most important factor affecting early marine and brackish-water larval mortalities up to 10- 15 days of age (Jones and Houde, 1986). It is almost totally dependent on a very few species of live-food organisms, namely the Rotifer *Brachionus plicatilis*, the Branchiopod Crustacean *Artemia salina*, Copepods from wild plankton

collections, the Harpacticoid Copepods, ***Tisbe*** and ***Tigriopus spp.***, and the Naked Dinoflagellate ***Gymnodinium splendens***. The literature on these live-food organisms is vast, and wide-ranging. In this section, perception and recognition, handling and ingestion, and nutritional value of live-foods and artificial diets will be considered.

Larval perception and recognition, as related to feeding, focuses on several issues including light intensity and photoperiod effect, colour of background (tank internal wall), colour of live food and live-food concentration. Most of the marine and brackish-water larvae are visual feeders with lesser reliance on chemo-reception and mechano-reception (Iwai, 1981). Blaxter (1981) recommended that a desirable illumination for successful feeding would be between 200-1000 Lux. Continuous light during the first week of larval rearing has been recommended by several authors to increase the time of contact between larvae and food organisms. A matt black wall in the larval rearing tank gives high contrast with the live-food, so enhancing larval vision and recognition. In repeated trials, white larval rearing tanks ($4m^3$) gave very low survival after 8-10 days from stocking (Shehadeh, personal comm.). Other background colours which give good contrast may also be helpful in enhancing visual feeding. Corazza and Nickum (1981) worked on Walleye larvae (***Stizostedion vitreum vitreum***) and found that the larvae were evenly distributed in grey containers than in white, yellow or greens ones.

The colour of the live-food is also important in perception and recognition. Arthur (1976) working on the larvae of Jack Mackerel (***Trachurus symmetricus***) suggested that these larvae may select the more brightly coloured Copepods such as ***Microstella*** which occurs in their stomach in greater abundance than in the wild plankton. Rotifers conditioned with ***Chlorella sp.***

tend to have a greenish colour which may also enhance perception during first feeding.

To be perceived, a prey item must be relatively near. First-feeding Herring (*C. harengus*) larvae react to prey at 0.7-1.0 body length (L) (Rosenthal and Hempel, 1970), or 0.4 L (Blaxter and Staines, 1971). This observation could be exploited by increasing live-food concentration, so as to ensure 100% chance of contact. Houde and Taniguchi (1979) found that survival rate is directly related to the amount of food available. Houde (1978) showed that survival rate increased as a function of food concentration, but with a diminishing return at high food levels. Hunter (1981), quoting several authors, concluded that, live-food concentration is strongly related to its size, where a lower food concentration is required with a large-sized organism than with a small one.

Live-food size, motion and texture will affect handling and ingestion. In relation to the mouth size of larvae, small live-food organism are handled and ingested more efficiently than larger ones. Hunter (1981) reported that the prey-width to mouth-width ratio of 0.76, is found to be responsible for 50% feeding success among Anchovy (*Engraulis mordax*) and Mackerel (*Scomber japonicus*) larvae. The Rotifer *Brachionus plicatilis* as first live-food organism with its two size types, large (L) and small (S), provides a satisfactory prey size range of 70-300 µm in length (without eggs) for most marine and brackish-water larvae. The percent of the small rotifers (70-80 µm) among the total supplied is very important during the first feeding and learning period and hence in ultimate feeding success. Theilacker and Mcmaster (1971) used the rotifers as the only food for rearing *Engraulis mordax* and found that only 7% of the rotifers used were of the acceptable size range for first feeding of anchory larvae.

The nutritional value of live-food has been substantially reviewed by Gatesoupe and Robin (1982), Watanabe *et al.* (1983). The mass culture of rotifers originally depended on marine *Chlorella sp.* as food organisms until baker's yeast, *Saccharomyces cerevisiae* was found to be better, producing very high culture densities of rotifers (about 10 times that with marine *Chlorella*). However, rotifers cultured solely on baker's yeast frequently produce heavy early larval mortalities in Red Sea Bream, *P. major* (Kitajima and Koda, 1976; Kitajima *et al.*, 1979). These high early mortalities can be avoided by "conditioning" the rotifers for 12 hours or more with a moderate cell density of marine *Chlorella* before supplying them to the larval tanks (Kitajima *et al.*, 1979). The marked improvement of larval survival is thought to lie in the provision of essential long chain highly unsaturated fatty acids (HUFA) from the algal debris in the rotifer gut and tissue (Watanabe, *et al.*, 1983). This technique was also used by Gatesoupe and Luquet (1981) who found that survival or European Sea Bass larvae (*D. labrax*) was enhanced by feeding enriched rotifers. The most important fatty acids among the HUFAS appear to be the 20:5W3 and 22:6W3 and this has been demonstrated in Turbot, *S. maximus*, by Howell (1979) and Scott and Middleton (1979) and by the Japanese in Red Sea Bream, *P. major*, (Kitajima *et al.*, 1980). Watanabe *et al.* (1989) worked Red Sea Bream and found that 22:6w3 is superior to 20:5w3 in larval growth, survival and prevention of hydrops incidences.

Variability and inconsistencies in the nutritional value of rotifers can be easily and rapidly reflected in early larval survival. Standards of hygiene in the rotifer culture tank and the age of the culture may also effect the consistency of cultured rotifer batches. The

standardization of production procedures for both algal and rotifer cultures is thus very important in reducing variability.

The use of artificial diets for larval rearing has some advantages over live-food organisms, as discussed by Adron *et al.* (1974) and Girin (1979b). The detailed knowledge of the nutritional requirements of marine and brackish-water larvae and hence the optimal values for feed formulation pose less problems than the manufacturing technology, storage, stabilization in water, specific gravity (neutrally buoyant) and the effects on larval osmoregulation. Other major associated problems are the presentation of these artificial diets and hygiene of the rearing water.

Marine fish larvae generally prefer living food, but will accept non-motile food as can be seen in the occurrence of copepod eggs and other non-motile food items in the stomach of wild-caught and laboratory-reared larvae (May, 1970). Similarly, Artemia cysts and dust (sand) particle have been found in the stomach of laboratory- reared larvae (the author's observation). This shows very clearly that movement is not completely essential. It is also possible that the ingestion of these non-motile objects is a result of the larvae drinking water (Tytler and Blaxter, 1988). Most of the experimental trials with complete replacement of live-food have given very low survival. Barnabe (1976) quoted by Girin 1979b, obtained 4% survival after 70 days of rearing European Sea Bass (*D. labrax*) on artificial microdiets, while under the same conditions but with live-food, a doubled growth rate and six times higher survival rate were obtained after 60 days of rearing. Adron *et al.* (1974) working with Plaice (*P. platessa*) and artificial diet obtained 17.5% survival rate at the age of 50 days, while almost double was obtained with live-food. It should be kept in mind that first-feeding plaice larvae are bigger than those of European Sea Bass.

Kanazawa *et al.* (1982) working on Red Sea Bream (*P. major*) and Aye (*Plecoglossus altivelis*), tested three artificial microdiets; nylon-protein microencapsulated diet, agar-polyacrylate micro-binding diet and zein micro-coated diet. They found that both survival rate and growth were poor in all the diets. However, combination of rotifers with any one of these artificial microdiets gave high survival and good growth in both species.

To conclude, early larval survival in marine and brackish water hatcheries is affected by a long series of biological, nutritional and environmental events all of which must be optimized to achieve good survival. Good gamete quality with bad handling, incubation and larval rearing procedures will give results similar to using bad gametes with good incubation and larval rearing procedures. An additional complication is that surviving larvae may have abnormalities of various types and causes. Some of these individuals are of little use for on-growing due to their low survival, low growth and gross appearance. They also, of course, represent an ultimate financial loss to the aquaculture venture.

B. <u>Abnormalities</u>

Abnormalities occur in most marine and brackish-water hatcheries and can cause mortality. The types and percentage of incidents vary from batch to batch or from year to year with little conclusive evidence of origin or causation. Many causes have been proposed but in most cases the authors admit to great uncertainty (Paperna, 1978; Kanazawa, *et al.*, 1981; Barahona-Fernandes, 1982; Newsome and Piron, 1982; Mckay and Gjerde, 1986). However, the causes for some abnormalities have been clearly determined (Kitajima *et al.*, 1981; Tave *et al.*, 1983). Abnormalities are always associated with survival rates as these combined affect total hatchery

production. Abnormal larvae surviving to become a juvenile can represent a financial loss for a hatchery manager and an early death for this abnormal larva would be better economically. Fish abnormalities can be considered in two stages, larval and juvenile.

B.1. Larval abnormalities

The main objective of a hatchery is to achieve high survival rate and maximum possible growth and this is done through the application of optimal rearing conditions. However, these "Optimal rearing conditions", along with lack of predators, completely eliminate any natural selection that would otherwise occur in the natural environment. Therefore, the appearance of a high abnormality rate is not surprising, especially if the rearing conditions are sub-optimal, as they often will be.

The majority of larval abnormalities which appear in hatcheries are lethal before day 40 of age. By contrast, those which start in the larval form but become apparent only in the post-larval or juvenile fish (after 40 days) do not generally interfere with larval survival, even though they are more susceptible to diseases and cannibalism as they grow. This non-lethal characteristic of some larval abnormalities poses a real problem in hatcheries. Larval abnormalities may or may not be noticeable during the larval rearing stage, but can be very pronounced in the juvenile stage as the abnormality develops and increases in severity. Larval abnormalities are of different types and some of those most frequently occurring are axial bending, jaw deformation, absence of pigmentation, abnormal yolk sac, abnormal oil globule position and number, non-inflated swimbladder and the occurrence of foreign bodies in the urinary bladder.

B.1.a Parental effect

The parental influence on larval abnormalities is not well documented. Effects can be induced through the broodstock husbandry and feeding regime because of their influence on egg quality, as mentioned previously (Table 1.2). The use of large, very fecund broodstock which are old may also increase the frequency of abnormalities. Larval abnormalities can thus be of genetic origin. Inbreeding and hybridization are two other sources known to produce abnormalities (Aulstand and Kittelsen, 1971; Hickey *et al.*, 1977). Tave *et al.* (1983) established that "saddleback" deformity in *Sarotherodon aureus* is due to a dominant lethal gene in its heterozygous state. Mckay and Gjerde (1986) working on Salmon (*S. salar*) found that a spinal deformity was genetically heritable but could not determine what had caused it initially among the group of the fish tested. Nankee (1981) described and illustrated physical anomalies among collected wild marine larvae of 12 families and he suggested the possible cause to be genetic.

B.1.b Non-parental effect

Many workers have tested the effect of salinity and temperature on egg incubation and larval performance, and hence found that abnormal larvae do appear in certain conditions (Volodin, 1960; Doroshev and Aronovich, 1974; Fonds *et al.*, 1974; Santerre, 1976; Gulidov and Popova, 1979; Akatsu *et al.*, 1983; Camus and Koutsikopoulos, 1984). Malformations are also induced when eggs are incubated in excessive densities (Hempel, 1971; Devauchelle, 1976) or when eggs or larvae are exposed to mechanical shocks (Pommeranz, 1974). Many abnormalities undoubtedly result from mechanical injury incurred through contact with the rearing tank wall and

other solid materials used in hatcheries. Mechanical injury is usually manifested by malformed snouts or jaws. Mouth deformity of Sea Bass (*D. labrax*) larvae, appearing a few days after hatching, was reported by Barahona- Fernandes (1982), and this persisted for 60 days. The progressive reduction in the prevalence of this larval mouth deformity, especially severe forms, show that it is lethal. Surprisingly many larvae with these injuries survive to the juvenile or adult stage under rearing tank conditions. Mortality from such injuries could be high, but this is probably not recognized and these injuries almost certainly prohibit feeding resulting in mortality from starvation.

Larval bending (notochord deformity) was described by Barahona-Fernandes (1982). She described many varieties of this abnormality in newly-hatched European Sea Bass (*D. labrax*) and suggested an environmental cause. Lumare and Villani (1973) suggested that these early notochord abnormalities could be lethal. Plaice (*P. platessa*) larvae often developed a condition that Shelbourne (1956) referred to as oedema of the yolk sac with associated distortions of the axial musculature causing larval bending. He believed that osmotic stress, caused by high salinity and high metabolites level might be responsible. The occurrence of "foreign bodies" in the urinary bladders of Red Sea Bream (*P. major*) may also be lethal (Ueda *et al.*, 1970). These "foreign bodies" contain calcium (Ishioka *et al.*, 1970) and are most common in larvae reared at high salinities.

The nutritional factors causing larval abnormalities are restricted to the quality of the live-food used during early larval rearing, e.g. rotifers and artemia nauplii. It was found that, Red Sea Bream (*P. major*) larvae fed on rotifers having low content of W3 HUFA become weak, having low swimming activity and a lack of reflex responses. Consequently,

these larvae had difficulty in gulping atmospheric air at the rearing tank water surface, where some larval fitness is required to break the water surface tension and hence were unable to inflate their swimbladder (Watanabe, 1986a).

It has been reported that some of the geographical strains of *Artemia* nauplii contain agricultural insecticides, pesticides or heavy metals (Olney *et al.*, 1980) and that these could lead to some abnormalities of the larvae. These contaminants are now prevalent in many natural bodies of waters and have been reported by several authors to be a possible cause of abnormalities (Amin, 1968; Meade and Harvey, 1969; McCann and Jasper, 1972; Hirose and Kitsukawa, 1976).

B.2. Juveniles abnormalities

Juveniles abnormalities may be induced either during the larval or post-larval stage and become obvious at different times depending on developmental stage and severity. Commercial hatchery output and hence production cost is affected by the percentage of abnormality, which tends to make farm managers ask for a guarantee to assure fry quality. There are two types of juvenile (fry) abnormalities, i.e., non-spinal and spinal.

B.2.a Non-spinal abnormalities

Some of the most pronounced non-spinal deformities occurring during rearing are: pughead, operculum abnormalities, deformed jaws, pigmentation abnormalities such as albinism and fin and spine disorders. Pughead is a reduction of the frontal skull and upper jaw bones and it is well documented in Sea Bass (*D. labrax*) (Barahona-Fernandes 1982; Paperna 1984), and Ayu (*P. altivelis*) (Kanazawa *et al.*, 1981; Takashima *et al.*, 1976)

hatcheries. This abnormality has also been seen among Brown-Spotted Grouper (*Epinephelus tauvina*) and Blue-Finned Sea Bream (*A. cuvieri*) juveniles produced in MFD, KISR. Kanazawa *et al.* (1981) suggested that the lack of phospolipids and lecithin is one of the possible cause for this abnormalities.

The reduction or twisting of the opercular bone(s) or the malformation of the gill arches are referred to as operculum abnormalities. These are very common in marine and brackish-water hatcheries and have been reported in Sea Bass (*D. labrax*) (Barahona-Fernandes, 1982; and Paperna, 1984); in Gilthead Sea Bream (Paperna *et al.*, 1977) and in Ayu (*P. altivelis*) (Takashima *et al.*, 1976). It has also been seen in MFD, KISR in *E. tauvina, A. cuvieri* and *Acanthopagrus latus* (Yellow-Finned Sea Bream). There is some evidence that it may be due to nutritional deficiencies, such as vitamin C (Kitamura *et al.*, 1976).

B.2.b Spinal abnormalities

There are many varieties of deformed vertebral column but the major types are scoliosis, lordosis, brachiospondylosis (fusion of vertebrae) and twisted caudal peduncle. Spinal deformities in fishes can be induced by a variety of factors including: toxic substances (Valentine, 1975; Bengtsson, 1975; Nesome and Piron, 1982) nutritional deficiencies, e.g. vitamin D, C or Lecithin (Roberts and Shepherd, 1974; Ashley *et al.*, 1975; Lim and Lovell, 1978; Kanazawa *et al.*, 1981), Pathogenic agents, e.g. Myxosporidian, *Myxosoma cerebralis* (Rogers and Gaines, 1975), and inbreeding (Aulstand and Kittelsen, 1971). The lack of a functional swimbladder is also found to result in a spinal deformity (Fukusho, 1985; Watanabe, 1986a).

B.2.c Swimbladder-related spinal deformity

The spinal deformity described as V-shaped vertebral column bending (lordosis) is common in artificially produced juveniles. Some of the most important cultured species known to have this type of abnormalities are; the Red Sea Bream (*Pagrus major*), Gilthead Sea Bream (*Sparus aurata*), Sea Bass (*Dicentrarchus labrax*), Striped Bass (*Morone saxatilis*) and the Blue-Finned Sea Bream (*Acanthopagrus cuvieri*). All these species are of prime value and of high potential in the fish farming industry. This abnormality is originally developed very early in the larval stage (6-15 days from hatching). The association between this abnormality and the failure of initial swimbladder inflation has been widely reported (Kitajima *et al.*, 1977, 1981, Paperna, 1978). It appears that nutritional and/or environmental factors are responsible for the lack of initially inflated swimbladder in these larvae. This failure to initially inflate the swimbladder, results in abnormal larvae with a "temporary" normal notochord. It is believed that a larval fish without a functional swimbladder will have difficulties in maintaining station in the upper or middle layer of the water and that the deformity result due to body development and muscle action in the absence of a swimbladder (Watanabe, 1986a).

The Blue-Finned Sea Bream (*A. cuvieri*) is one of those species affected by this abnormality. It is one of the local species of the state of Kuwait, and has been shown to have good potential for commercial farming. Much research is being currently conducted in Kuwait to finalize hatchery techniques, concentrating on higher survival and better fry quality.

III FISHERIES AND AQUACULTURE IN KUWAIT

The state of Kuwait is a sub-tropical desert area at the northwestern tip of the Arabian Gulf. It is about 15,000 square Kilometers in area and has almost 200 Kilometers of coast-line. A sub-tropical desert climate dominates with a lowest air temperature range of 0-10°C in winter and a highest of 45-50°C in summer. The projected estimate of the population was 1.8 million in 1987 with a 15 Kg per capita consumption of fresh and processed marine food (Afzal and Hayat, 1977).

There is no commercial aquacultural activity in Kuwait as yet, but aquacultural research was first introduced by the government through the Kuwait Institute for Scientific Research (KISR), Mariculture and Fisheries Department (MFD) in the early 1970s as a potential research area. This was stimulated by the shortfall in local supply of prime fresh fish and its relatively high price compared to the neighbouring countries.

A. Supply and demand in Kuwaiti fisheries and projected future needs.

The supply of fresh fish and shrimp from local catches during the past decade has ranged from about 6,000 to 9,000 tonnes per annum. The major species of prime value that dominate the landings, are; Silver Ponfret (*Pampus argenteus*), Brown-Spotted Grouper (*Epinephelus tauvina*), Green Shrimp (*Penaeus semisulcatus*) and Silver Grunt (*Pomadasys argenteus*). The remainder of the landings comprise another 20 species, some of which have a high market value but very small landing, such as the Blue-Finned Sea Bream (*A. cuvieri*). The rate of increase of local fisheries catches has averaged around 2% per year during the period 1977-1987, while the Kuwaiti population has increased annually at an average of 6.2% for the same period (Anonymous, 1988a). This has resulted in an increase in demand for fisheries products.

As a consequence, the quantities of imported fresh fish have increased from 1302 metric tonnes in 1983 to about 4008 metric tonnes in 1987, while the frozen, canned and processed product importation has remained stable at around 4000 metric tonnes per annum for the same period. It can be seen from these figures that there is strong consumer preference in Kuwait towards fresh fish rather than frozen or processed fish or related products.

The projected demand for 1988 to 2008 was assessed by the Ministry of Planning, central statistical office, from the statistics of fisheries landings and catches collected monthly by their workers. It was found that local shrimp fisheries landings will still be in excess of the local demand; whereas the projected demand for fresh finfish will probably rise from an anticipated 4000 metric tonnes in 1988 to 7500 metric tonnes by 2008. The fisheries management authority cannot see the possibility of increasing the local landings of finfishes to 7500 metric tonnes without seeking other fishing grounds nearby or endangering the local finfish stocks. They consider that maximum sustainable yields from the local finfish stocks have already been reached. Imports and finfish farming are thus the two areas for consideration to cover the shortage of fresh fish for the next twenty years.

B. <u>Species development at MFD, KISR</u>

Research on aquaculture has been carried out exclusively by MFD on several local species of finfish and shrimps. A planned screening program was established in the early 1970's to select and develop one or two species for commercial application. The screening program included five marine species namely; a Mullet (*Liza macrolepis*), Rabbit Fish (*Siganus oramin*), Yellow-Finned Sea Bream (*Acanthopagrus latus*), Brown-Spotted Grouper

A

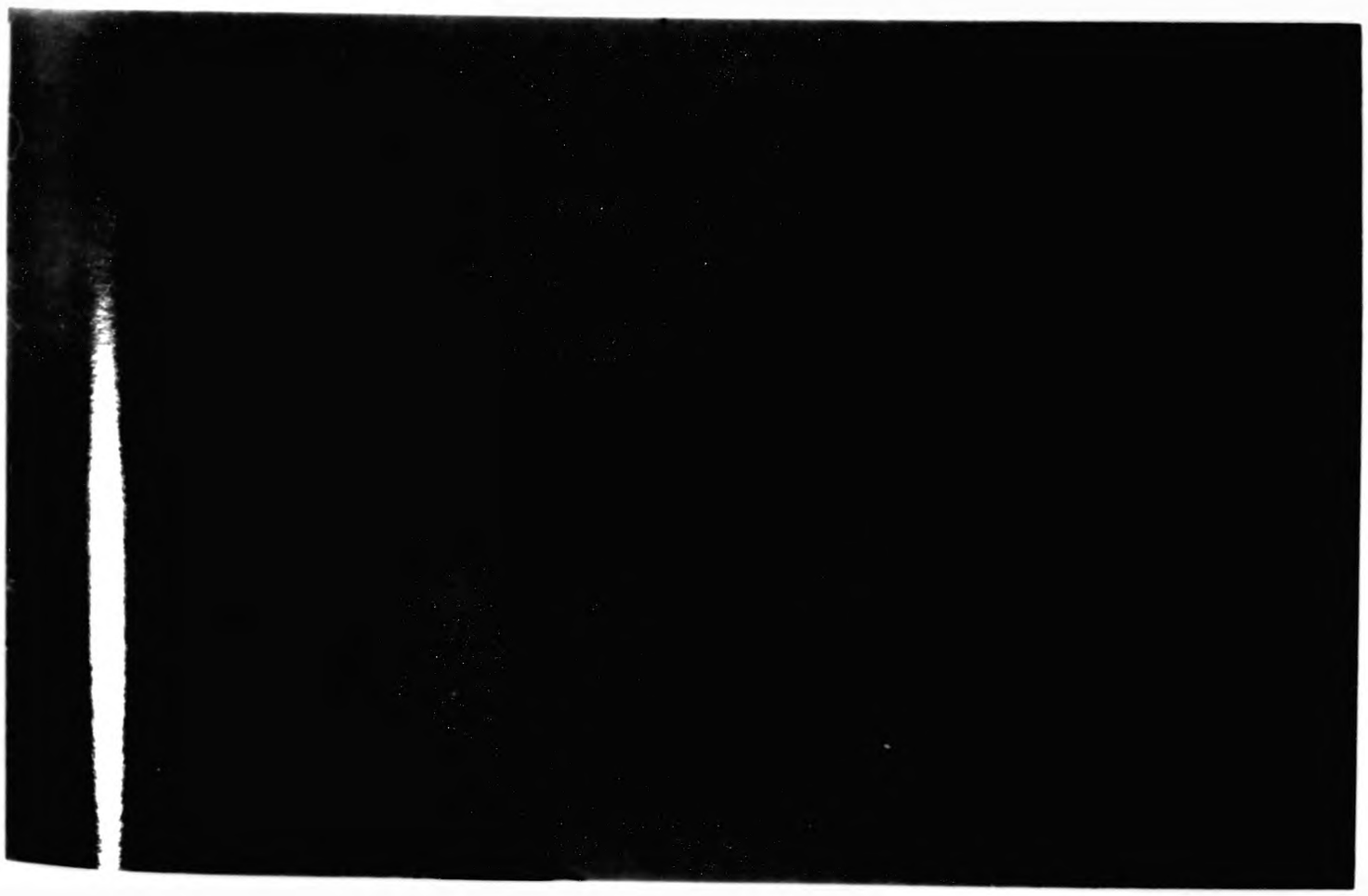


Plate (1.1) *Acanthopagrus cuvieri* (A) a 4-year-old specimen (TL = 520 mm; b.wt. = 2850 g) and (B) 8-hour post-hatching larva (TL = 2.2 mm; b.wt. = 1.5×10^{-4} g).

(*Epinephelus tauvina*) and Blue-Finned Sea Bream (*A. cuvieri*) (Al-Abdul-Elah *et al.*, 1985). By the late 1970's a decision was made to select *A. cuvieri* and *E. tauvina* as first priority species for research in two areas, namely, fry production and on-growing. Subsequently, *A. cuvieri* was given priority over *E. tauvina* as better results were being obtained and there were less problems in fry production. As this fish has a higher market price also, outside funding was secured from the United Fisheries of Kuwait.

C. Blue-Finned Sea Bream (*Acanthopagrus cuvieri, Day*)

A full research program on broodstock collection, spawning, egg incubation and larval rearing was started in 1978 and the first scientific publication on *A. cuvieri* appeared in 1980 (Hussain *et al.*, 1981) on egg and early larval development. Recently, Kuronuma and Abe (1986) renamed the species *Sparidentex hasta* (Valenciennes) shown in Plate (1.1). It is also named *Chrysophrys cuvieri* Day (= *Dentex hasta*, Valenciennes), *Sparus cuvieri* and *Mylio cuvieri* (Kuronuma and Abe, 1986 and FAO species identification sheet for fishing area No. 51, Volume IV, 1984). Common English names given to this species are; Black Porgy, Sobaity, Sea Bream, Silvery Black Sea Bream and Blue-Finned Sea Bream. In Kuwait this species is called Sobaity and its landings from local waters ranged from 20-60 metric tonnes per year during the period 1982-1986 (Anonymous, 1988b).

A. cuvieri is a protandrous hermaphrodite (Hussain and Abdullah 1977, Abu-Hakima, 1983). About 96-98% 1+ *A. cuvieri* will be matured males, while the remainder will be "virgin females" (unpublished data). These one year "virgin females" are significantly larger than the males (unpublished data). The sex ratio within a sibling group tends to approach 1:1 by the 3rd or 4th year of age (the author's observation), although confirmation of this

fact is required. *A. cuvieri* females mature after two years of cultivation, but only produce low quality and quantity of eggs. The best females for use as broodstock, with least age-related problems are from 3 to 6 years old (Teng *et al.*, 1984). The fecundity of a 3 to 4 year-old cultured *A. cuvieri* (2.0 to 3.5 kg) would be 1.5 to 2.4 x 10^6 eggs per fish (Teng *et al.*, 1984). Abu-Hakima *et al.* (1983) found the fecundity of wild fishes from Kuwaiti waters to be about 0.31 to 1.7 x 10^6 eggs for fish of 47.1 to 63.5 cm in total length (1.9 to 5.0 kg) respectively. The maximum size of *A. cuvieri* seen in Kuwait is about 100cm in total length (about 11 to 13 kg). This species is mainly caught by hook and line and due to its jumping and fast swimming, it is also considered one of the best sport fishing species in Kuwait.

D. Larval rearing and its major problems

The first trials of larval rearing of *A. cuvieri* in Kuwait, MFD were done in 1979 and a production of 1000 fingerlings was achieved. In 1985 and 1986 the production of *A. cuvieri* fry had reached 100,000 and 250,000 respectively, these fry being used in pilot-scale on-growing operations in sea-cages. The history of Sobaity larval rearing and fry production can be looked at in more details in KISR final reports (1979-1988). The main problems faced during the last 8-9 years of larval rearing have been low survival due to mass mortalities during the first two weeks of rearing, and abnormalities. The main type of abnormality was the spinal deformity of fingerlings of 5-10 gram in weight.

IV AIM AND RATIONALE OF PRESENT RESEARCH

From this review it can be concluded that one of the main problems facing the advancement of marine and brackish-water finfish farming

is the production of fry. The two major problems facing for the artificial production of fry for any marine species, are the survival and larval abnormalities. The spinal deformity caused by the lack of a functional swimbladder, is of general concern and is also of interest to MFD, KISR, as it affected a large percentage of *A. cuvieri* fingerlings produced in the early trials.

The objective of this project is to describe the development of the swimbladder of *A. cuvieri* and to study the larval migration behaviour and development of buoyancy. The effects of some biotic and abiotic factors on initial swimbladder inflation are also considered. It is also intended to confirm that the V-shaped spinal deformity is due to the lack of a functional swimbladder that started in the larval phase. Finally, the growth performance of normal and abnormal (lacking functional swimbladder) juveniles is compared. The overall aim is to synthesize the developmental, behavioral and physiological data into a working hypothesis which may explain the variability in swimbladder inflation success observed in hatcheries.

CHAPTER 2

GENERAL MATERIALS AND METHODS

I. INTRODUCTION

The work described in this thesis was carried out in the State of Kuwait, at the research facilities of the Mariculture and Fisheries Department, Kuwait Institute for Scientific Research. The experiments were highly seasonal, depending on the natural spawning of the Blue-Finned Sea Bream *Acanthopagrus cuvieri*, which takes place between January and March. Work on fingerlings usually started in May and extended to August. This chapter describes the general methods and techniques used in broodstock culture and spawning, egg collection, incubation and hatching and finally larval rearing systems and procedures. These techniques were common to all the experiments described. More specific materials and methods relating to the detailed tests on larvae or fingerling are given separately in the relevant chapters.

II. BROODSTOCK CULTURE AND SPAWNING

A. Broodstock maintenance

Acanthopagrus cuvieri (Blue-Finned Sea Bream) broodstock at the Mariculture and Fisheries Department (MFD), Kuwait Institute for Scientific Research (KISR), are either wild fish caught from the sea and acclimatized to tank culture conditions for 8-10 months before spawning or cultured fish of 3 to 6 years of age grown from eggs at MFD. The two groups are kept separately. All the eggs used for this project were obtained from the wild broodstock. The broodstock were held at a density of about 1 kg/m^3 and a female:male ratio of about 1:1. Table 2.1 shows the size, number, sex ratio, stocking density and other spawning data for the broodstock used throughout this study (1986 to 1988).

Table 2.1 Spawning data of *Acanthopagrus cuvieri* for the years 1986 to 88.

	Year 1986	1987	1988
Spawning Period	14 Jan-28 Feb	12 Jan- 1 Mar	18 Feb-22 Mar
Broodstock Body weight (kg ± SD)			
Females	5.6±0.8	4.4±0.4	5.94±1.09
Males	4.7±0.7	3.8±0.5	4.45±0.41
Stocking density*			
Fish/m^3	0.27	0.33	0.25
Kg/m^3	1.43	1.40	1.18
Sex ratio (Male:Female)	9:7 (1.3:1)	10:10 (1:1)	9:4 (2.2:1)
Total Eggs Spawned (millions)	28.6	28.5	32.3
Floating Eggs	23.1	14.8	19.9
Sunken Eggs	5.5	13.7	12.4
Hatching % (Floating Eggs)	80.0	83.2	81.6

*The Broodstock Tank Volume 60 m^3

The broodstock were fed to satiation with chopped small pelagic fish, mainly Mullet and *Caranx spp.*, once daily, six days a week. In addition, the broodstock were fed with chopped pieces of Cuttlefish (*Sepia* spp.) two to three meals per week. This additional feeding is started three to four months before the spawning season, and is continued until the end, to enhance the level of HUFA in the spawned eggs (Watanabe *et al.*, 1984b). Commercial feed supplements known as "Paramix M" and "Juvela Food 50" were mixed with the chopped fish at a dosage of 30 to 40 g/kg of the former and

3 to 6 g/kg of the latter. The main ingredients of the "Paramix M" are vitamins C, E, B2, B6 and some minerals while the "Juvela Food 50" mainly contains vitamin E (Teng *et al.*, 1984).

B. Broodstock tanks

The broodstock were maintained in large circular concrete tanks of $60m^3$ volume (6.0m in diameter and 2.2m in depth). The tanks were supplied with seawater at a continuous rate of about $10-14m^3/h$. Strong aeration was applied continuously using air-lifts. Seawater (salinity 38-40 parts per thousand:ppt) was pumped from concrete sea- wells (2m in diameter and 3-4m deep) in the intertidal area adjacent to the laboratory. The well seawater is very clear and is quite low in suspended slit. The temperature of the seawater ranged from 19-21°C during winter (December to March) and 26-28°C during summer (June-September). The dissolved oxygen content of the well seawater was 2.8-3.0 mg/L (Teng *et al.*, 1984).

Due to the low dissolved oxygen, strong aeration is necessary. Each tank was fitted with six air-lifts made of 8 cm diameter PVC pipes of about 160 cm in length. These air-lift were fitted on the inner wall of the tank with an elbow on top of it to direct the water flow to enhance water mixing, aeration and circulation (Figure 2.1A). With these air-lifts a stable and high dissolved oxygen content of the tank water was attained (5.0-5.5 mg/l during summer and 6-7 mg/l during winter).

The broodstock tanks were covered with UV-resistant shade netting which achieves 60% reduction of sunlight. This was necessary to reduce the algal growth and build up of dead algal matter on the tank bottom and hence to extend the duration for which a tank can be used for holding broodstock. The fish waste and dead algae accumulation in the broodstock tanks were

removed once every two weeks by a bilge sweeper. In addition the broodstock were moved to clean tanks once every 3-4 months and the evacuated tanks were cleaned with a high pressure water jet and left to dry for a few days.

C. Broodstock spawning

A. cuvieri broodstock were found to mature and spawn naturally starting from mid of January and stopping by end of February under local tank culture conditions (Hussain *et al.*, 1981; Teng and Higuchi, 1981). By mid-December 1986, 87 and 88, the broodstock were transferred to clean tanks for spawning. At the time of transfer, the fish were anaesthesized (6 ppm quinaldine), measured for total length and body weight, sexed, and egg maturity determined by cannulation. The fish density and sex ratio were adjusted to around 1 kg/m^3 and 1:1 respectively. The water flow arrangement was changed from a bottom- drain (Figure 2.1.A) to a top-draining arrangement (Figure 2.1.B) designed to assist egg collection. Based on information obtained from examining the eggs obtained by cannulation and daily observation on the swimming activities of the broodstock, egg collection trials were started to determine the beginning of spawning. The fish generally spawned just before sunset and continued for a few hours. During the spawning time, all spawners showed intense swimming activity. A spawning female will be closely followed by two or three males who are aggressive to each other. A non-spawning female will chase off the males.

Spawning started on 14th of January in the 1986 season and on 12th January in the 1987 season. In the 1988 season the fish spawned one month later than normal. This delay in spawning was most probably due to the increase in well water temperature which was caused by the land reclamation of the intertidal area where the sea wells are located. The average monthly

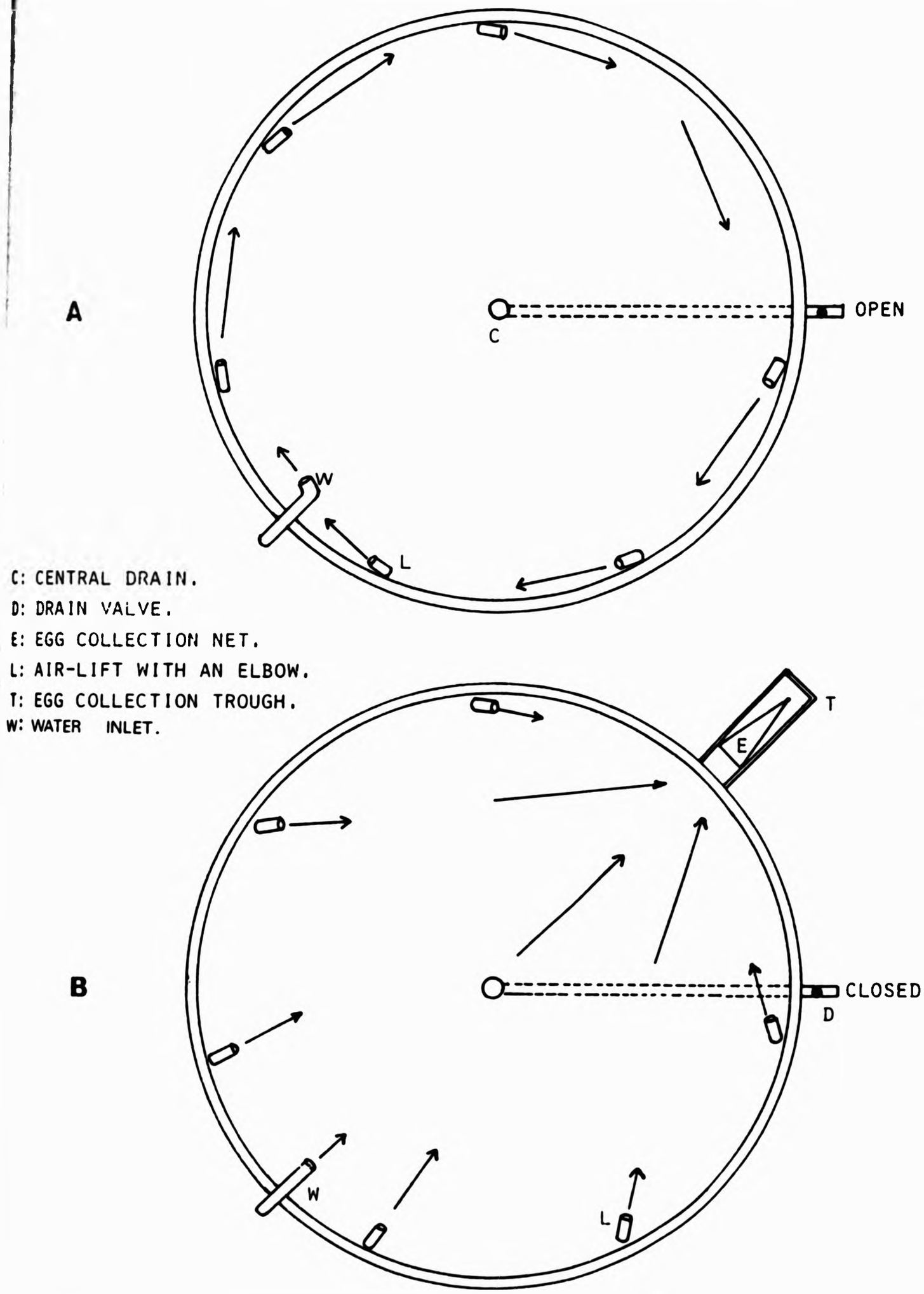


Figure (21). Broodstock circular concrete tank of 60m³.
A: Broodstock maintenance arrangement.
B: Broodstock spawning arrangement.

water temperature and photoperiod during the three spawning seasons is shown in Table 2.2.

III. EGG COLLECTION, INCUBATION AND HATCHING

A. Egg collection

Egg collection was started by closing the bottom drain of the broodstock tank and allowing water to overflow from one side into a fiberglass trough (40 x 40 x 160 cm). This trough was suitably placed beneath the overflow of the spawning tank to achieve a gentle flow of water with little turbulence. A conical plankton net of 200-400 um mesh size was fitted on a square wooden frame (39 x 39 cm) and set into the trough. The water outlet was switched from bottom to upper side as described above for egg collection at around 1600-1700 hr. The spawned eggs which are initially buoyant were collected the following morning (0800-0900 hr). The continuously overflowing water from the spawning tank carried the pelagic eggs into the trough and then into the egg collection net. The drain from the trough was adjusted such that the eggs collected inside the conical net were in water during the egg collection period.

The collected eggs were gently washed through a flat scoop net made of 1 mm plastic mesh into a conical plankton net (400 um) suspended in a plastic bucket. The algae and all debris were removed by this washing process. The washed eggs were placed into one-litre graduated cylinders (not exceeding 100 ml of eggs in each cylinder) to determine their volume. In the graduated cylinders, the egg usually segregated into three distinct layers according to their buoyancy in seawater of 38-40 ppt. The three egg layers were; an upper layer of floating eggs and a bottom layer of sunken

Table 2.2 Mean monthly water temperature and photoperiod of the broodstock tanks during the years, 1985-1988. Rectangles represents spawning period.

Month	Water temperature (°C) 1985	1986	1987	1988	Photoperiod (Daylight in hrs) 1985 to 88.
JAN	-	19.9 (18.0-20.8)	19.4 (18.5-20.0)	24.0 (23.5-24.7)	10.35 (10.15-10.50)
FEB	-	19.8 (18.0-21.0)	19.0* (19.0-19.0)	23.0 (22.5-23.4)	11.16 (10.55-11.40)
MAR	-	20.5 (18.5-22.1)	19.0 (19.0-19.0)	22.8 (22.3-23.0)	12.02 (11.45-12.25)
APR	22.0 (21.0-22.7)	22.1 (21.0-22.8)	20.3 (20.0-21.0)	22.6 (22.3-22.7)	12.51 (12.30-13.15)
MAY	23.9 (21.4-25.6)	23.8 (21.1-25.5)	23.0 (23.0-23.0)	22.8 (22.7-23.0)	13.35 (13.15-13.50)
JUN	25.5 (24.0-27.5)	25.4 (24.0-27.6)	23.5 (23.5-24.0)	22.8 (22.5-23.1)	13.57 (13.15-14.00)
JLY	26.3 (24.7-28.0)	26.4 (24.6-28.2)	24.0 (24.0-24.0)	23.2 (22.7-23.7)	13.50 (14.00-13.45)
AUG	26.8 (25.2-28.3)	26.8 (25.0-28.2)	25.7 (25.0-26.0)	24.5 (24.1-25.0)	13.26 (13.45-13.10)
SEP	26.8 (25.1-28.6)	26.7 (25.0-28.6)	26.9 (26.0-27.5)	-	12.52 (13.10-12.25)
OCT	25.9 (24.0-27.3)	25.8 (24.2-27.1)	26.6 (26.0-27.5)	-	11.55 (12.25-11.35)
NOV	24.6 (23.0-26.3)	24.5 (23.1-26.2)	25.5 (25.0-26.0)	-	10.50 (11.35-10.40)
DEC	21.5 (19.6-23.1)	21.5 (19.5-23.0)	24.8 (24.5-25.0)	-	10.17 (10.35-10.15)

*Beach reclamation started.
Figures in brackets represent the range

eggs and sometimes a mid-layer of suspended eggs. The volume of floating and sunken eggs was recorded and the number of suspended eggs was estimated (four sub-samples were taken for counting from all the suspended eggs collected on a day). The number of collected eggs (floating and sunken) was estimated by multiplying the total volume collected by the number of eggs per ml. The number of both floating and sunken egg/ml is usually about 2000. The mean egg diameter of *A. cuvieri* is 0.81 ± 0.01 mm (Hussain *et al.*, 1981).

B. Egg incubation and hatching

Only the floating eggs were used for incubation and hatching. The sunken eggs were discarded as they are known to be of poor quality and show very low hatching rates (Teng *et al.*, 1984). The eggs were incubated in cylindrical hatching nets (30 cm in diameter and 35 cm in depth) made of fine black cloth (60-160 µm) with a plankton net bottom (400 µm). The hatching nets were suspended in fiberglass troughs (200 x 50 x 50 cm) supplied with continuous running well seawater. By means of a partition near the inlet, the water was forced to flow to the bottom of the trough near the bottom of the hatching net (400 µm in mesh size) on its way out. Gentle aeration was supplied inside each hatching net to help circulate the eggs and to provide sufficient dissolved oxygen. Four hatching nets were suspended in each trough and each net was stocked with around 50 ml of floating eggs (100,000 eggs). The eggs normally hatched within 32-40 hours from spawning, at a water temperature of 19-22°C; 4-5 ml/l dissolved oxygen, 7.7-8.0 pH and 38-40 ppt salinity. The mean total length of just hatched *A. cuvieri* larvae is 1.87 ± 0.08 mm (Hussain *et al.*, 1981). The newly hatched larvae were transferred, after removing the aeration stone from the hatching net and allowing the newly-hatched larvae to float. The transfer was carried out by

siphoning off the larvae with a small flexible hose (10 mm in diameter) to a 30-litre bucket.

The number of newly-hatched larvae was estimated by counting four random sub-samples of 100 ml each from the bucket. The larvae were mixed thoroughly in the bucket before each sample.

The percentage hatching was calculated as follows:

$$\text{Percent hatching (\%)} = \frac{Nt}{No} \times 100$$

Where Nt = total number of hatched larvae per net

No = total number of eggs stocked per hatching net.

The hatching percent and other spawning data for the three seasons 1986-88 are given in Table 2.1.

IV. LARVAL REARING SYSTEM AND PROCEDURES

A. Larval rearing facilities

The facilities used for larval rearing consisted of six concrete tanks lined by glazed ceramic tiles (5 x 2.3 x 1 m each) which were used as waterbaths for the larval rearing tanks as shown in Figure (2.2). A large room was constructed over these concrete tanks using wooden supports covered with light-proof PVC liner. The room was 17 meters long, 6.2 meters wide and 2.2 meters high. The room was provided with eight exhaust fans. Artificial illumination was provided by eight 5-feet fluorescent day-lights (80 watt each) per concrete tank. The lights were suspended 165 cm above the water surface of the larval rearing tanks. Light intensity and photoperiod were controlled separately in each of the concrete tanks which could be separated from each other by a wall of PVC liner sheet.

Three sizes of fiberglass larval rearing tanks were used during the course of this project: 500 litre (103 cm in diameter x 74 cm in depth), 100 litre (48 cm in diameter x 80 cm in depth) and 30 litre (38 cm in diameter x 29 cm in depth). Each tank was supplied with one air-stone (3 cm in diameter x 5 cm in length), producing air bubbles of 2-5mm in diameter at the surface, preferably placed centrally. The external walls of the tanks were either covered with black plastic sheets or painted black. All the 500 l tanks were fitted with gap-meters to adjust and control the level of air-flow.

Two sizes of waterbath tanks were used to control larval rearing water temperature: 10m^3 concrete tanks (5 x 2.3 x 1 m) and 0.33 m^3 galvanized iron tank (1.45 x 0.92 x 0.25 m). Each 10 m^3 waterbath was fitted with two (2 KW) glass immersion heaters. Each heater was connected to a temperature controlling unit which had a contact thermometer as its sensor placed in one of the larval rearing tanks. Three large airstones (5 x 5 x 17 cm) were used to circulate the water in the 10 m^3 waterbath. Each heater had one airstone underneath it and the third one was placed in the middle of the tank. The arrangement of the heaters and airstones was the same in the 0.33 m^3 galvanized iron waterbath tank, but with two (1KW) glass immersion heater and smaller airstones (3 cm in diameter x 5 cm in length). The water level was always kept constant in the waterbath tanks.

The larval rearing tanks were carefully cleaned and dried for several days before stocking with newly-hatched larvae. On the day of stocking, the tanks were filled with seawater and strongly aerated for 1-2 hr. The aeration level was later adjusted to about 200 ml/min in the 500 litre tanks and 50 ml/min in the 100 and 30 litre tanks. The larvae in each

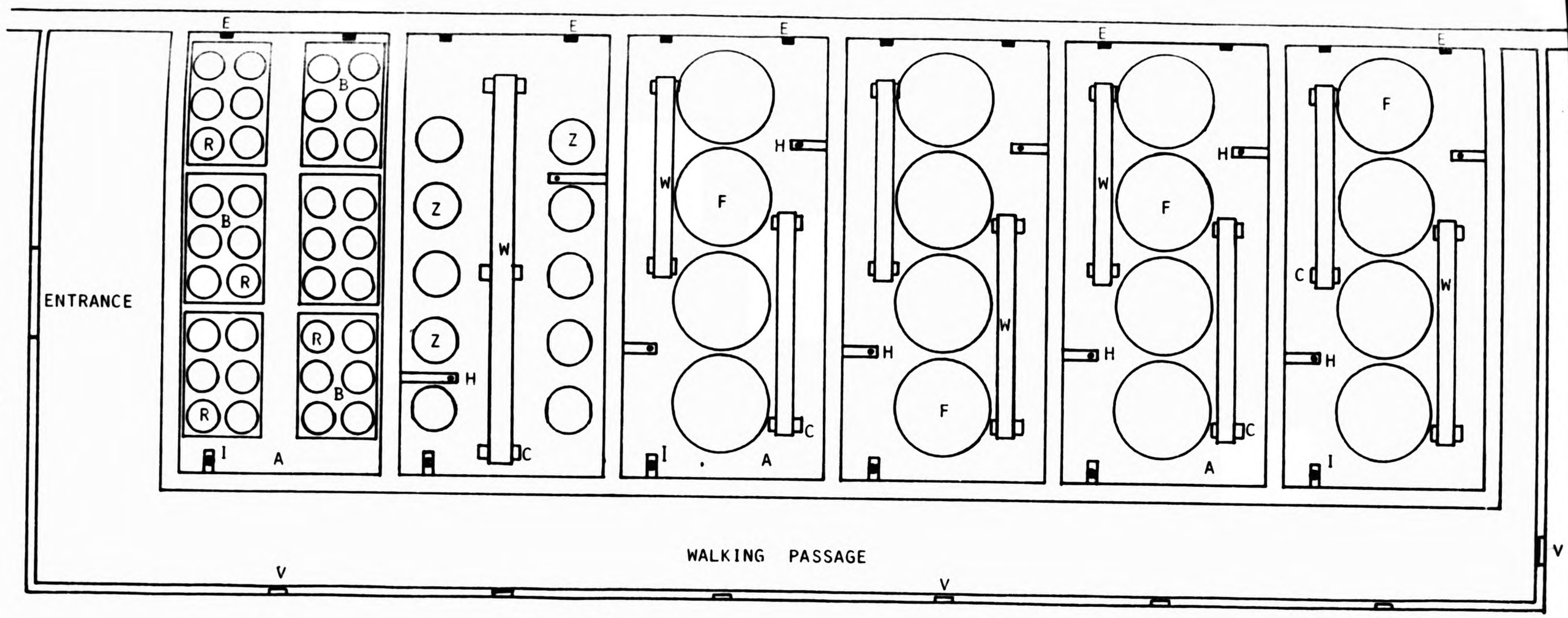


Figure (2.2). Outlines of the larval rearing research facilities.

A: CONCRETE TANK OF 10M³ USED AS WATERBATH.
B: GALVANIZED IRON TANK OF 0.33M³ USED AS WATERBATH.
C: CONCRETE BLOCKS.
E: 15 AMP - 3 PIN SOCKETS.
F: 500 LITER FIBERGLASS TANK USED FOR LARVAL REARING.
H: WOODEN ARM FITTED WITH GLASS IMMERSION HEATER.
I: WATER INLET VALVE OF 2" IN DIAMETER.
R: 30 LITER FIBERGLASS TANK USED FOR LARVAL REARING.
V: VENTILATION FAN.
W: WOODEN WALKWAY.
Z: 100 LITER POLYCARBONATE TANK USED FOR LARVAL REARING.

hatching net were checked before a decision was made whether or not to include them in the stocking. The criteria used to judge for good larvae were; good swimming activity, posterior position of oil globule, low percentage of bent or curved larvae and good hatching rate (around 80%). The stocking of larvae was done volumetrically in such a way that all the larval rearing tanks of a certain experiment would receive an equal number of the larvae hatched in the same net. Usually four to five hatching nets would be required to stock sixteen 500 litre tanks at a density of 60 larvae/litre, while one or two hatching nets would be enough for the 30 litre and 100 litre tanks.

B. Larval rearing procedures

The larval rearing procedures used here are based on the green-water system first adopted by the Japanese in 1960's for rearing the Red Sea Bream *P. major* larvae. There are some slight changes which were made to fit the experimental conditions of the tests.

Feeding of stocked larvae was started on the morning of the third day of rearing at a water temperature of 23-25°C. At this time, the larvae have already attained a functional digestive system. Cultured rotifers (*Brachionus plicatilis*) of the L-type were the only larval food used in this project due to the short duration of the tests. Rotifers were used as the initial larval feed at a density of 5 rotifers/ml of larval rearing water during the first week. During the second week the rotifer density was increased to 7-8 rotifer/ml. The density of rotifers remaining in each larval rearing tank was estimated once daily during the first week, and twice daily during the second week. Checking of the remaining rotifer number was done by counting four one millimeter samples from each of the larval rearing

tanks. This is done by dipping a one millimeter pipette at random in the rearing tank to sample rotifers for counting. The rotifers in the sample is counted by eye by looking through the pipette against black background. The daily addition of new rotifers was thus adjusted accordingly.

Normally the rotifers were cultured in large fiberglass tanks (6-7 m^3 in volume) on baker's yeast and *Chlorella spp.* (James *et al.*, 1986, 1987). The harvested rotifers were conditioned with *Chlorella* (25 x 10^6 *Chlorella* cell/ml) for 16-18 hours at a density of 500 rotifers/ml before feeding them to fish larvae.

In almost all tests carried out in this project, the larval rearing tank water was static (no water change) throughout the rearing period (16 or 21 days). The single-celled green alga *Chlorella* was added daily to the larval rearing tank as a "conditioner" to control the water quality and as a supplementary feed for the remaining rotifers in the rearing tank. The number of *Chlorella* cells was adjusted daily by addition of new *Chlorella* to maintain a density of 200-300 x 10^3 cell/ml. Water temperature, dissolved oxygen and pH of the larval rearing tanks were monitored daily, using an oxygen meter (YSI Model 57) and pH meter (Hydro-Bios CG 728).

All the data were computed and analysed statistically using Lotus 123, SPSS$^+$ and Minitab. Different groups were compared according to one way ANOVA and Duncan's Multiple range test. Graphs were prepared by using Cricket graph.

CHAPTER 3

SWIMBLADDER DEVELOPMENT AND INITIAL INFLATION MECHANISM

I. INTRODUCTION

A large number of the known species of teleost possess a swimbladder at some stage of their life cycle. Its function depends upon the lifestyle of the species and it can act as a hydrostatic, respiratory, sound producing, sound receiving, or pressure detecting organ (Jones and Marshall, 1953). Powers (1932) presented a long argument on the function of the swimbladder, and concluded that its primary function is hydrostatic as was first suggested by Needham (1667) who was quoted by him. He considered that all other functions are secondary. Jones and Marshall (1953), Blaxter and Tytler (1978) and Fange (1983) further discussed the theory of hydrostatic function of the swimbladder.

Two types of swimbladders have been described in teleosts. These are, the physostomous type, which opens into the foregut through the pneumatic duct, and the physoclistous type which has no connection with the foregut, except for some species which have a temporary pneumatic duct in their larval stage. Generally, the more ancient soft-rayed fishes (*Malacopterygii*) are physostomous, while the more modern, spiny-rayed fishes (*Acanthopterygii*) are physoclistous (Lagler *et al.*, 1962). In physoclistous fishes, there are two structural varieties of swimbladder according to the position of the gas gland and oval; the paraphysoclistous and the euphysoclistous. The former refers to fishes where the resorbent (oval) and secretary (gas gland) parts of the swimbladder are not sharply separated from one another, while in the latter (Plate 3.1), the two areas are well separated (Steen, 1970). Adult *A. cuvieri* have an euphysoclistous swimbladder with a well developed oval, gas gland and rete mirabile (Plate 3.1 and Plate 3.2).

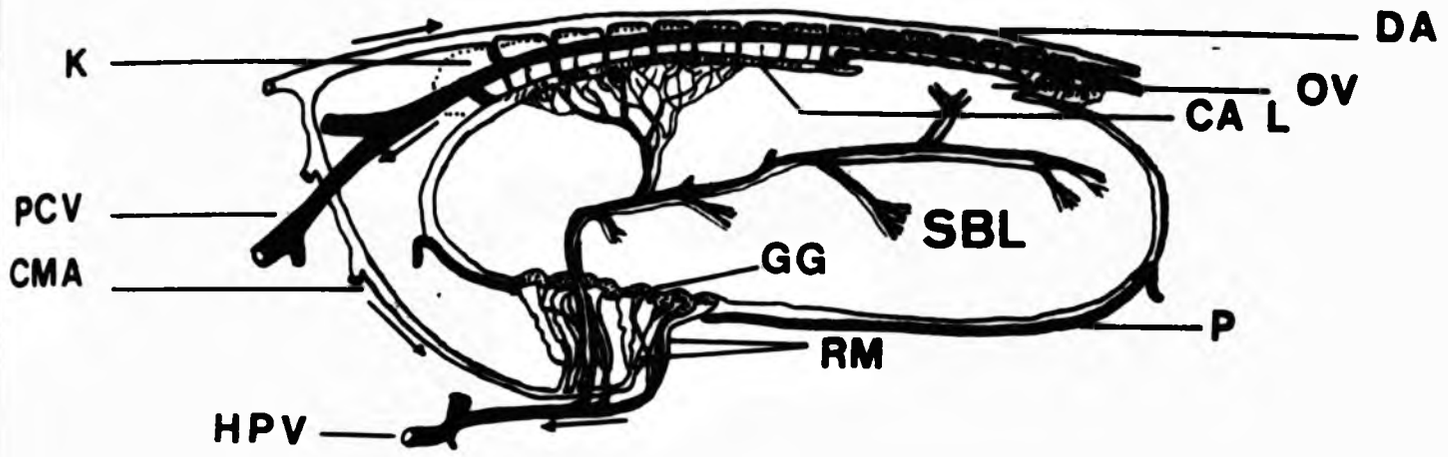


Plate (3.1). A general diagram of a one-champer euphysoclistous swimbladder. (After Saupe, 1939; quoted by Jones and Marshall, 1953). Arrows indicate blood circulation. CAL, capillary layer; CMA, Coeliaco-mesentric artery; DA, dorsal aorta; GG, gas gland; HPV, hepatic portal vein; K, kidney; OV, oval; P, peritoneum; PCV, posterior cardinal vein; RM, rete mirabile; SBL, swimbladder lumen.

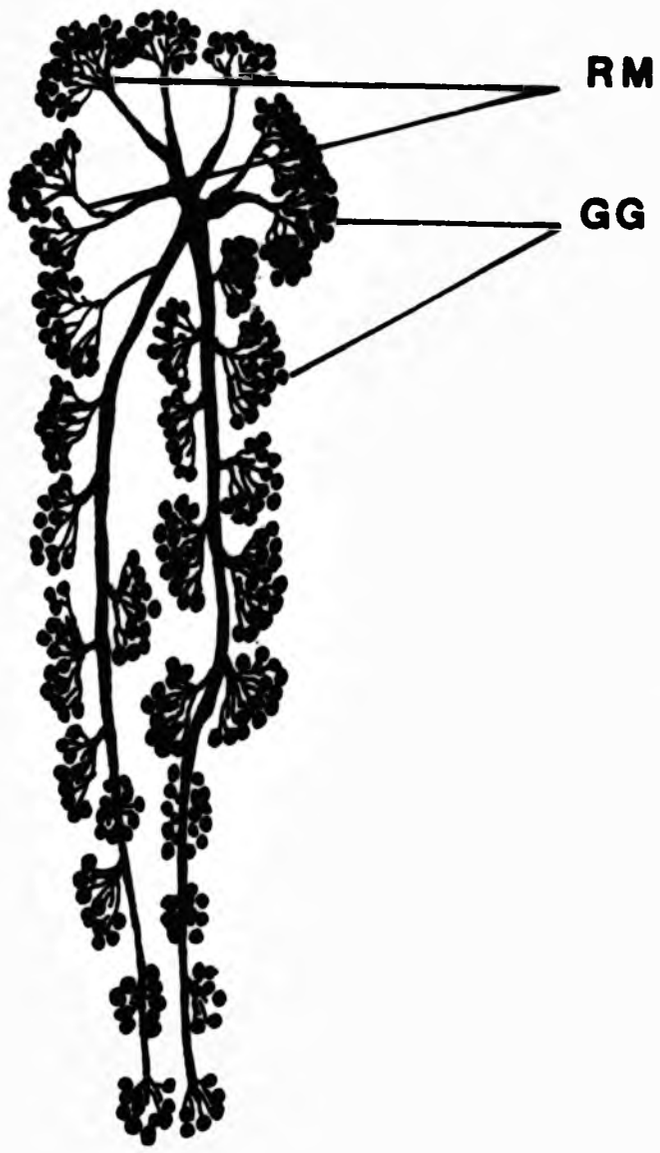


Plate (3.2). Gas glands and rete mirabile of adult *A. Cuvieri* of 60 cm in total body length. The diagram represent approximately actual size. The abbreviations are as in the above Plate.

Most of the very early work on the teleostean swimbladder concentrated on its evolutionary relationship to the lungs (Spengel, 1904; Makuschok, 1913; Ballantyne, 1927 as quoted by Schwarz, 1971) or its usage as a taxonomical index (Lagler *et al.*, 1962). Others noted that it is an organ unique to teleosts (Tracy, 1911; Maier and Scheuring, 1923 quoted by Schwarz, 1971). Latter investigations on swimbladder development included studies of its initial inflation mechanism (Ledebur, 1928; Ledebur and Wunder, 1937; Jacobs, 1938 quoted by Jones and Marshall, 1953; Powers, 1932; McEwen, 1940; Johnston, 1953; Schwarz, 1971). More recently, it has been realized that swimbladder development and the initial inflation mechanism are important in the artificial rearing of some marine and brackish-water species. A large number of incidences of improper inflation of larval swimbladder affecting survival rate and fry quality have been reported, (Yamashita, 1966 and 1982; Doroshev, 1970; Nash *et al.*, 1974, 1977; Spectorova and Doroshev, 1976; Doroshev and Cornacchina, 1979; Bulak and Heidinger, 1980; Doroschev *et al.*, 1981; Kitajima *et al.*, 1981).

Most commonly, the swimbladder develops as a dorsal or lateral diverticulum of the foregut as in *Lepomis macrochirus macrochirus* (Duwe, 1952). The epithelia of both the swimbladder and pneumatic duct are generally considered to have an endodermal origin (McEwen, 1940; Johnston, 1953), but Hoar (1937) reported that the presumptive swimbladder is mesodermal in origin while the pneumatic duct is endodermal. The connection of the swimbladder to the gut is by the pneumatic duct and this may originate on the right or the left side.

In physostomous fishes, the swimbladder is usually initially inflated by gulping atmospheric air at the water surface and passing it into the swimbladder lumen via the oesophagus through the pneumatic duct (Tait,

1960). The same mechanism of initial swimbladder inflation is used in some of the early physoclistous larval stages, which have a temporary pneumatic duct, as seen in *P. major* (Kitajima *et al.*, 1981 and Yamashita, 1966; 1981), *M. saxatilis* (Chapman *et al.*, 1988) and *S. aurata* (Chatain and Ounais-Guschemann, 1990). Several theories have been advanced to explain how the swimbladder is initially inflated in fishes that do not gulp atmospheric air, or which are totally physoclistous prior to initial inflation. These theories range from the production of gases by the disintegration of organic materials (Powers, 1932), to production of gases as a result of food digestion (Johnston, 1953), vacuolation of the swimbladder epithelia (McEwen, 1940), and the functioning of the rete mirabile as a gas gland (Schwarz, 1971). The proposed mechanisms are summarized in Table 3.1.

The aim of this chapter is to describe the larval swimbladder development of *A. cuvieri* and to investigate its initial inflation mechanism. The work concentrates on the timing of major events in swimbladder development. This information will help in designing the later experiments dealing with larval rearing procedures, where initial inflation of the swimbladder and larval survival are the major objectives.

II. MATERIAL AND METHODS

A. Anatomy and histology of the developing swimbladder

A group of 24,000 newly hatched larvae were initially stocked in a 500 liter fibreglass tank and reared for three weeks. These larvae were used for the daily sampling. Larval rearing procedures and tank maintenance were according to the general materials and methods presented in Chapter 2. The initial water temperature of the larval rearing tank was 21°C which is the same as in the egg hatching nets in the KISR hatchery. The temperature

Table 3.1. A summary of the data on initial inflation mechanism in larval swimbladder of some physoclistous fishes.

Species	Initial inflation mechanism	Reference
Morone saxatillis (Striped bass)	Gulping of atmospheric air.	Chapmain *et al.* (1988).
	Lumen dilation, followed by atmospheric air ingestion.	Doroshev *et al.* (1981)
Pagrus major (Red sea bream)	Gulping of atmospheric air.	Kitajima *et al.* (1981)
Gasterosteus aculeatus (Sticklebacks) and *Lebistes reticulatus* (Guppies)	Gulping of atmospheric air.	Ledebur (1928), Ledebur and Wunder (1937), quoted by Jones and Marshall, (1953).
Melanogrammus aeglefinus (Haddock) and *Gadus morhua* (cod)	Gas secreting glandular epithelium produce first filling gas. Glycogenolysis is the source of energy for such process.	Schwarz (1971), Fange (1953) quoted by Schwarz (1971)
Stizostedion vitreum (Walleye)	Gulping atmospheric air oil film (from formulated feed) on water surface prevent initial swimbladder inflation.	Colesante *et al.* (1986)
Hippocampus sp. (Seahorse)	As soon as young leave the brood pouch, they swim to water surface for air gulp.	Jacobs (1938) quoted by McEwen (1940).
Hemichromis bimaculata (Cichlid)	Swimbladder initially inflated by the liberation of gas formed by highly vacuolated epithelial cells of the inner wall of the swimbladder.	McEwen (1940)
Sarotherodon mossambica (Tilapia)	Gas secreting glandular epithelium produce first filling gas for initial inflation.	Doroshev *et al.* (1981), Cornacchia (1982)

Table. 3.1. Cont'd.... 2

Micropterus salmoides (Black bass)	Initial inflation by a gas released from the vacuolated columnar cells in the swimbladders' ventral wall epithelium. In addition, digestive gas from the stomach passing through the pneumatic duct could also be contributing to the initial inflation of the larval swimbladder.	Johnston (1953).

was gradually increased within 24 hours to 25°C and was then kept constant within ± 0.5°C throughout the trial. The larvae usually hatch at about 4-5 a.m. after an incubation period of about 36 hr.

A daily sample of 20-30 live larvae was taken at 1300-1400 hr for 21 days. The larvae were initially collected in a 250 ml glass beaker and were gently transferred into a 5 cm-diameter, 2 cm-deep, wire dip net made from 110 μm nylon mesh. The dip net was then inverted into a full 100 ml sampling bottle of 10% buffered neutral formalin. The sampling bottles were kept in the refrigerator at about 4°C. Another sample were obtained to measure the total body length of larvae and percentage of swimbladder inflation.

Specimens of fixed larvae were dehydrated and cleared in alcohol and xylene and latter embedded in paraplast plus (purified paraffin and plastic polymers, manufactured by Monoject Scientific Inc.). Serial longitudinal and cross sections were cut at 4-7 μm and were routinely stained with Harris haematoxylin and eosin. In addition, thin longitudinal section of 1 - 2 μm were cut using Glycol Methacrylate Monomer as the embedding medium. These sections were stained with toluidine blue.

B. Initial Swimbladder Inflation Mechanism.

This trial is a part of a larger experiment, which consist of 20 tanks of 30 litres capacity. The trial tested the mechanical effect of different aeration rates, which will be presented in Chapter 5.

About 1500 newly hatched larvae were stocked in eight circular, 30 liter fibreglass tanks containing 25 liters of water. All of the eight tanks had an air-stone each diffusing about 50-70 ml of air/min. Four tanks were mixed with 0.5 ml of silicon based surfactant (silicone antifoaming

agent, an aqueous emulsion containing 30% W/W silicone, BDH, U.K.) per liter of rearing water. This surfactant is non-toxic and forms a thin unbroken mat like an oil film covering the whole surface area of the rearing tank thus preventing access to the air. The other four tanks were left as such with an open water surface (without anything added). Larval rearing procedures and tank maintenance were as described earlier. Water salinity and temperature was kept constant at 38-40 ppt and 25 $\pm$ 0.5°C respectively, while 24 hr illumination of 1000 Lux was provided at the water surface. Larval total body length and incidence of initial swimbladder inflation were recorded at day 6, 12 and 16 of the rearing period. Final larval survival was taken at the termination of the trial at day 16.

Water quality parameters in both treatments were kept similar. Dissolved oxygen was maintained in both treatments close to 90% saturation during the first week, this dropped to 80% and 75% saturation at the end of first and second week respectively as a result of increased bacterial activities. The pH of the water was 7.8-8.2. There were no significant differences between the treatments as far as water characteristics are concerned.

III. RESULTS

A. Anatomy and histology of the developing swimbladder

The swimbladder of *A. cuvieri* larvae was first observed on the second day after hatching as an outgrowth protruding upward from the foregut. It grew into more complex structure developing from a kidney-shaped appearance with a hollow center on day 3, to an oval shaped organ with more specialization of cell type and tissue on day 4 and 5 of age. Expansion of the swimbladder due to initial inflation with air was observed on day 6-7.

A full description of the development of the pneumatic duct, gas gland and rete mirabile will be given in the following time series:

Day 1, 10-12 hrs after hatching (total length of larvae; 2.59-2.72 mm).

No differentiation of the swimbladder was observed at this age. The digestive tract appears as a straight tube curving downward posteriorly with both ends closed. The yolk sac is still large with an oil globule at its posterior end. The mouth and eyes are not yet developed.

Day 2, 34-36 hrs after hatching (total length of larvae; 2.81-3.26 mm).

The first differentiation of the swimbladder was observed at this stage as an outgrowth protruding upward from the foregut (Plate 3.3A). No morphological difference between the epithelial cells of the germinal swimbladder and those of the oesophagus was recognized, except that the former is slightly smaller than the later. A mass of undifferentiated cells was found to surround the developing swimbladder. These cells are probably of mesenchyme origin.

Day 3, 58-60 hrs after hatching (total length of larvae; 3.13-3.48 mm).

By day 3 the swimbladder appears kidney-shaped with a hollow center (35 X 12 μm). It has a thin dorsal wall (3-7 μm) but with much thicker ventral and lateral walls (16-28 μm) (Plate 3.3B). The ventral and lateral walls of the swimbladder seem to consist of dense, eosinophilic cellular structures with large nucleated, possibly cuboidal cells. These may be the rudiment of the gas gland, as they appear to be secretary in nature. The epithelial cells forming the dorsal wall of the swimbladder are flattened with a V-shaped fold. Mesenchymal cells were no longer observed at the periphery of the swimbladder except for some connective tissue which may be residual mesenchyme. A clear line of melanophore tissue was seen on the dorsal side of the swimbladder.

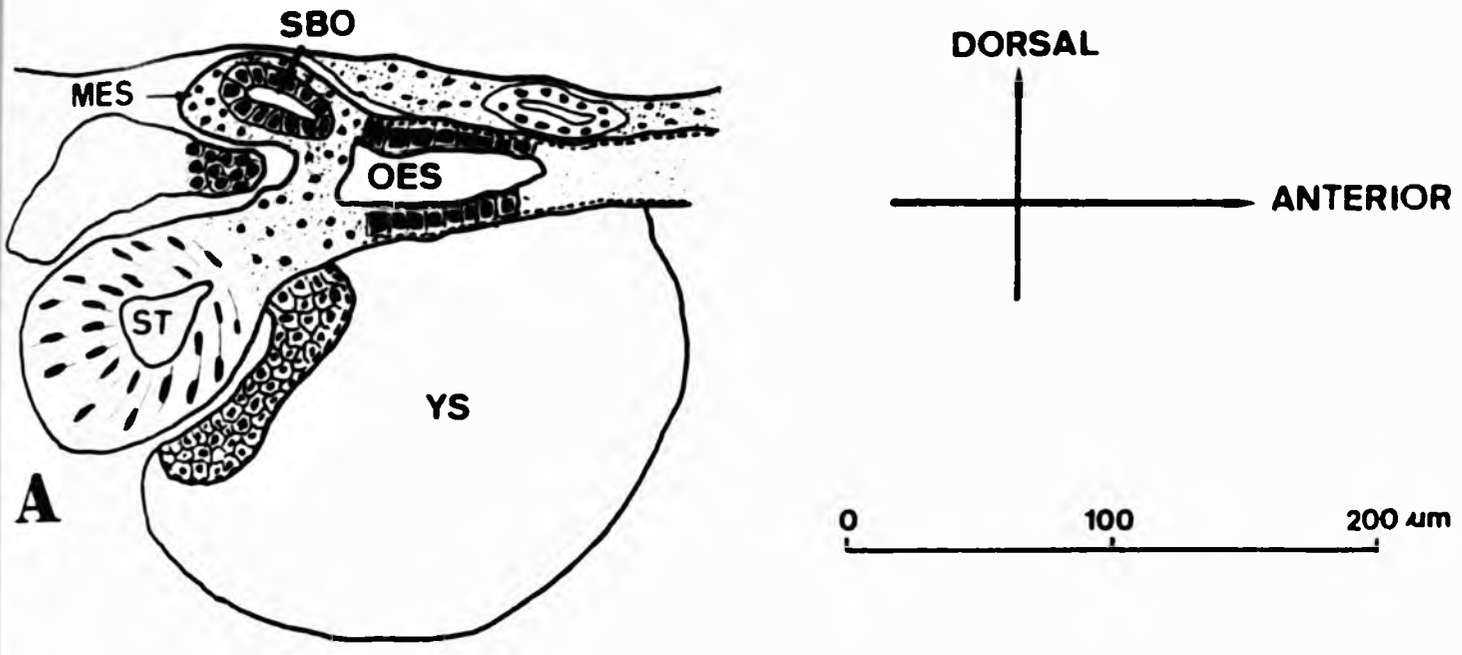


Plate (3.3) Longitudinal section through the swimbladder of *A. cuvieri* larvae of different ages.

A. 34-36 hr after hatching.
B. 58-60 hr after hatching.
B̄. Through the pneumatic duct.

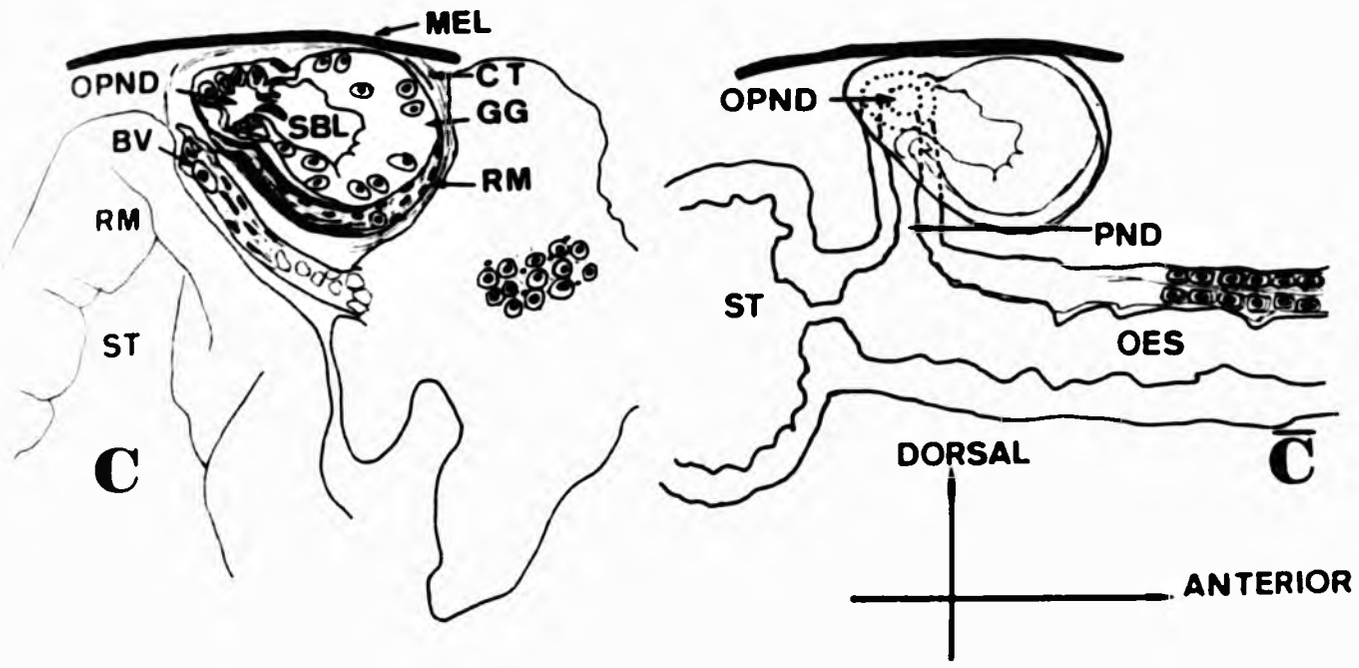
CT, connective tissue; MEL, melanophore; MES, mesenchyme; OES, oesophagus; OPND, opening of the pneumatic duct; PND, pneumatic duct; SB, swimbladder; SBL, swimbladder lumen; SBO, swimbladder outgrowth; ST, stomach; YS, yolk sac.

Day 4-5, 82-108 hrs after hatching (total length of larvae; 3.24-3.50mm)

There are two main features occurring at this stage of swimbladder development. Firstly, the rotation of the swimbladder 90 degree anti-clockwise, so that its ventral side becomes anterior and its dorsal becomes posterior (Plate 3.3C̄). The pneumatic duct attachment to the swimbladder also changes from its centro-dorsal position to the posterior most position. At this stage the future rete mirabile is located on the ventral side of the swimbladder extending anterio-posteriorly as shown in Plate 3.3C. The rete mirabile consists of elongated cells with small nuclei arranged in a manner resembling the structure of capillaries. Even though the incidences of inflated swimbladders was about 5-10% of the larvae examined under dissecting microscope, none were observed among the specimens examined histologically.

Day 6-7, 130-156 hrs after hatching (total length of larvae; 3.28-4.12 mm).

By this stage the swimbladder lumen expands from about 29 X 36 μm to 71 X 90 μm resulting principally from its dilation and filling with air (Plate 3.3D). The frequency of larvae with an inflated swimbladder is about 30-40% at this age. The gas gland has already taken its final position in the larva at the anterior cap of the swimbladder(Plate 3.3D and D̄). The rete mirabile further increases in size and complexity and it appears as two long bands of tissue with a structure similar to blood capillaries. One of these runs from the anterior of the swimbladder, where it is connected with the gas gland, to its posterior end, while the other runs from the posterior of the swimbladder along its ventral side to approach a major blood vessel (perhaps the hepatic vein). Blood corpuscles were first seen in the rete mirabile at this stage. A small patch of dense eosinophilic cellular tissue appeared at the posterior end of the swimbladder connected to the upper part



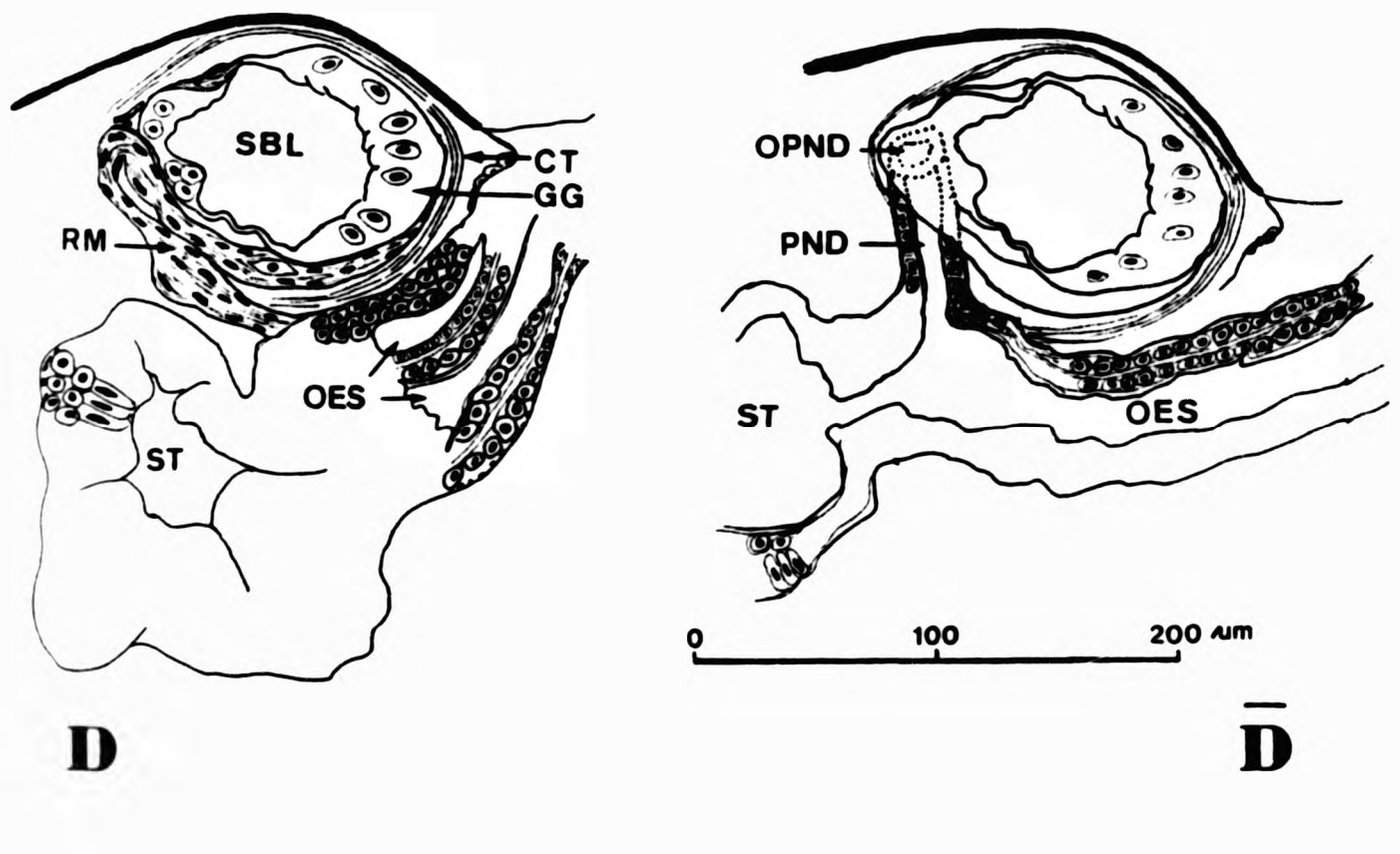


Plate (3.3) Longitudinal section through the swimbladder of *A. cuvieri* Larvae of different ages.

C. 82-108 hr after hatching .
C̄. Through the pneumatic duct.
D. 130-165 hr after hatching.
D̄. Through the pneumatic duct

BV, blood vessel; GG, gas gland; RM, rete mirabile, the rest of abbreviations as in Plate (3.3 A and B).

of the rete mirabile. Well developed connective tissue was observed surrounding the swimbladder walls.

Day 8-9, 178-202 hrs after hatching (total length of larvae; 3.37-4.39 mm).

By the 8th or 9th day the swimbladder increased in size and became more elongated in shape (Plate 3.3E and $\bar{E}$). The gas gland became more defined and the rete mirabile increased in size. The epithelial cells of the swimbladder wall tend to become membranous in appearance, with few nuclei. The first signs of atrophy of the pneumatic duct were observed and its width, as seen from the oesophageal side, started to decrease slightly (Plate 3.3E and $\bar{E}$).

Day 10-12, 226-276 hrs after hatching (total length of larvae; 4.12-5.02 mm).

The major developmental event at this age is the atrophy of the section of the pneumatic duct connected to the oesophagus, which results in the closure of access to the oesophagus and hence to the external environment, as shown in Plate (3.3G). Although the oesophageal end of the pneumatic duct is still open, the swimbladder at this stage has been transformed from a physostomous to a physoclistous type by losing the connection to the oesophagus. The internal surface of the gas gland tends to become smoother than in the earlier stages. The rete mirabile has broken its connection with the posterior end of the swimbladder and forms a U-shaped structure connecting the anterior part of the gas gland with a ventral source of blood supply (Plate 3.3F).

Day 19-21, 442-492 hrs after hatching (total length of larvae; 5.41-7.38 mm).

By the end of the third week, the pneumatic duct has become completely rudimentary, and is attached only to the posterior of the

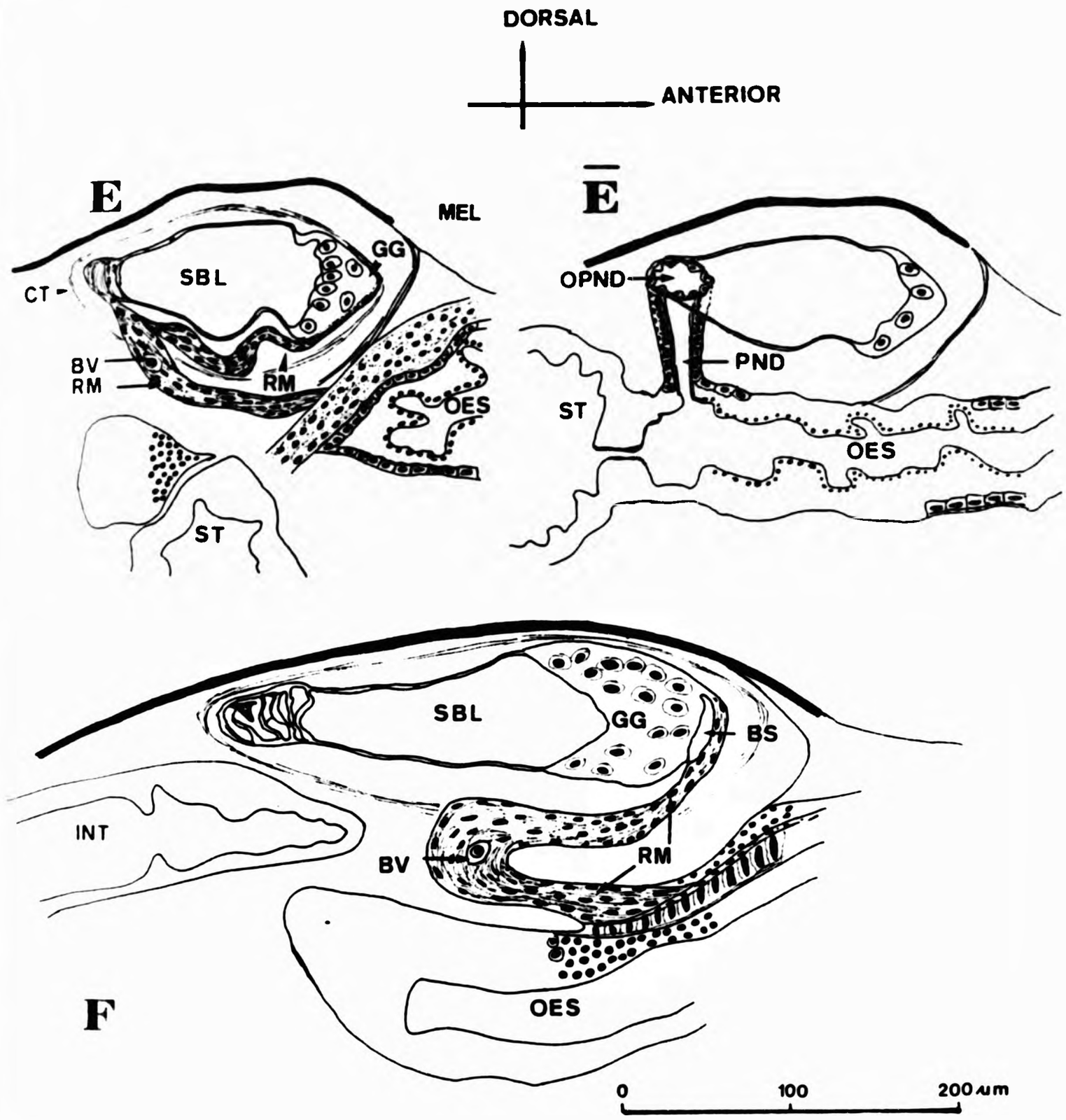


Plate (3.3). Longitudinal sections through the swimbladder of *A. cuvieri* larvae of different ages.

E. 178- 202 hr after hatching.
Ē. Through the pneumatic duct.
F. 226-276 hr after hatching.

BS, blood sinus; INT, intestine, the rest of the abbreviations as in plate (3.3 A-D).

swimbladder (Plate 3.3H). The gas gland has reduced in size in relation to the total volume of the swimbladder due to the overall expansion of the swimbladder. Complicated folds appear within the gas gland and the structure of the swimbladder wall becomes more strongly differentiated. The rete mirabile appears as a straight bundle of blood vessels connected to the anterior part of the gas gland and supplied with blood from a ventrally-situated blood vessel (Plate 3.3H). The relative position of the swimbladder in relation to other organs of *A. cuvieri* larvae is shown in Plate 3.4.

A summary of swimbladder development is given in Table 3.2 focusing on its general appearance, and the development of the gas gland, rete mirabile and pneumatic duct.

B. Initial swimbladder inflation mechanism

The results obtained from the initial swimbladder inflation test are summarized in Tables 3.3, 3.4 and 3.5. *A. cuvieri* larvae clearly require access to atmospheric air to initially inflate their swimbladders. Table 3.3 shows clearly that the four replicates which had their water surface covered with the silicon-based surfactant (anti-foam) achieved zero initial inflation on Days 6, 12 and 16. By contrast, those with access to the air had a high initial inflation percentage even on Day 6. The two treatment results are significantly different ($P<0.01$) at all the three ages studied (percentages were transformed using arcsine for statistical testing).

Total larval survival, and survival of larvae with and without an inflated swimbladder is shown in Table 3.4. Total survival in both

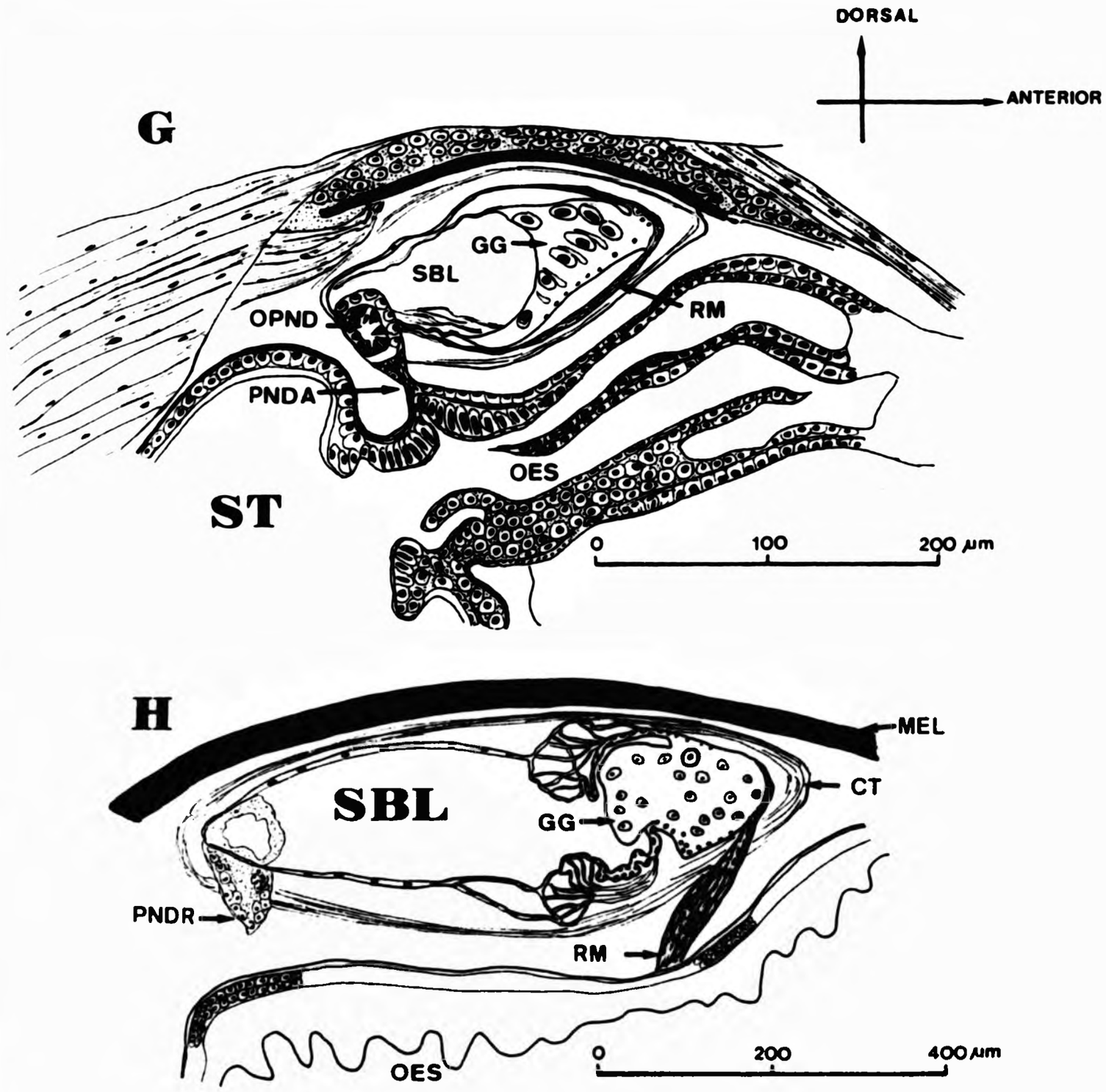


Plate (3.3). Longitudinal sections through the swimbladder of *A. cuvieri* larvae of different ages.

G. 226-276 hr after hatching showing the atrophy of the pneumatic duct.

H. 442 -492 hr after hatching.

PNDA, pneumatic duct atrophy; PNDR, pneumatic duct rudiment, the rest of the abbreviations are as in Plate (3.3 A-F).

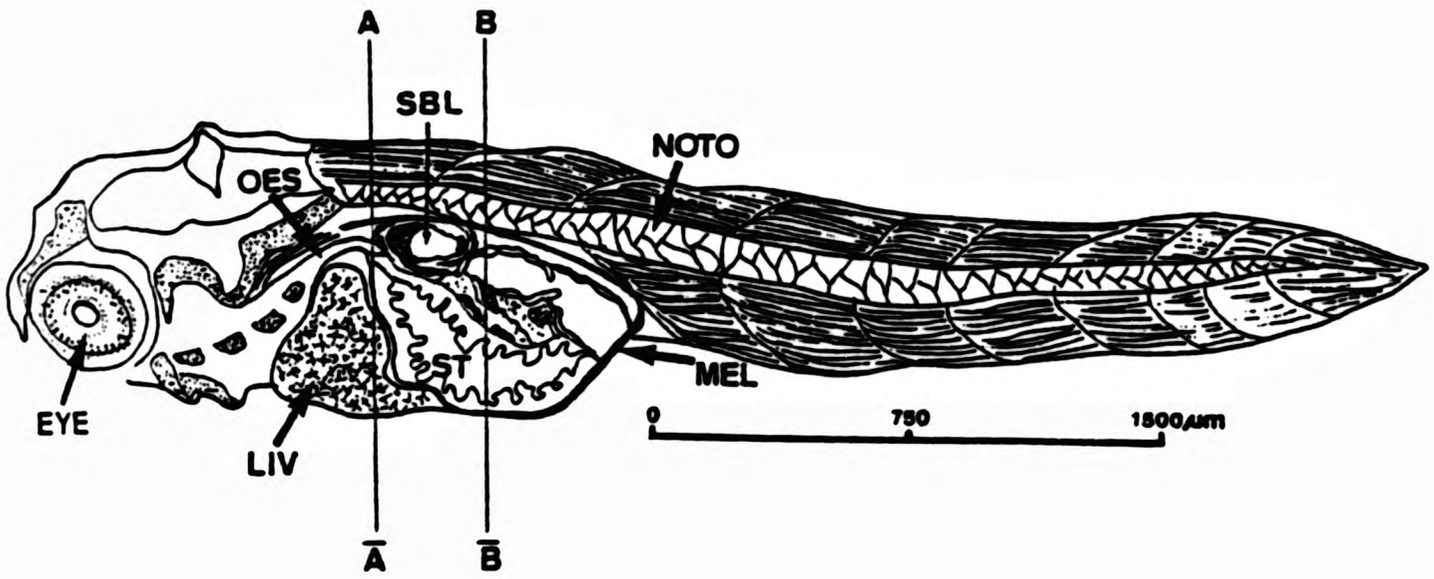


Plate (3.3) Longitudinal section through the swimbladder of *A. cuvieri* larvae of different ages.

I. 226-276hr after hatching showing the swimbladder (between line AĀ and BB̄) with respect to its location among other organs.

NOTO, notochord, the rest of the abbreviations are as in Plate (3.3 A-H)

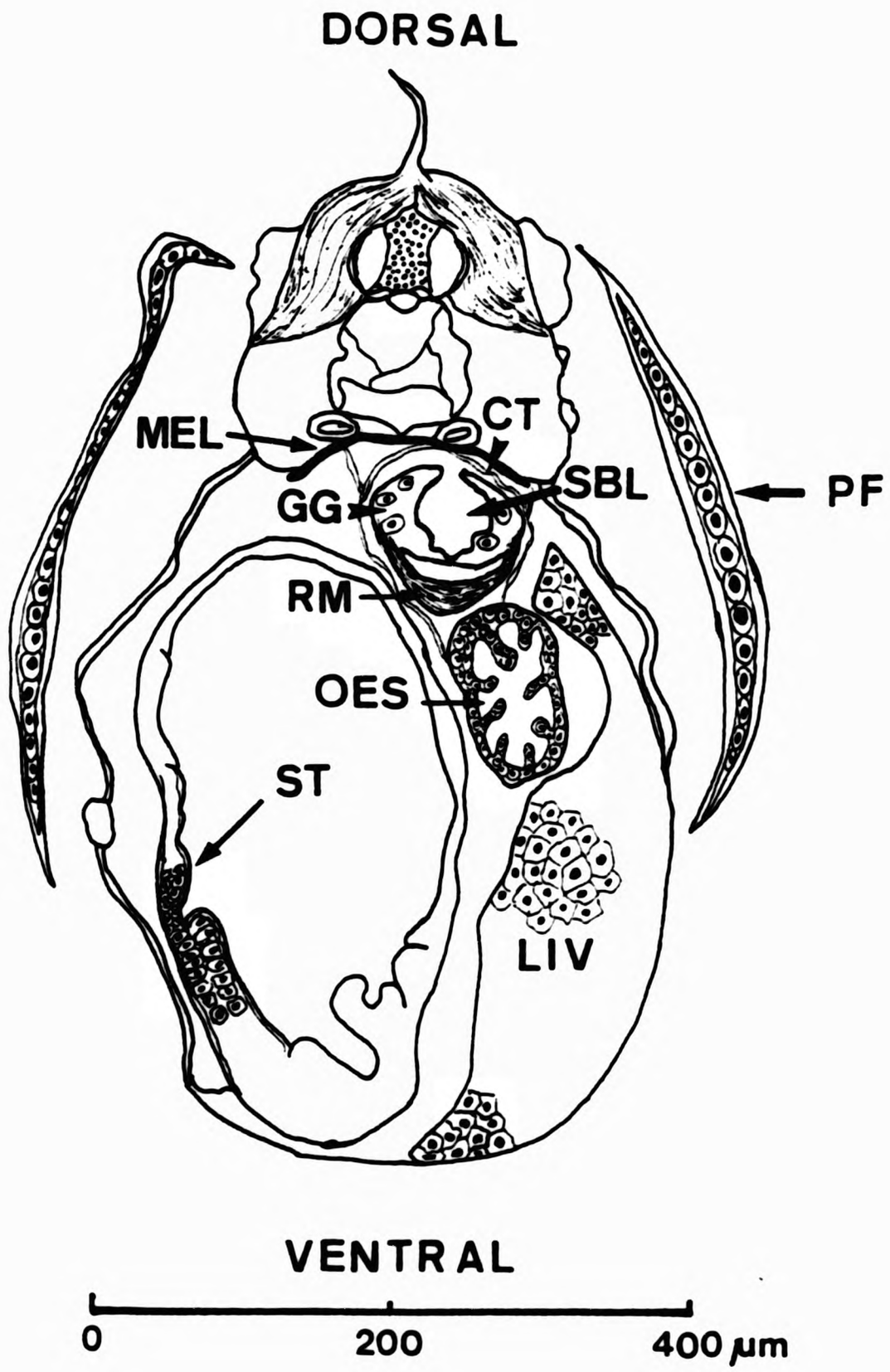


Plate (3.4). A cross section through the swimbladder of *A. cuvieri* larvae of 178-202 hr after hatching; showing the relative location of the swimbladder to other organs. LIV, liver; PF; pectoral fin, the rest of the abbreviations are as in Plate (3.3 A-H).

Table 3.2. A summary of swimbladder development in *Acanthopagrus cuvieri* larvae during the first three weeks after hatching.

Age of larvae after hatching		Total body length range (mm)				
(Day)	(Hr)	(1)[a]	Pneumatic duct	General Appearence of the swimbladder	Gas gland	Rete Mirabile
2	34-36	2.81-3.26 (0)	Foregut outgrowth represents the early pneumatic duct.	First appears as an outgrowth of the dorsal wall of the foregut.	Not visible	Not visible
3	58-60	3.13-3.48 (5)	Connects the oesophagus with the left dorsolateral wall of the swimbladder.	Kidney shaped, with a hollow cavity. Thin dorsal wall making V-shape fold, while thick ventral wall secretory in nature.	First appears as the ventral portion of swimbladder, as large dense cells, secretory in nature.	Not visible
4-5	82-108	3.24-3.50 (17-33)	Changes its position of connection to swimbladder to a posterior one.	Swimbladder appears to turn 90° anticlock wise.	Occupies the anterior portion of the swimbladder as a result of the rotation of the swimbladder.	First appears as a small collar of the anterioventral portion of the gas gland.
6-7	130-156	3.28-4.12 (43-48)	Short, straight and thick in appearance.	Dilation and expansion as a result of being inflated with air. Well developed surrounding connective tissue.	Increases in area, with reduction in thickness as swimbladder expands.	Grows in size and complexity, blood corpusles first observed.
8-9	178-202	3.37-4.39 (48-50)	Early signs of atrophy at the oesophageal connection.	Becomes elongated in shape with more membranous walls.	Uneven internal surface.	Well developed structure of capillaries connecting the anterior of gas gland to the posterior of the swimbladder.
10-12	228-276	4.12-5.02 (50-60)	Oesophageal connection is lost, complete atrophy observed.	Doubles in length with membranous foldings at the posterior end.	Smoother internal surface.	Broken attachment with the posterior end of the swimbladder, for a U-shaped arrangement with gas gland and a ventral source of blood supply.
19-21	442-492	5.41-7.38 (50-60)	Disconnected from the oesophagus, but still some rudiment attached to the posterior end of the swimbladder.	Increases in size to about 8-10% of the total length of the larvae.	Two folds wing-like in appearance branching dorsally and ventrally from the gas gland forming a tubular net work structure which is connected to swimbladder wall.	A more vertical appearance of the blood capillaries connecting the anterior gas gland with a ventral blood supply.

(1)[a] = % of larvae having an inflated swimbladder.

Table 3.3 Initial inflation success (%) of larval swimbladder of *Acanthopagrus cuvieri* after 16 days of rearing with and without access to air.

Replicate	Covered water surface			Open water surface		
	Day 6	Day 12	Day 16	Day 6	Day 12	Day 16
1	0	0	0	49	74	96
2	0	0	0	65	70	90
3	0	0	0	77	84	100
4	0	0	0	73	100	100
Mean	0_a	0_a	0_a	66.0_b	82.0_b	96.5_b

Table 3.4 Percent survival of total, normal and abnormal (lacking inflated swimbladder) larvae of *Acanthopagrus cuvieri* after 16 days of rearing of covered and open water surface tanks.

Replicate	Covered water surface			Open water surface		
	Total	Abnormal	Normal	Total	Abnormal	Normal
1	11.1	11.1	0	16.8	0	16.8
2	22.4	22.4	0	15.9	0	15.9
3	11.8	11.8	0	11.3	0.4	10.9
4	15.4	15.4	0	23.4	2.3	21.1
Mean	15.2_a	15.2_a	0_b	16.9_a	0.68_c	16.2_a

Table 3.5 Larval growth expressed as total length (mm) of *Acanthopagrus cuvieri* during 16 days of rearing of covered and open water surface tanks.

Replicate	Covered water surface			Open water surface		
	Day 6	Day 12	Day 16	Day 6	Day 12	Day 16
1	3.26	4.21	6.05	3.31	4.60	6.43
2	3.56	4.69	6.24	3.23	4.76	5.30
3	3.47	4.83	5.74	3.30	4.74	6.45
4	3.20	4.21	6.69	3.37	4.65	5.54
Mean	3.37_a	4.48_b	6.18_c	3.30_a	4.69_b	5.93_c

Different subscripts are significantly different at $P<0.05$.

covered and open water surface treatments are not significantly different at $P>0.05$ at Day 16. However, the survival of larvae having an inflated swimbladder of both treatment are significantly different at $P<0.01$. Measurements of larval total length of both treatments at Day 6, 12 and 16 are presented in Table 3.5 and show no significant differences between treatments ($P>0.05$).

IV. DISCUSSION

The embryonic origin of the teleost swimbladder is well documented in the literature for several species. Generally, it may originate in three ways. It is formed from a dorsal or lateral diverticulum of the foregut in ***Lepomis macrochirus macrochirus*** (Duwe, 1952), ***Sarotherodon mossambica*** (Cornacchia, 1982), ***Percina caprodes*** (Grizzle and Curd, 1978), ***Melanogrammus aeglefinus*** (Schwarz, 1971), ***Pagrus major*** (Yamashita, 1982) and many others. Secondly, it may originate from the oesophagus as a solid mass of cells in which a cavity appears later. This cavity then extends downward and establishes communication with oesophagus. This type has been described in ***Coregonus palaea*** (Vogt, 1842 quoted by Schwarz, 1971). Lastly, the swimbladder may originate as a solid mass of cells which is later invaded by an endodermal evagination from the gut, and an example of this type is *Salmo salar* (Hoar, 1937).

In this work, the histological study of *A. cuvieri* larvae has shown that the swimbladder originates as a diverticulum of the dorsal wall of the foregut on Day 2 after hatching. It differentiated further and increased in size and complexity on Day 4-5 of age. At this time, the pneumatic duct, gas gland and rete mirabile were all well-developed and probably functional. The appearance of blood corpuscles in the rete mirabile on Day 6-7 strongly suggest that it is functional.

The rotation of the swimbladder through 90° observed in *A. cuvieri* on Day 4-5 after hatching, Plate 3.3, B and C, was also observed by Yamashita (1982) working with *Pagrus major*. He reported that the shape of the swimbladder 5-6 days after hatching (120-140 hrs) changed remarkably in comparison with the previously examined sample (72-96 hrs) and it appeared as if the swimbladder had turned 90° from its previous position. Since the pneumatic duct is attached to the swimbladder, this rotation also affects the relative position of the pneumatic duct. It is assumed therefore, that the final (posterior) point of connection of the pneumatic duct to the swimbladder is a result of this turning.

The experiments performed by different authorities to date to test initial inflation mechanisms of larval swimbladder have not all been conclusive in their results, as noted by Bulak and Heidinger (1980) and Doroshev and Cornacchia (1979) working on *M. saxatilis*. Chapman *et al.* (1988) obtained more conclusive results on the initial inflation mechanism of *M. saxatilis* and they rejected the other theories of internal gas secretion proposed previously by other workers (Doroshev *et al.*, 1981). Many experimental approaches have been used by researchers, as shown in Table 3.6, to prevent larvae from having free access to atmospheric air at the water surface. Chapman's aquarium design, where he used a glass ceiling to deny Striped Bass larvae free access to atmospheric air at the water surface, was clearly very effective. Some of these methods, however, did not work and caused massive mortality giving inconclusive results (Doroshev and Cornacchia, 1979). In the present study the choice of a silicon-based surfactant as the material used to obstruct larvae from free access to atmospheric air at the water surface was an effective one, and caused no apparent side effects such as mortality, difficulties in tank maintenance or

Table 3.6. A summary of materials and methods used by different workers in preventing free access of larvae to atmospheric air.

Species	Materials and Methods	Reference
Pagrus major	A layer of liquid paraffin covering larval rearing water surface Electric fan blown vertically at water surface of rearing tank	Kitajima *et al.* (1981)
Morone saxatilis	Used a glass-roofed aquarium	Chapman *et al.* (1988)
Morone saxatilis	Nylon mesh screen under water surface of rearing tank	Doroshev and Cornacchia (1979)
Sarotherodon mossambica	5-7 cm layer of mineral oil covering rearing water surface	Doroshev and Cornacchia (1979)
Engraulis mordax	0.5 cm layer of mineral oil covering rearing water surface	Hunter and Sanchez (1976)
Hemichromis bimaculata	Submerged glass tube covered with cheescloth at each end	McEwen (1940)
Acanthopagrus cuvieri	0.5 ml of silicon-base surfactant per litre of rearing water, gives unbroken thin mat covering the entire larval rearing tank surface	The Present Study

difficulties in larval observation or sampling. In the first run of this test colourless liquid paraffin was used, but high larval mortality was found in the tanks with liquid paraffin, as larvae appeared to collect within the paraffin layer. Sampling of larvae from tanks covered with paraffin layer was also very difficult.

Reviewing the work published on the different modes of the initial swimbladder inflation of physoclistous fishes (Table 3.1), one may conclude that no generalization can be made. It is fairly clear, however, that demersal fry tend to have a totally internal type of initial inflation through gas produced in the swimbladder, as in *Hemichromis bimaculata* (McEwen, 1940), *Sarotherodon mossambica* (Doroshev *et al.*, 1981) and in *Micropterus salmoides* (Johnston, 1953). Their initial larger size than the marine pelagic larvae and probably their well developed and larger rete mirabile and gas gland could help in forming the initial gas bubble in the swimbladder lumen which is probably filled with fluid. The formation of a gas bubble requires extremely high partial pressure. The pressure required to form a gas bubble is inversely proportional to its radius and directly proportional to the surface tension of the fluid (Blaxter and Tytler, 1978). The pelagic larvae tend to have an external type of initial inflation and the gulping of atmospheric air at the air-water interface is a necessity. Once a small air bubble is obtained in the swimbladder lumen, there is no difficulty to increase its size by means of the gas gland and rete mirabile and then much lower partial pressure is required to do so. In this study, *A. cuvieri* larvae were clearly shown to require access to the water surface to gulp atmospheric air to initially inflate their swimbladder (Table 3.3). The question to be asked here is, if initial inflation is achieved by a simple air gulp at the water surface, why is the state of development of the swimbladder so highly advanced at the time of initial inflation?

The early development, relatively large size and early functioning of the gas gland and rete mirabile in larval *A. cuvieri*, observed from the histological work, is worth considering more carefully. The possibility of relating such early manifestations of adult swimbladder structure to the initial inflation mechanism is inevitable. Yamashita *et al.* (1982) working on *P. major* found a similar early development of the gas gland and rete mirabile. *P. major* is a sparid and is closely related to *A. cuvieri*. It starts to inflate its swimbladder on the 8th day after hatching at 18°C and at the same time blood corpuscles were seen in both the gas gland and the rete mirabile indicating that they are probably functional. *P. major* has been confirmed by several authors to require access to atmospheric air to initially inflate its swimbladder (Kitajima *et al.*, 1981; Iseda, 1982). Schwarz (1971), working on *Melanogrammus aeglefinus* larvae, also reported that the rete mirabile was well developed just before inflation. Similarly, Bulak and Heidinger (1980) and Chapman *et al.* (1988) working on *Morone saxatilis*, also found that the gas gland and the rete mirabile were developed before or just at initial inflation. Table 3.7 summarizes these findings.

Overall, it seems most probable that when *A. cuvieri* larvae swallow atmospheric air at the water surface, the gas gland and rete mirabile complex will be triggered to function, to complete the filling of the swimbladder. The sequence of structural development, the timing of initial inflation, and the requirement for access to the air all contribute to this hypothesis in this species. This proposal is not a new theory on the initial inflation of larval swimbladder of physoclistous fishes and Jacobs (1938) had the same idea (quoted by Jones and Marshall, 1953). Keeping in view the literature discussed above, it can safely be concluded that the mechanism of initial inflation of larval swimbladder of physoclistous fishes is a more sophisticated process than a simple gulping of air at the water surface.

Table 3.7 A summary of the timing (in days after hatching) of major events in the larval swimbladder development of some marine and brackish water species that have pelagic eggs

Species	Yolk sac absorbed	Oil droplet absorbed	Swimbladder			Gas gland appeared	Rete mirabile appeared	Presence of pneumatic duct	Atrophy of pneumatic duct	Mechanism of inflation	References
			Appeared	Begin to inflate	Inflated						
Pagrus major (Red sea bream)	Day 5 at 18°C	—	Day 2 (48 hr)	In * physostomous: Day 8 at 18°C Day 4 at 23°C In ** physoclistous: Day 40 at 18°C	In * physostomous: Day 10 at 18°C In ** physoclistous: Day100 at 18°C	Day 6 at 18°C	Day 6 and it is functional on Day 8-9	Yes	Day 10-12 at 18°C Day 8-10 at 21-23°C	Atmospheric air gulp as in physostomes ,and through gas gland as in physoclists	Yamashita (1982), (1966) Chatain (1982)
Morone saxatilis (Striped bass)	Day 5-7 at 18°C	55 % resorption by Day 10	Day 3	Day 4 at 18°C	20-27 % by Day 7 80 % by Day 10	Day 3	Day 4-8	Yes	Inflation of swimbladder triggers atrophy	Atmosphric air gulp	Chapman *et al.*; (1988) Doroshev *et al.*; (1981) Bulk and Heidinger (1980)
Melanogrammus aeglefinus (Haddock)	Day 6 at 13°C	—	Day 11 before hatching at 5.5°C	Day 6 at 13°C	—	—	Day 6 at 13°C	Yes	Day 12 at 13°C	Cellular gas production	Schwarz (1971)
Acanthopagrus cuviere (Blue-finned sea bream)	Day 3 at 24-25 °C	Day 6-7	Day 2	Day 3-4	Day10-12	Day 3	Day 4	Yes	Day 10-12	Atmospheric air gulp	The present study

* Larvae before losing the pneumatic duct
** Metamorphosed larvae after losing the pneumatic duct

CHAPTER 4

THE BUOYANCY BUDGET OF *A. CUVIERI* LARVAE

I. INTRODUCTION

Several comprehensive reviews on buoyancy in adult marine fishes have been published, e.g., Denton and Marshall (1958), Bone (1973) Denton (1974), Tytler (1976), Love (1980), Bone and Marshall (1982) and Gee (1983). In contrast, very little information is available on the subject of buoyancy in the marine pelagic fish larvae. However, recently some studies have appeared on the buoyancy of eggs and larvae (Blaxter and Ehrlich, 1974; Coombs *et al.*, 1985; Nielson *et al.*, 1986; Craik and Harvey, 1987; Yin and Blaxter, 1987).

Buoyancy is scientifically defined as the "*resultant vertical force exerted on a body by a static fluid in which it is submerged or floating*". It can also be defined as "*an upward force generated by the displacement of the ambient medium by a lower density substance*". In fish, this upward force can in some cases neutralize the downward force generated by the fish weight, and thus neutral buoyancy is achieved. Buoyancy in fish can also be assessed by dividing the density of the whole intact fish by the ambient water density, then positive buoyancy is <1.0, neutral buoyancy = 1.0, and negative buoyancy is >1.0. This definition is developed from the term "sinking factor" which was introduced by Lowndes (1937) who was quoted by Jones and Marshall (1953).

Neutral buoyancy is advantageous for fish, since it means that all the power generated by the locomotor muscles during swimming can be used to provide forward thrust. Furthermore, if the fish is neutrally buoyant it is able to rest motionless in the water column, thus conserving a considerable amount of energy (Alexander, 1972).

Buoyancy mechanisms in fishes can be summarized in two general categories of upward lifts; hydrodynamic and static (Gee, 1983). The hydrodynamic lift is generated by the movement of the body and the fins through water. Fish can control this hydrodynamic lift by manipulating their swimming speed, and angle and area of fins exposed to the water (Magnuson, 1978; Gee, 1983). The second category, the static lift, can be further divided into two main groups namely, fixed and adjustable buoyancy. Fixed buoyancy usually involves the inclusion of low-density metabolically derived substances, such as lipids (Lewis, 1970) or ammonium ions (Denton and Marshall, 1958) in soft body tissue such as fresh or liver. It can also be based on lipid or wax ester-filled swimbladders (Nevenzel *et al.*, 1969) or as poorly ossified bone with reduced musculature having a high water content (Denton and Marshall, 1958). The possession of oil-filled bones is another variety of fixed buoyancy mechanism adopted by some fishes (Lee *et al.*, 1975). Fixed buoyancy mechanisms provided a long-term fixed value of an upward force. One of the great advantages of this mechanism is that it is virtually independent of pressure and remains constant during vertical movements. However, the nutritional status of those fishes adopting such a mechanism may alter its upward force values (Tytler, 1976).

The second category of static lift is the adjustable buoyancy mechanism which involves the development of an internal gas-filled chamber and this technique is usually adopted by fishes with regular vertical movements. The short-term variable upward forces generated result from the fish ability to control the swimbladder gas volume (Tytler, 1976). Jones and Marshall (1953) generalized that neutral buoyancy for freshwater fishes will be attainable with 7% of the fish total volume being gas and 5% for marine fishes, assuming that fresh water and seawater have densities of 1.000

and 1.026 g/cm^3 respectively, and that fat-free fish density is 1.076 g/cm^3. Alexander (1966) reported that the gas volume of the swimbladder can be 5.6 - 8.3% for fresh water fishes and 3.1 - 5.6% for marine fishes of the fish total volume. Andriashev (1944), who was quoted by Jones and Marshall (1953), worked on Black Sea fishes and found that the density of fishes with swimbladders ranged from 1.012 to 1.021 g/cm^3 as compared with 1.061 to 1.085 g/cm^3 for those without, noting that the density of the surface water of the Black Sea is about 1.013 g/cm^3.

The positive buoyancy of marine pelagic eggs is mainly the result of the high amount of water of low salinity in the embryo and yolk (Riis-Vestergaard, 1984; Craik and Harvey, 1987). In certain species the oil globule plays an additional role in achieving buoyancy. The perivitelline water (water between the Chorion and perivitelline membrane surrounding the embryo, which is in osmotic equilibrium with the outside water) does not contribute to the buoyancy of the egg (Hempel, 1979). Although the chorion is highly permeable, the embryo is protected against osmotic change by the vitelline membrane which is either impermeable or osmoregulatory active (Hempel, 1979).

Buoyancy in marine pelagic larvae destined to develop swimbladders in their early life history can be considered chronologically in two stages. The first stage is a temporary one, acquired upon hatching and its upward force is derived mainly from the large amount of low-salinity water stored in the yolk sac and secondly from the lipids stored in the yolk sac and oil globule as a nutritive source (Blaxter and Ehrlich, 1974; Riis-Vestergaard, 1984; Craik and Harvey, 1987). The second stage begins with the appearance of an air-filled swimbladder approximately at the end of the first stage, when most of the low-salinity water and lipid stored in

the yolk sac and oil globule have been utilized by the growing larvae (Doroshev *et al.*, 1981; Chatain, 1986). The generation of dynamic lift by muscular activity and coordination of fin movement will also enable the larval fish to explore the water column since its density will still be close to that of the environment. However, as bone becomes ossified and muscular growth increases, so the density increases and a buoyancy mechanism is needed.

The development of a functional swimbladder as a buoyancy mechanism usually takes place before or during the initiation of external feeding. Hydrostatic regulation and the achievement of neutral buoyancy are essential for efficient swimming, capture of prey and maintenance of position in the water column. Thus, the energetic costs of all these activities is greatly reduced and this is reflected in the improvements in survival and well being of larvae (Hunter, 1972).

The objective of this section was to study and analyse different factors contributing to the larval buoyancy of *A. cuvieri*. Moreover, in the present study an attempt has also been made to construct a buoyancy budget sheet which would help in understanding the developmental sequence related to initial swimbladder inflation. The individual parameters taken into consideration of different ages are; total length, body weight, yolk sac volume, oil globule volume, swimbladder volume, and total density of *A. cuvieri* larvae. The calculated and measured changes in buoyancy were correlated amd compared with fish behaviour, in particular, with the vertical migration of the larvae.

II. MATERIALS AND METHODS

A. Morphological Measurements

About 60,000 just-hatched larvae were stocked in a 1000 litre fiberglass tank and reared according to the procedures described in Chapter 2. Two samples of about 100-200 larvae were randomly collected daily for the first two weeks after hatching, while a smaller weekly sample of 30 to 50 post-larvae and fry was taken up to the Day 80.

One of the two samples (of 100-200 larvae) was divided into three further portions, excess water was dried from each portion and the larvae were then weighed using a sensitive electronic balance to the nearest five decimal places. Individual body weight was then calculated by dividing the bulk weight of each portion by the number of individuals in the sample. Individual body weights by direct weighing were done only after the 40th day of age.

The second sample was used to measure total length, yolk sac length and height, oil globule diameter, and swimbladder length and height. These measurements were taken from live specimens, anaesthesized with MS-222, using a Nikon dissecting microscope. Swimbladder volume of metamorphosed larvae of total body length between 25-35 mm could only be measured with difficulty due to the formation of the scales on their skin.

The volumes of the yolk sac and the swimbladder were calculated using the formula for a prolate spheroid, $4/3\pi LH^2$, where H is half of the height and L is half of the length of the prolate spheroid. The oil globule volume was calculated using the formula for a sphere, $4/3\pi r^3$, where r is the radius. Since the oil globule is within the yolk sac, the yolk volume was finally corrected by subtracting the oil volume from it.

B. Density

A density gradient column (Techne, DC-1, DC-2 and DC-3) was used to determine the density of *A. cuvieri* larvae, post larvae and metamorphosed fry. The gradient column and associated equipment are shown diagrammatically in Figure 4.1a. Essentially it consists of a graduated glass tube (850 mm in length, 50 mm internal diameter) closed at its lower end and immersed in a temperature-controlled water bath (± 0.02°C). Mounted on top of the column is a clearing device consisting of mesh basket which can be raised or lowered at a low speed by a nylon thread attached to a slowly revolving spindle. The gradient mixture comprises a base plate on which are mounted two reservoir chambers, the combined capacities of which are equal to the volume of the gradient tube (1.6 litres). The mixing reservoir carries a stirrer (250 r/min) and blade. Fitted to the base plate of the mixer is a flow control valve controlling a small-bore passage connecting the two reservoir chambers. A second passage of the base plate leads from the mixing reservoir via a peristaltic pump to a flexible capillary tube used for filling the gradient tube. A fully detailed description of this apparatus, calibration and operation are presented by Coombs (1981), who used it to measure the density of Mackerel eggs (*Scomber scombrus*). Coombs used two salinities (23.3 and 40.2%) to make up a density gradient column which ranged from 1.017 to 1.030 g/ml. Kuo *et al.* (1973) used the same apparatus to measure the density of *Mugil cephalus* larvae. They used a density gradient made up with 15% calcium nitrate and distilled water to obtain a density range of 1.005 to 1.047 g/ml. Both of the above mentioned density gradient salts (calcium nitrate and sodium chloride) were tested with *A. cuvieri* larvae in this study and poor results were obtained. The use of these two salts as density gradient media was considered unsatisfactory due to the fact that after the larvae were

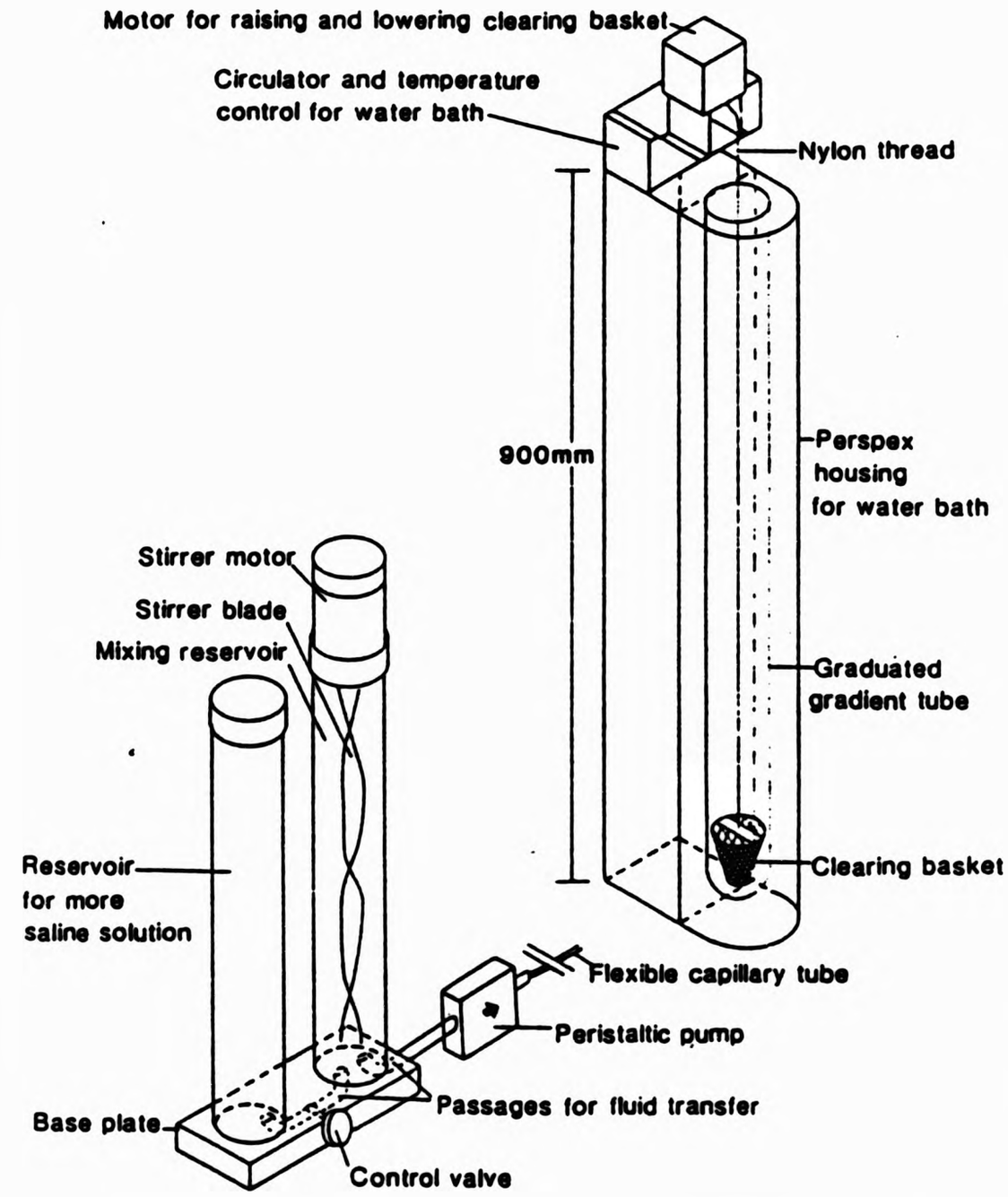


Figure 4.1a Diagrammatic illustration of density-gradient column, gradient mixer, filling system and clearing device (After Coombs, 1981).

introduced into the density column they did not reach a phase of constant minimal descent rate, indicating neutral buoyancy (Coombs 1981). In addition, the larvae also soon became curved and white in colour as they approached denser salt concentrations, indicating strong osmotic activity due to the difference in salt concentration between the larvae internal fluid and the column media. This condition was not seen with fish bigger than 15 mm in total length.

Because of this, Glycerol was selected to produce the required gradient densities, mixed with seawater of one salinity 19 ppt. The choice of 19 ppt seawater was to reduce any possible osmotic activity that could interfere with the larval or fry descent rate. The density of Glycerol is 1.2613 g/ml and it is miscible with seawater, this made it possible to prepare different sets of density gradient columns with different density ranges. By mixing different proportions of Glycerol with 19 and 40 ppt seawater to make the stock solutions A & B (Coombs, 1981), it was possible to cover a density range of 1.0207 to 1.1137 g/ml. This range was sufficient to cover all possible measurements of larval and fry density, with or without a swimbladder. Table 4.1 shows, in detail, all the density gradient columns prepared, stock solutions A & B and the size and type of fish they were used with. The column was calibrated with the aid of sixteen, 6 mm diameter, colour-coded glass spheres (Martin Instrument Co.) of known density (1.0207 - 1.1137 g/ml) as marker floats.

The temperature of the density gradient column was kept constant at 23°C by circulating the water of the required temperature in the a jacket around the density gradient column tube (see Coombs, 1981). Before each measurement, the heights of the calibration glass-floats were checked and compared with the calibration curve for linearity and only after securing

linearity, were fish samples introduced into the column to be measured. A density gradient column made up from Glycerol and 19 ppt seawater could be used for several days, maintaining a stable density gradient.

The larvae and fry used for measurement were reared in a 1000 litre fiberglass tank according to procedures mentioned in Chapter 2. A daily sample consisting of 6 - 9 larvae was selected at random during the first five days of age. After that the samples were taken on Day 7, 10, 14, 18, 22, 30, 40, 50, 60 and 70. Each sample was divided into 3 subsamples of 2 to 3 fish and each was measured separately. The fish were lightly anaesthesized with MS-222 for measuring total length and swimbladder volume under the dissecting microscope. The measured fish were picked up and introduced individually and very gently into the density gradient column, 30

Table 4.1: Data on the Preparation of the Density Gradient Column Solutions for Measurement of the density of *A. Cuvieri* larvae and fry of different sizes, with and without swimbladder.

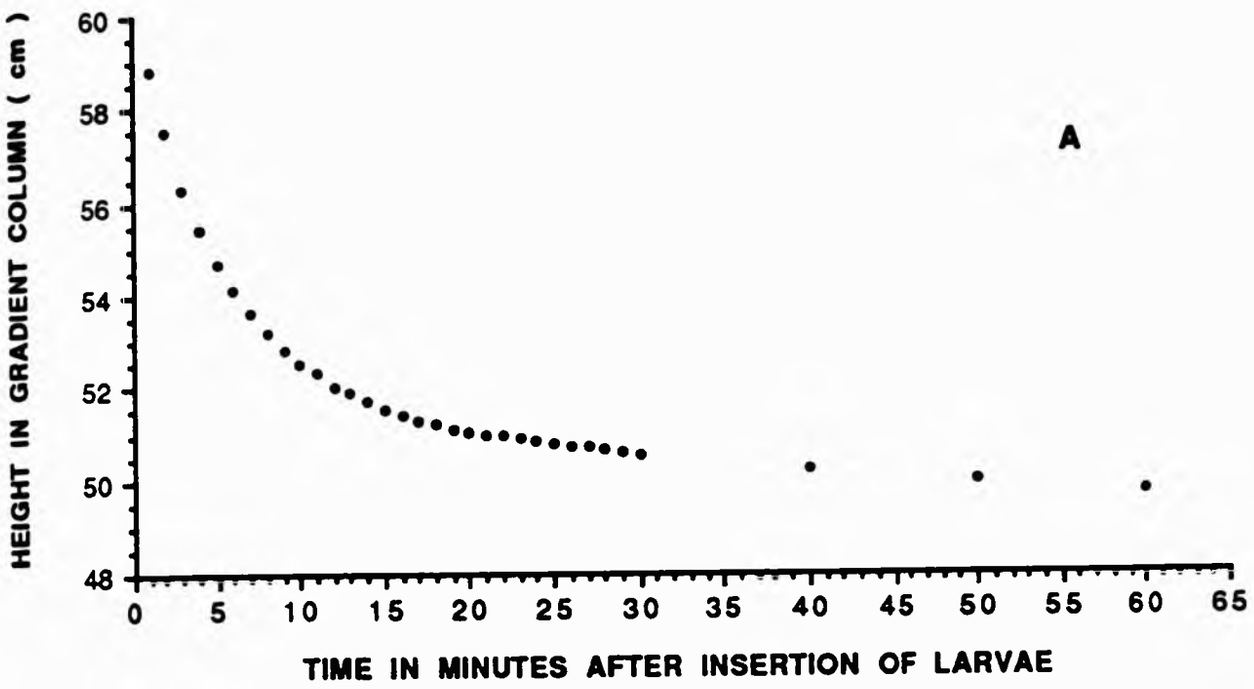
Size range T.L. (mm) Status of Swimbladder	Solution A (Low Density) Glycerol (%)	Seawater (%)	Solution B (High Density) Glycerol (%)	Seawater (%)	Range of density gradient column obtained (g/cc)
1.87 - 4.5 with and without swimbladder	2.3	97.7 19 ppt.	17.6	82.4 19 ppt.	1.0207 - 1.0399
4.8 - 35.0 only with swimbladder	4.7	95.3 19 ppt.	22.7	77.3 19 ppt.	1.0262 - 1.0451
10.3 - 26.64 only without swimbladder	0.0	100.0 40 ppt.	28.6	71.4 40 ppt.	1.0339 - 1.0707
27.8 - 34.7 only without swimbladder	0.0	100.0 40 ppt.	58.3	41.7 40 ppt.	1.0451 - 1.1137

seconds apart. The larvae were usually picked up by a small pipette using 19 ppt seawater was introduced with the larvae into the column. The column contained about 40 ppm MS-222 which was mixed with the density gradient elements, i.e., solutions A and B. The addition of the anesthetic was necessary to prevent any swimming or jerking activities which may disturb the gradient. Individual descent rate was assessed by recording the position in the column every minute for each fish for the first 30 minutes and then every 10 minutes, for another 30 minutes. Between 6 - 9 individual descent rates were recorded for each sample. The column height readings were always taken at the tip of the fish head which was pointing downward as the larva was sinking.

The descent rate of each fish was plotted on graph paper and a straight line was drawn through the final points of constant minimal descents (Figure 4.1A). This line was then extended to cross the y-axis (height of fish in the column) to give the height at which neutral buoyancy was attained. This height was converted to a density value from the calibration curve made for the column, as shown in Figure 4.1B.

C. Vertical Movement of Larvae

Observations of vertical movements of larvae were carried out in two tanks constructed from transparent acrylic sheet. The tank dimensions were 200 X 30 X 15 cm and black horizontal lines were marked every 20 cm of its height. The two tanks were placed in a room (250 x 150 x 100 cm) made from thick, opaque, PVC-lining walls with a transparent top made from clear plastic sheet. Indirect sunlight coming through the fiberglass roofing of the building was the only source of illumination used in this study. The light intensities were measured at a height of 0, 100 and 200 cm of the



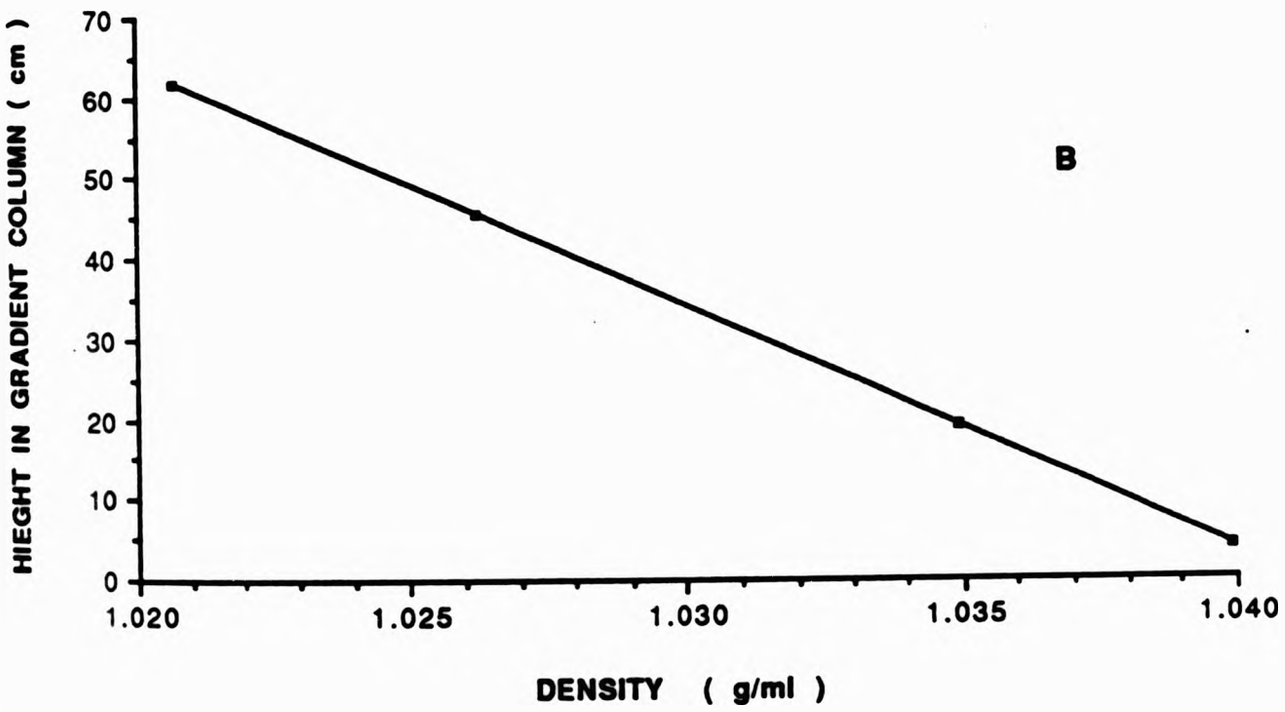


Figure (4.1b) A: A plot of larval descent rate against height in the density gradient column. A straight line is drawn through the points of constant minimal descent which indicate neutral bouyancy. B: A calibration curve showing linearity of the gradient through the use of marker floats of known density. The height at which neutral bouyancy was indicated in graph A is converted to a density value using graph B.

column and are shown in Table 4.2. The larvae used in the observation tanks were reared in two 1000 litre fiberglass tanks according to the procedures description given in Chapter 2.

Table (4.2) The light intensities of the diffused natural light measured during the vertical movement study of *A. cuvieri* larvae in a 200cm water column.

Time of measurement (24hr)	Light intensities (lux) at different height of the column		
	0cm (bottom)	100cm (middle)	200cm (top)
8.15	700	2500	7000
12.00	1800	3800	8100
15.30*	300	680	1650

* sunset is at 17.00

The observation tanks were evacuated and restocked daily with larvae transferred from the rearing tanks as the age of larvae progressed. Temperature, salinity and dissolved oxygen were kept constant both in the observation and rearing tanks. The density of the larvae in the observation tanks was about 10 per liter, a total of about 900 - 1000 larvae per tank.

For a period of two weeks, daily observations were carried out in the morning (9-10 hrs) two hours after restocking and in the evening before and after sunset (15-22 hrs). Each observation included recording the position of larvae in the water column, their orientation, apparent buoyancy (sinking or floating), aggregation patterns and swimming activity in addition to total length.

D. Buoyancy Budget Sheet

A buoyancy budget sheet was prepared for two groups of fish, with and without swimbladder. The sheet was programmed using Lotus 1- 2-3. A set of total lengths were selected to cover the first 70 - 80 days of rearing ranging from 1.87 to 35 mm as follows: 1.87, 2.73, 3.00, 3.20, 3.34, 3.37, 3.40, 3.50, 3.60, 3.70, 4.00, 4.31, 4.83, 6.15, 6.85, 10.00, 15.00, 20.00, 25.00, 30.00 and 35.00. For each of these selected total body length, a series of measurements were complied as follows:

(1) Wet fish weight (g)

(2) Yolk sac volume (ml)

(3) Oil globule volume (ml)

(4) Swimbladder volume (ml)

(5) Fish density (g/ml)

For each size of fish these measurements were obtained from the calculated regression equations with the exception of (2) and (3) where the mean value of yolk sac and oil globule volume were used. The use of these measurements made it possible to subsequently calculate the following:

(6) Total volume of fish (ml) = (1)/(5) [1]

(7) Volume of body tissue (ml) = (6) - [(2)+(3)+(4)] [2]

(8) Weight of oil globule (g) = (3) x 0.90 (g/ml) [3]

Oil globule density is not directly available and so the value of 0.90 g/ml has been assumed, following Craik and Harvey (1987).

Yolk is a multiplicity of different components mainly; water, inorganic salts, protein and lipid. The density of yolk of a particular species depends on the present ratio of these components. Since there are no available values for the density of the yolk of pelagic marine larvae, it is assumed that the values given by Craik and Harvey (1987) for the composition (% of wet weight) of a typical pelagic egg without oil globule, and the yolk density assumed for it, would roughly represent the situation in a just-hatched *A. cuvieri* yolk sac larvae in which over 80% of their body volume is yolk. The figures given by Craik and Harvey (1987) for the composition (% of wet weight) of a typical pelagic egg without oil globule are, 92% water, 3% amino acids, 0.3% inorganic salts and 1.1% lipid. The aqueous and inorganic salts components will have a density of about 1.016 g/ml. This value is corroborated by Riis-Vestergaard's (1982) work on plaice and halibut eggs which he showed to have an osmolarity of about 300- 400 m-osmol, corresponding to a salinity of 10-13 ppt and a density of 1.0146-1.0183 g/ml at 25°C. Assuming densities of 0.90 g/ml for the lipid and 1.05 g/ml for the amino acids (this figure was given by Lowndes, 1955,

and Alexander, 1959a, as the density for fish muscles, both were quoted by Alexander, 1972), then the assumed net yolk density would be about 1.016 g/ml. Hence, the yolk sac weight can be derived;

(9) Weight of yolk sac (g) = (2) x 1.016 g/ml [4]

(10) Weight of body tissue (g) = (1) - [(8) + (9)] [5]

$$\text{(11) Re-calculated density (g/ml)} = \frac{(10) + (9) + (8)}{(7) + (2) + (3) + (4)} \quad [6]$$

(12) Swimbladder volume (%) = (4)/(6) x 100 [7]

Finally, assuming a seawater density of 1.02635 g/ml, which was obtained from standard tables for seawater of 39 ppt at 25°C.

(13) Sinking factor ($\leq$ or $>$ 1) = (5)/1.02635 g/ml [8]

III. RESULTS

A. Morphological measurements

A.1. Total body length, body wet weight and age relationship

The relationships between total body length, body wet weight and age of the Blue-Finned Sea Bream larvae were plotted and are shown in Figure 4.2 A and B. The apparent growth of the larvae in terms of body length was fast during the first day after hatching, attaining a total body length of 3.0 mm which is equivalent to an increase of 60.4% of its original length (see Figure 4.2A). From day 2 to day 5 after hatching a semi-static phase ensued, in which larval body length remained more or less stable. Active feeding was established on Day 4 and gradual increase in total body length was then observed after Day 6. Change in larval body weight was quite

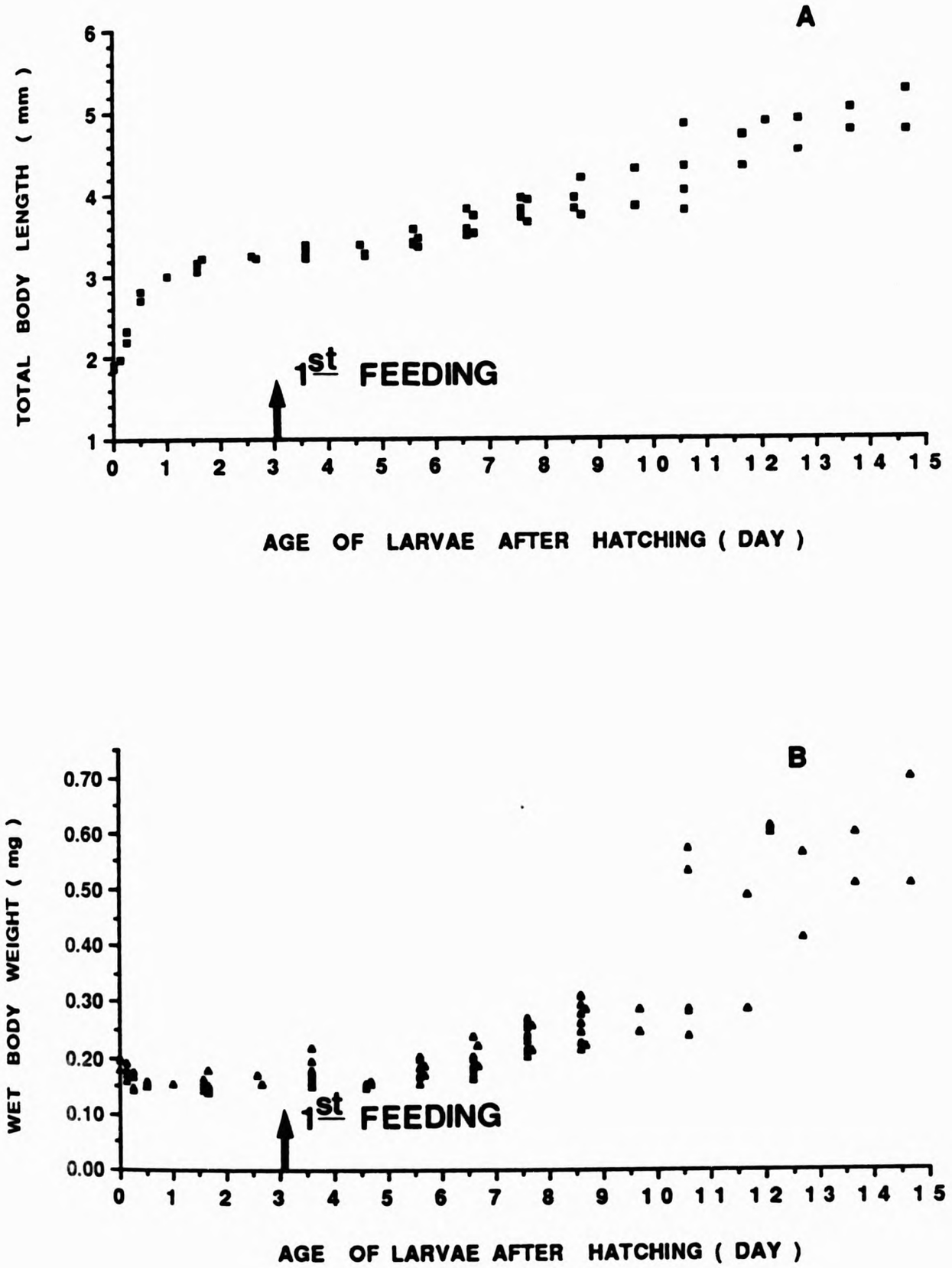


Figure (4.2) The relationship between age in days and total body length [A] and wet body weight [B] of *A. cuvieri* larvae.

different with a drastic fall being noted from 204 ug at hatching to 133 ug 17 hours after hatching. This fall in body weight is due to the fast consumption of yolk reserves. A static phase in larval body weight was observed to occur between 1 to 4.5 days after hatching. A gradual increase in larval body weight was observed to start after Day 5.

From these observations, it seems that there is a very rapid growth phase within the first day after hatching. There follows a relatively stable phase in which total body length and body weight do not change greatly. During this time yolk reserves are being used to construct body tissue. After external feeding became well established additional growth, both of body length and weight, was observed.

The relationship between larval total body length and wet body weight is shown in Figure 4.3A and can be summarized as follows:

$$Y = 0.82636 - 0.50380X + 0.091501X^2 \qquad [9]$$

$$R^2 = 0.988$$

where Y = wet body weight (mg)

X = total body length ($X \leq 6.8$ mm)

The relationship between total body length and wet body weight for metamorphosed larvae and fry is shown in Figure 4.3 B and can be summarized as follows,

$$Y = -0.096557 + 0.01657X - 0.00094466X^2 + 0.000028295X^3 \qquad [10]$$

$$R^2 = 0.965$$

where Y = wet body weight (mg)

X = total body length ($X \leq 40.0$ mm)

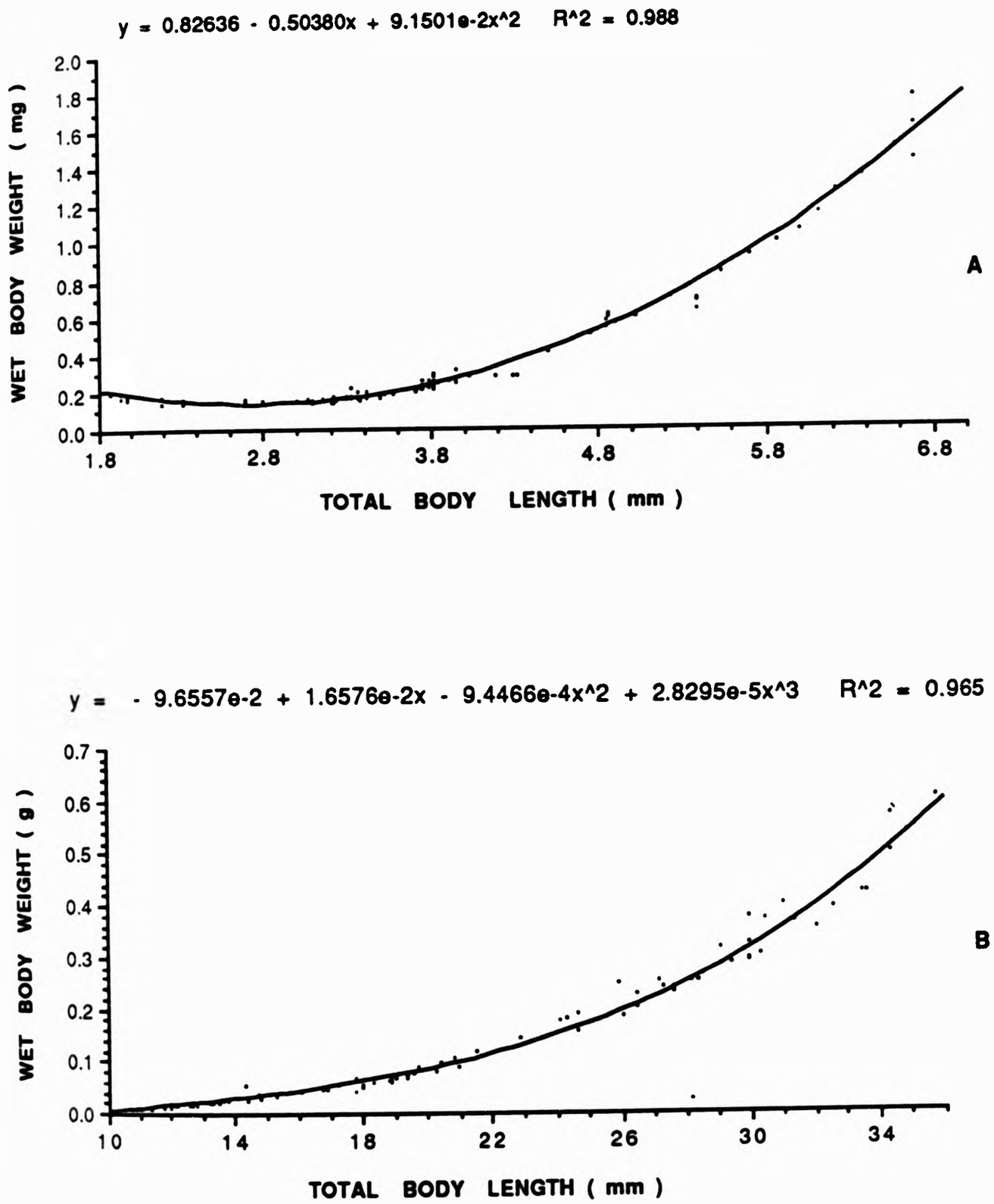


Figure (4.3) The relationship between total body length and wet body weight for *A. cuvieri* larvae [A] and metamorphosed larvae and fry [B].

A.2. Oil Globule and Yolk Sac Volume

A. cuvieri larvae possess a single oil globule, which has a spherical shape, located within the yolk sac at the most posterior end. On hatching, the oil globule volume ranges from 3.75 - 5.60 nl with an average of 4.21 ± 0.46 nl. The yolk sac has an oval shape with a volume range of 114.2 - 193.2 nl with an average of 163.5 ± 19.0 nl. The oil globule and yolk sac volume were plotted against larval total body length and are shown in Figures 4.4A and 4.4B respectively. Rapid yolk resorption was observed to occur up to a total body length of 3.0 mm (24 hour after hatching) by which time about 87.1% of the yolk reserve was consumed. This rapid and massive disappearance of yolk was clearly reflected in the steep initial growth phase of the total body length and in the sharp drop in wet body weight of the larvae. Complete yolk resorption was observed to occur at a total body length of about 3.33 mm (65 hours after hatching) (see Table 4.4). Oil globule resorption was observed to be very slow during the first 17 hours after hatching up to a larval total body length of 2.85 mm. This was followed by a gradual resorption and the oil globule disappeared completely at a total body length of about 3.6 mm (160 hours after hatching).

A.3. Swimbladder Volume

The swimbladder volume of *A. cuvieri* larvae increased exponentially with the total body length after Day 4, at which time initial inflation took place. This exponential relationship is shown in Figure 4.5A and is given by:

$$\text{Swimbladder volume (S.B.V) in nl} = 0.00197L^{4.64} \qquad [11]$$

$$R^2 = 0.96$$

Where L = Total body length ($L \leq 9.0$ mm)

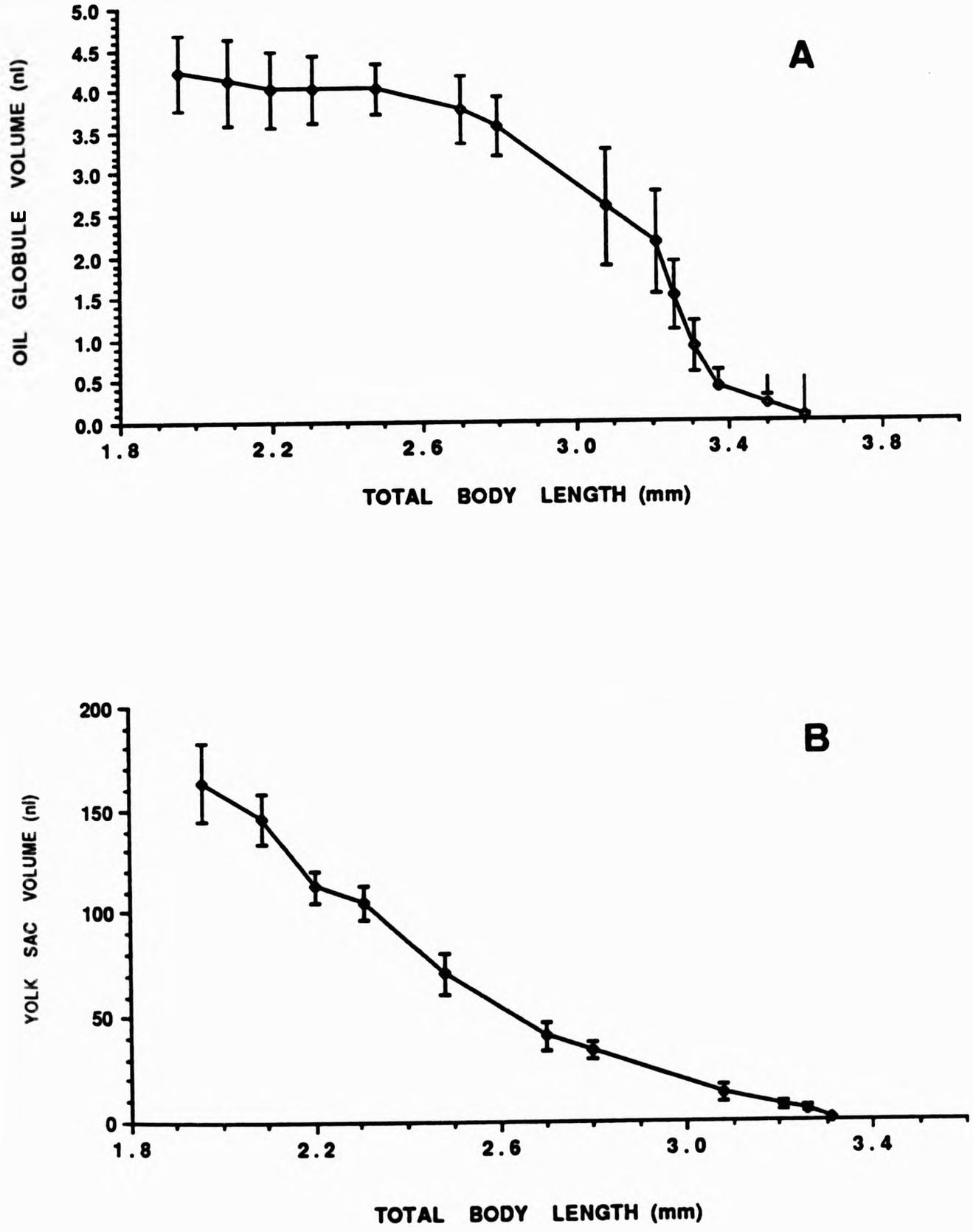
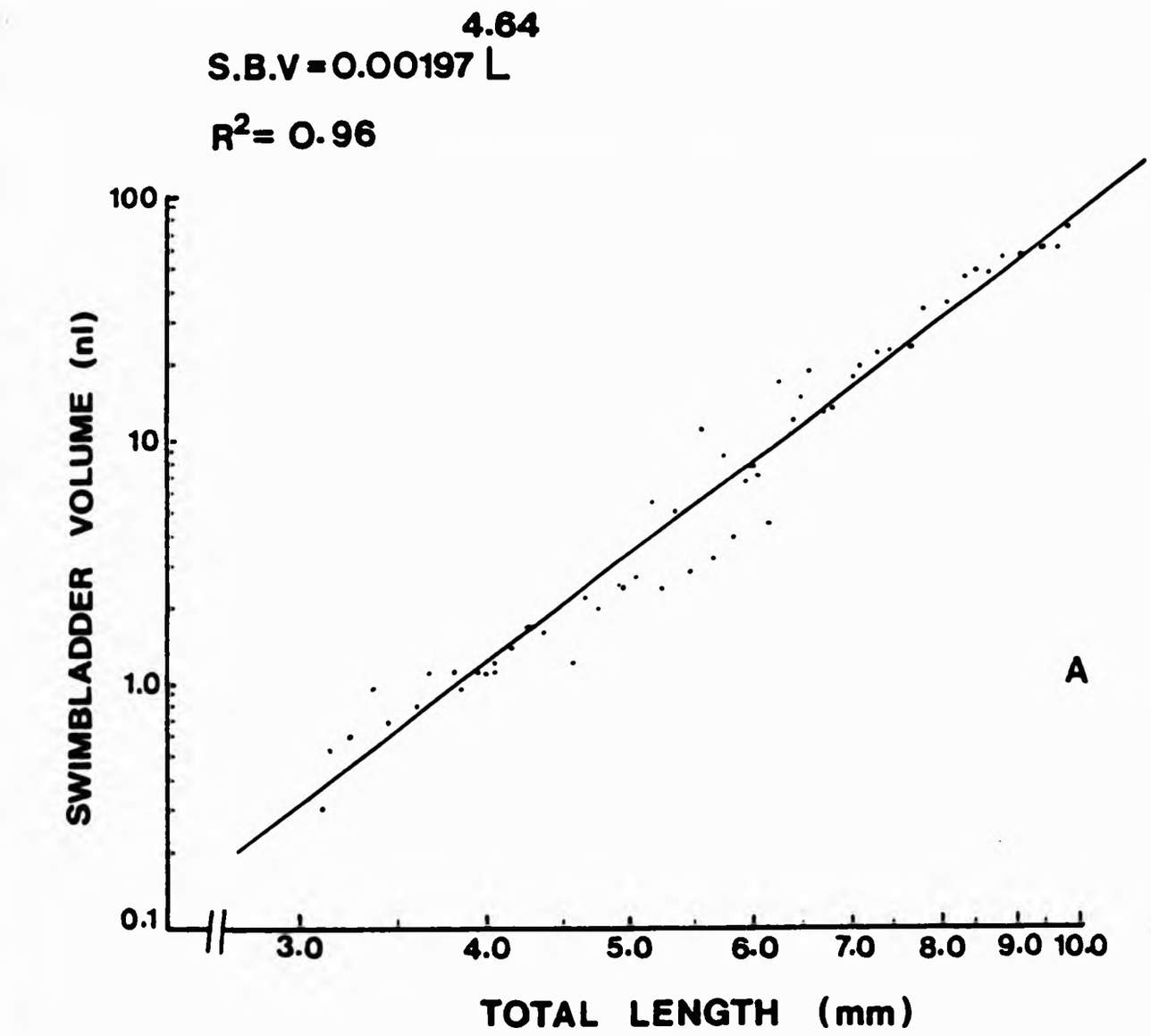


Figure (4.4) Oil globule volume [A] and yolk sac volume [B] versus total body length of *A. cuvieri* larvae. Vertyical bars represent ±1 standard deviation.



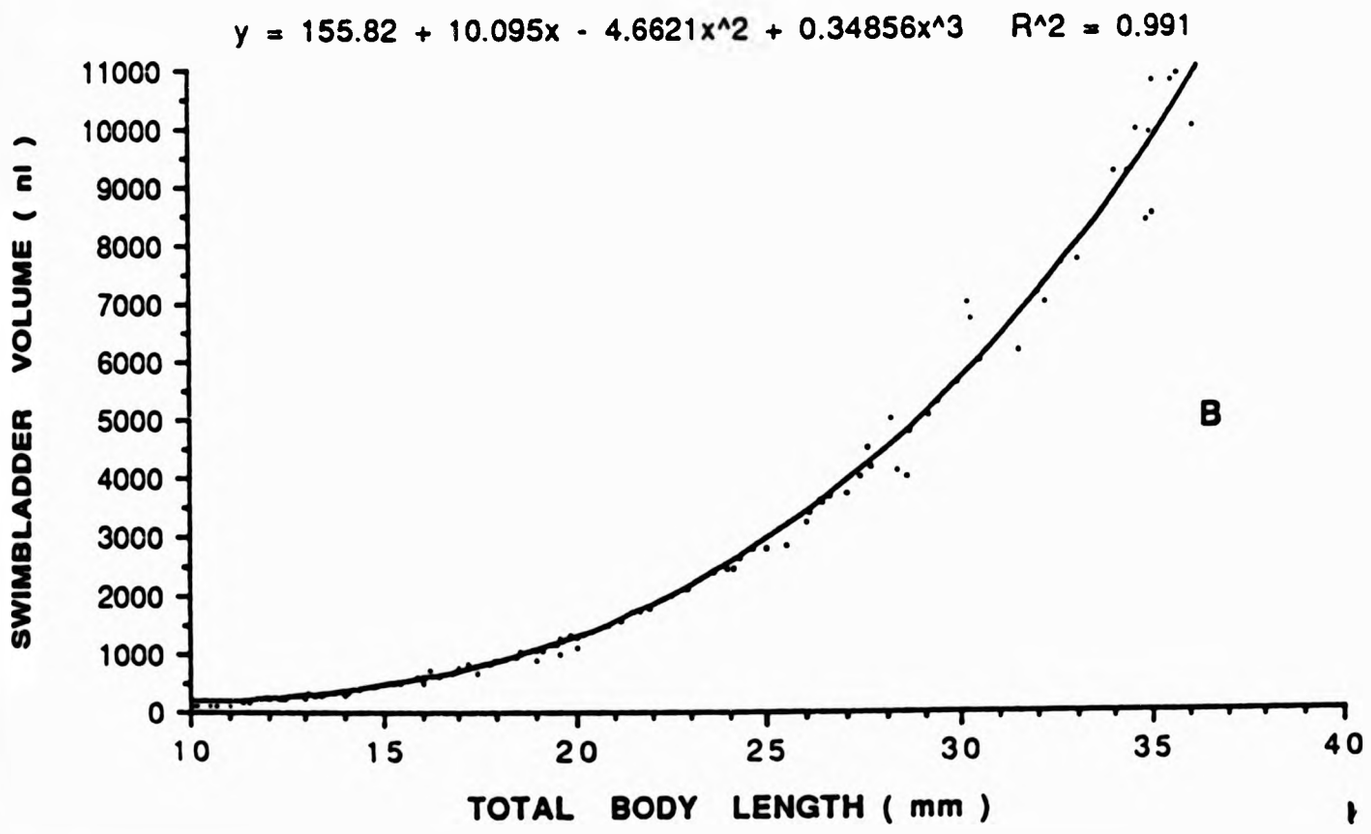


Figure (4.5) The relationship between the swimbladder volume and the total body length of *A. cuvieri* larvae [A] and metamorphosed larvae and fry [B].

The swimbladder volume and total body length relationship for larger, metamorphosed, larvae and fry is shown in Figure 4.5B which shows a steeper slope in the bigger fry (25 - 40 mm). This relationship can be summarized by the equation:

$$Y = 155.82 + 10.095X - 4.6621X^2 + 0.34856X^3 \quad [12]$$

$$R^2 = 0.991$$

Where Y = Swimbladder volume (nl)

X = Total body length ($X \leq 40$ mm)

The first shape of the swimbladder upon initial inflation was of a sphere, increasing in size and gradually elongating as the larvae grew and assuming the shape of a prolate spheroid. There was no clear distinction of when this transformation of swimbladder shape took place, but it can be seen from Figure 4.6 that no swimbladders with a spherical shape were observed above a total body length of 6.3 mm, while swimbladders with a prolate spheroid shape were observed to appear above a total body length of 4.0 mm. It is possible that the two different swimbladder shapes are indicative of the two different inflation mechanisms used by the larvae. The initial atmospheric air gulp used to first inflate the swimbladder, results in the sphere shape and the later internal filling by the gas gland and the rete mirabile, results in the final prolate spheroid.

The relationship between the two swimbladder shapes and the total body length of the fish can be expressed by the following;

For swimbladders of spherical shape:

$$Y = -2.8218 + 0.99285X \quad [13]$$

$$R^2 = 0.622$$

where Y = swimbladder volume (nl)

X = Total body length ($X \leq 6.3$ mm)

y = - 2.8218 + 0.99285x R^2 = 0.622 • ***SPHERE***

y = - 19.591 + 15.412x - 3.9429x^2 + 0.35220x^3 R^2 = 0.955 · ***PROLATE***

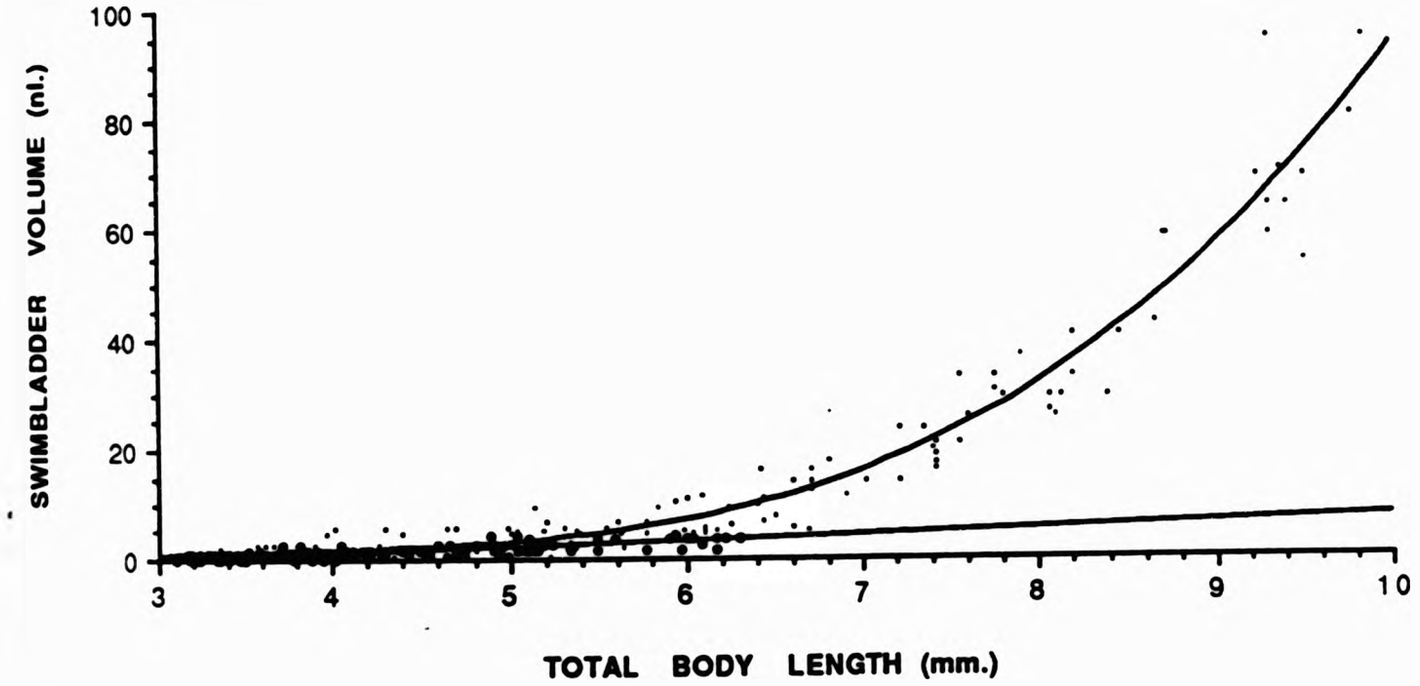


Figure (4.6) The relationship between total body length of larvae and their swimbladder volume of sphere and prolate spheroid.

For swimbladders of prolate spheroid shape:

$$Y = -19.591 + 15.412X - 3.9429X^2 + 0.35220X^3 \qquad [14]$$

$$R^2 = 0.955$$

where Y = swimbladder volume (nl)

X = Total body length ($X \leq 10.0$ mm)

The relationship between swimbladder volume (nl) and wet body weight (mg) of both larvae and fry was obtained by calculation using equations [9], [10], [11] and [12]. The relationship is shown in Figure 4.7.

The relationship between swimbladder volume as a percent of the fish total volume (%) and total body length of fish (mm) was also obtained by calculation, using equations [11] and [12] to obtain swimbladder volume for a set of selected total body lengths (see Figure 4.8). The calculated swimbladder volumes were then divided by the total body volumes of the fish and multiplied by 100. .The total body volume of the fish at those selected total body lengths were calculated according to the equation [1] adjusting for the units used. The calculated percentage swimbladder volume of the fish total body volume were plotted against the selected set of total body lengths of the fish as shown in Figure 4.8. This relationship can be summarized by the following equation:

$$Y = -0.60128 + 1.7462 * \text{Log } X \qquad [15]$$

$$R^2 = 0.982$$

Where Y = Swimbladder volume percent of the fish total body volume (%)

X = Total body length of the fish ($X \leq 35$ mm)

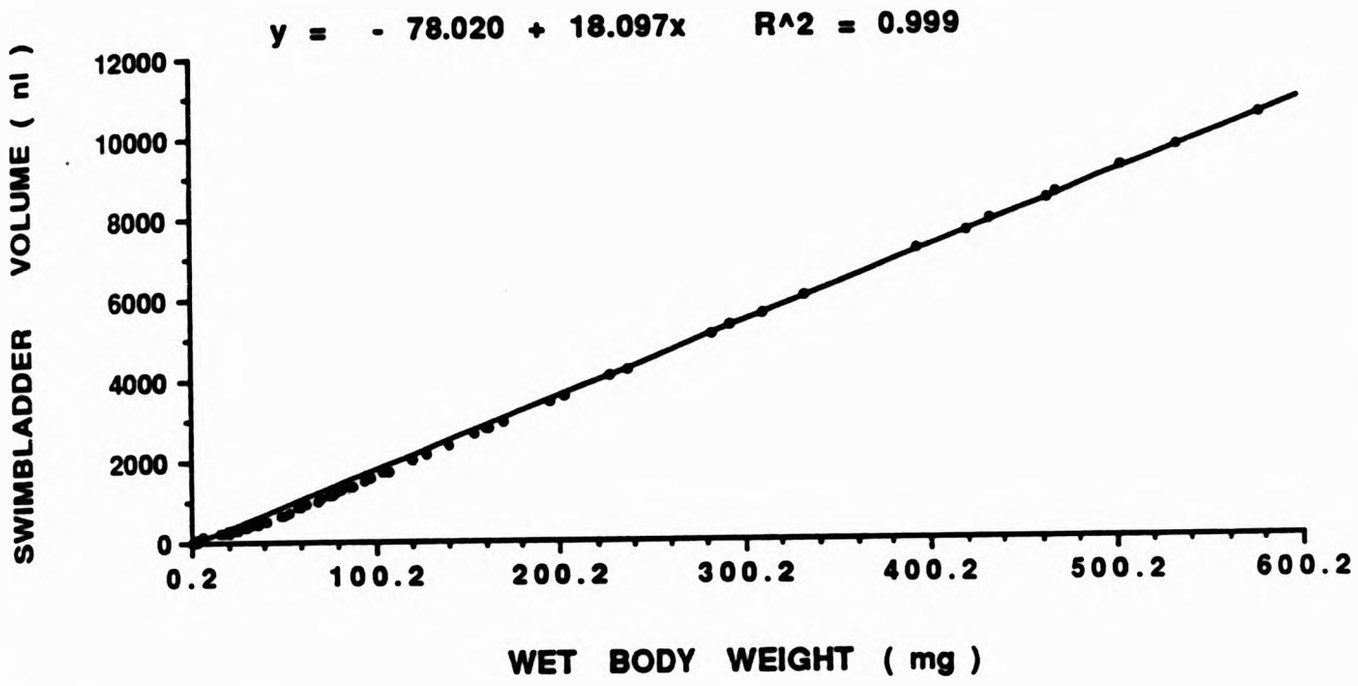


Figure (4.7) The relationship between fish wet body weight and swimbladder volume, based on calculated data.

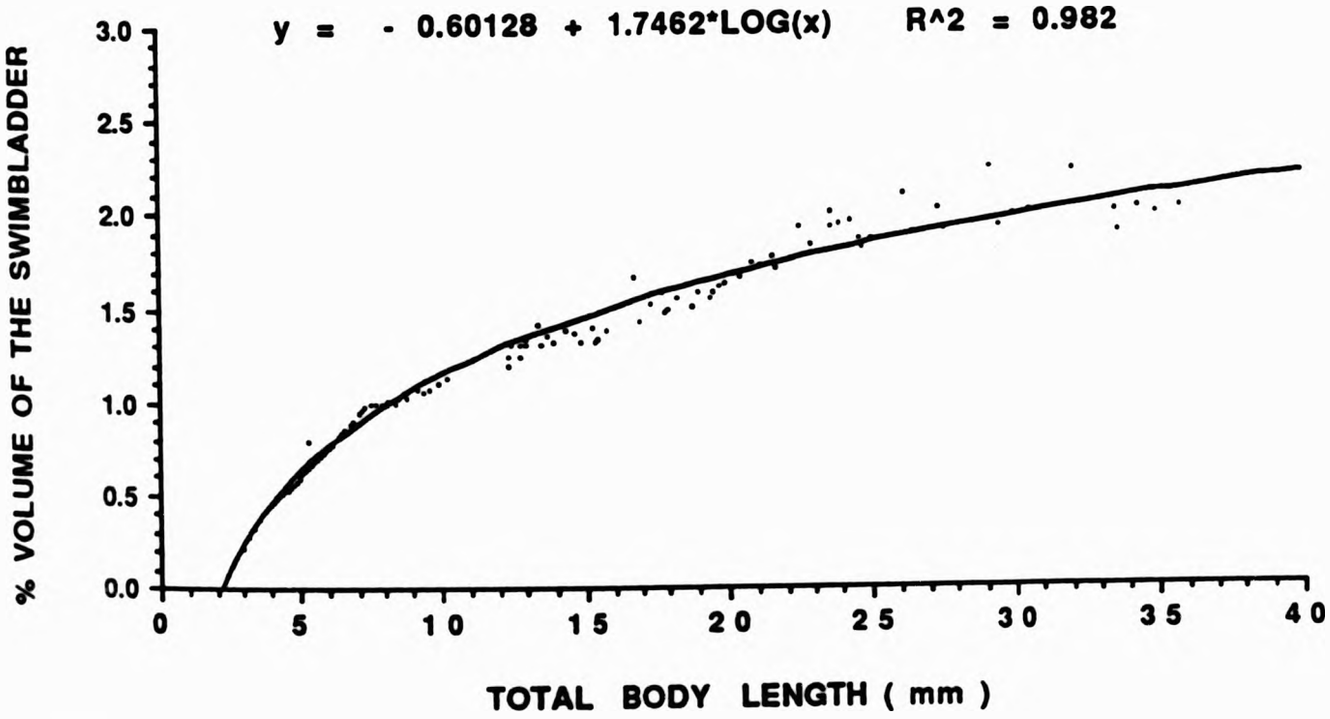


Figure (4.8) The relationship between the fish total body length and the swimbladder volume divided by the total fish body volume in percent, based on calculated data.

B. Density

The densities of larvae and fry with and without functional swimbladders are shown in Figure 4.9. The densities of the fish with functional swimbladders are represented by the curve B in Figure 4.9 which shows a steep increase in larval density from 1.0232 to 1.0340 g/ml during the first two weeks of age (from 3.37 to 6.00 mm in total body length). After this rapid increase, larval density settled at around 1.0320 g/ml. The densities of the fish without functional swimbladders are represented by curve A in Figure 4.9, and this shows a steeper increase than curve B. A plateau appears to be reached in curve A around the value of 1.0750 g/ml, corresponding to a total body length 25.00 to 35.00 mm. The relationship between fish densities and total body length can be expressed by the following regression curves:

For fish with functional swimbladder, the equation is:

$$Y = 1.0202 + 0.011377 * \text{Log } X \qquad [16]$$

$$R^2 = 0.815$$

For fish without a functional swimbladder, the equation is:

$$Y = 1.0026 + 0.047358 * \text{Log } X \qquad [17]$$

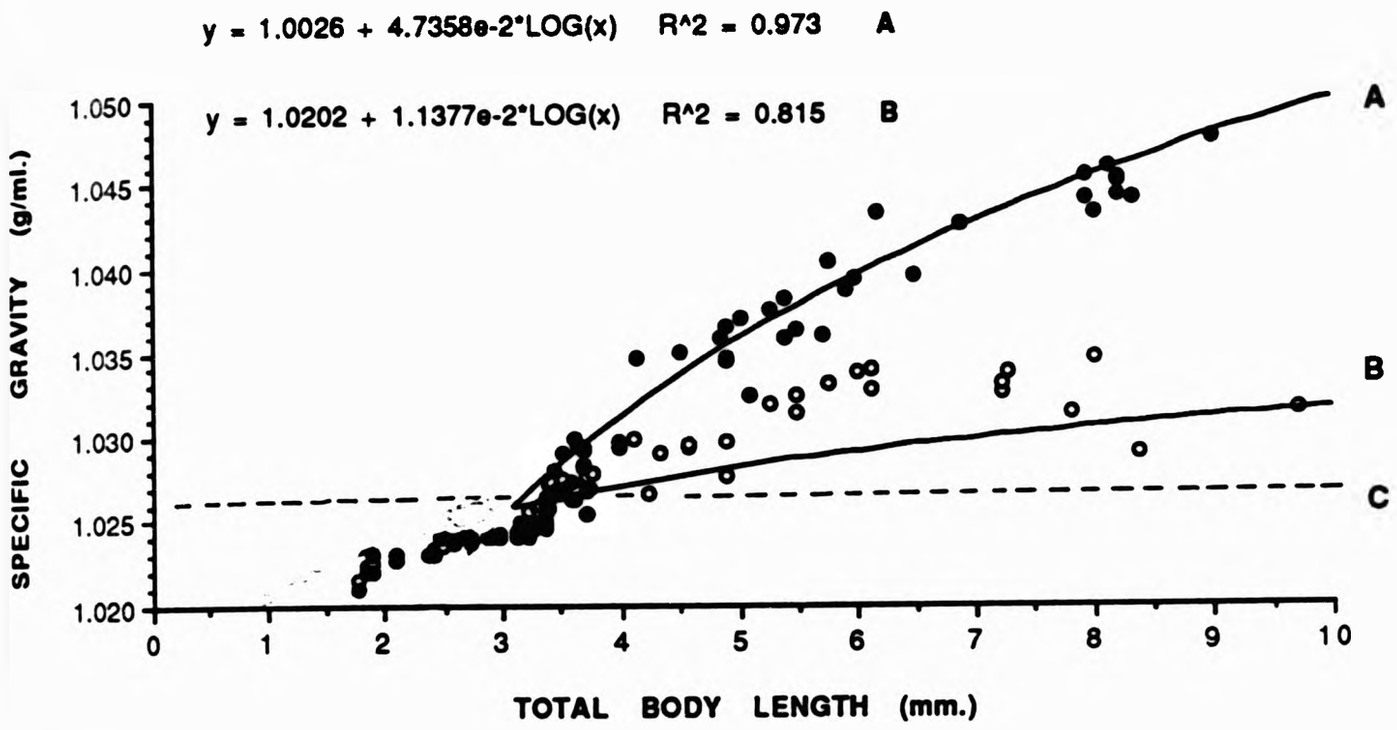
$$R^2 = 0.973$$

where Y = fish density (g/ml)

X = total body length of fish ($X \leq 35.00$ mm)

These regression equations do not truly predict the early part of the data.

The densities of fishes in Curve A and B in Figure 4.9 appear



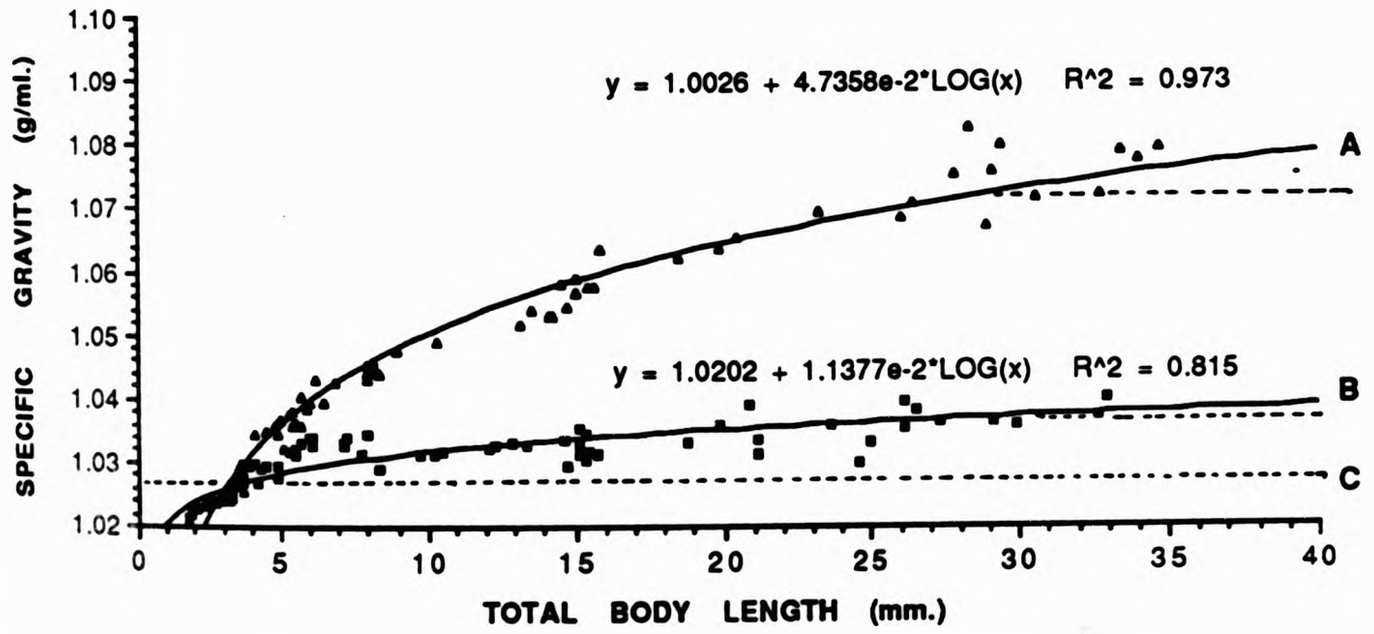


Figure (4.9). The body density of *A. Cuvieri* larvae and fry with functional swimbladder [B] and without [A] one. [C] is the density of rearing sea water (38-40 ppt at 25°C) of 1.0256-1.0271 g/ml. The upper figure is a magnification of first section of the lower figure.

to be different and this was confirmed using Student's t-test which was carried out on selected sections of the two curves as shown in Table 4.3. The test showed that there is no statistical difference between densities of fish with or without functional swimbladders at total body length of 3.4 to 3.8 mm. While a statistical difference ($P<0.01$) was observed to occur between fish densities for total body length of 4.0 mm to 35.00 mm of both Curves A and B, (see Table 4.3).

Table 4.3: T - Test results for the different selected sections of the two regression curves A and B from Figure 4.9.

Selected sections in terms of fish total body length (mm)	t-value	P-value
3.4 to 3.8	0.50	0.62 N.S.
4.0 to 6.6	7.23	< 0.01*
10.0 to 12.0	14.82	< 0.01*
20.0 to 24.0	30.83	< 0.01*
30.0 to 34.0	26.83	< 0.01*

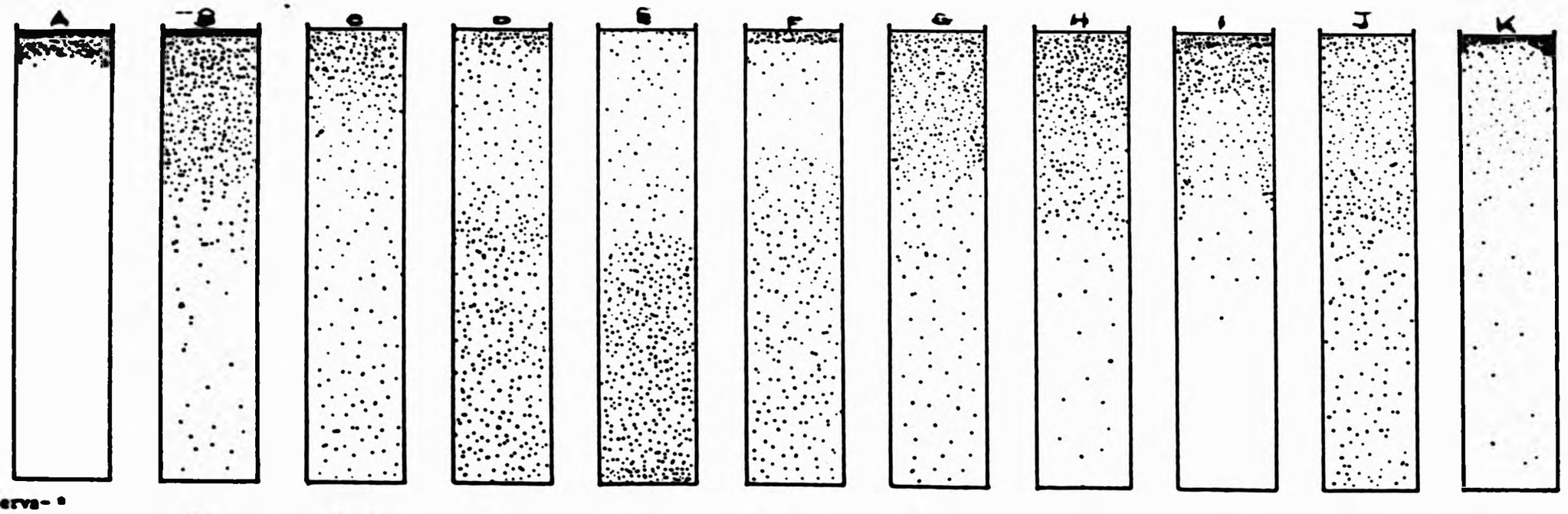
* = significantly different; N.S. = Not significant

The density of the rearing water (38-40 ppt at 25°C) would be about 1.0256 - 1.0271 g/ml (Cox, 1965) and this is represented in Figure 4.9 by the line C. The density of the rearing water is slightly less than that of the fish with a functional swimbladder making them very slightly negatively buoyant. The densities of the fish without functional swimbladders are, however, very much higher than that of the rearing water and this will clearly cause rapid sinking. Hence, these fish need to spend more energy in staying in the water column. An interesting inference may be

drawn from curve B in Figure 4.9 which effectively becomes horizontal. This suggests that the fish are adjusting their swimbladder volume and hence the uplifting force as they add more dense materials to their bodies, such as skeletal and muscular tissues. This phenomenon cannot be seen in curve A due to the absence of the compensating swimbladder, and the apparent plateau at 1.075 g/ml may represent the end of the ossification process and scale development.

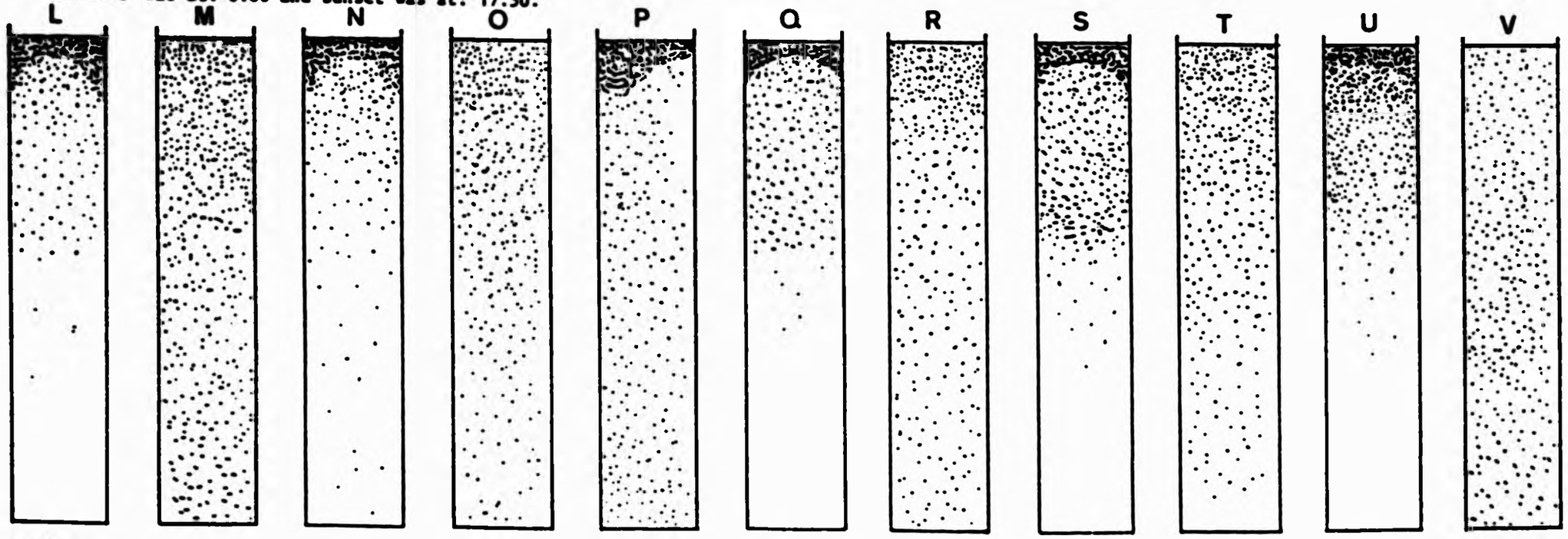
C. Vertical movement of larvae

The observations of the vertical migration pattern of *A. cuvieri* larvae are summarized in Figure 4.10 with additional details given in Table 4.4. Just-hatched larvae are positively buoyant in 38-40 ppt at 24-25°C, floating with their yolk sacs facing upward and their tail obliquely pointing downward (Figure 4.10A). Within a few hours, the newly hatched larvae begin to swim downward against their positive buoyancy force which pulls them upward as soon as they stop wriggling in the downward direction (Figure 4.10 B-D). The majority of the larvae managed to reach to the bottom of the observation tank forming dense patches at the approximate age of 29 hours after hatching (see Figure 4.10E). Some of the weak and dying larvae will stay near the water surface moving around sluggishly in a horizontal direction. On about the second day (Figure 4.10 F-G) an upward movement of some of the larvae resulted in an aggregation near the water surface. By the morning of Day 3 (Figure 4.10H) most of the larvae were near the water surface. It was observed that the ascending larvae on Days 2 and 3 had slightly negative buoyancy which was noticed when the larvae stopped wriggling upward and appeared to sink very slowly with their heads pointing downward. This sinking could be the trigger for the upward movements of the larvae on Days 2 and 3.



	A	B	C	D	E	F	G	H	I	J	K
Time Observation(24 hrs)*	6.00	10.00	17.30	22.00	10.00	18.00	21.00	9.00	15.00	22.00	10.00
Age (hrs)	0.00	4.00	12.00	17.00	29.00	37.00	40.00	52.00	58.00	65.00	77.00
Total length (mm)	1.87	2.07	2.70	2.85	3.10	3.16	3.20	3.28	3.32	3.33	3.35

*Sunrise was at: 6.00 and Sunset was at: 17.50.



	L	M	N	O	P	Q	R	S	T	U	V
Time Observation (24 hrs)*	16.00	21.00	10.00	21.00	9.00	15.00	20.00	14.00	21.00	A.M.	P.M.
Age (hrs)	83.00	88.00	101.00	112.00	124.00	130.00	135.00	153.00	160.00	7-15 days	7-15 days
Total length (mm)	3.37	3.37	3.38	3.39	3.40	3.42	3.44	3.50	3.55	4.01-5.51	4.01-5.51

Figure (4.10). Diel migration pattern of Acanthopagrus cuvieri larvae of different ages (0-15 days) in 2 meters seawater column (38-40 ppt and 24-25°C). More details on each age are given in Table (4.3).

Table (4.4) A collective summary of morphological, biological and behavioral events observed on the A. cuvieri larvae during the diel migration study shown in figure (4.10).

AGE (hours)	AGE (days)	B.T.LEN (mm)	B.WEIGHT (ug)	YOLK VOL (nl)	OIL VOL (nl)	SWB VOL (nl)	SWB INFLA (%)	DENSITY (g/ml)	GENERAL OBSERVATION
0	0.00	1.87	204.2	163.5	4.21	0	0	1.0233	Just hatched with upside-down position where the tail obliquely pointing downward.
4	0.17	2.07	175.6	145.8	4.10	0	0	1.0238	Wriggling downward with free lift at the end of each wriggle.
12	0.50	2.70	133.1	40.6	3.75	0	0	1.0251	The larvae are still wriggling downward.
17	0.71	2.85	133.7	33.7	3.50	0	0	1.0254	The weak larvae are staying near the water surface with sluggish movements.
24	1.00	3.00	138.5	21.1	3.00	0	0	1.0256	Appearance of pectoral fin buds and the start of eye pigmentation.
29	1.21	3.10	143.9	13.1	2.57	0	0	1.0258	The larvae densely aggregated near the bottom of the observation tank.
37	1.46	3.16	148.0	9.7	2.36	0	0	1.0259	The larvae start to wriggled upward and to aggregated densely near the water surface.
40	1.67	3.20	151.2	7.5	2.15	0	0	1.0259	
52	2.17	3.28	158.3	5.4	1.51	0	0	1.0261	Twisted gut and the beginning of mouth formation.
58	2.42	3.32	162.3	0.7	0.89	0	0	1.0261	The end of yolk resorption.
65	2.71	3.33	163.4	0	0.80	0	0	1.0261	The appearance of the opercula.
77	3.21	3.35	165.5	0	0.52	0	0	1.0262	Longer swimming period with completely pigmented eye and the opening of the mouth and anus.
83	3.46	3.37	167.7	0	0.48	0.55	5	1.0262	Phototaxis behaviour,the establishment of external feeding and initial swimbladder inflation
88	3.67	3.37	167.7	0	0.46	0.55	6	1.0262	
101	4.21	3.38	168.9	0	0.43	0.56	7	1.0262	Dense patches near the water surface during the day hours with the larvae showing vertical attaching behaviour.
112	4.67	3.39	170.0	0	0.40	0.57	10	1.0262	Even distribution throughout the tank at night with a vertical position of the larvae pointing their head downward.
124	5.17	3.40	171.2	0	0.33	0.58	16	1.0262	
130	5.42	3.42	173.6	0	0.27	0.59	20	1.0263	
135	5.63	3.44	176.1	0	0.23	0.61	22	1.0263	
153	6.38	3.50	183.9	0	0.20	0.66	27	1.0264	
160	6.67	3.55	191.0	0	0.10	0.70	35	1.0265	The end of oil globule resorption.
177	7.38	3.76	225.7	0	0	0.92	39	1.0267	
230	9.58	4.10	298.9	0	0	1.37	40	1.0272	
245	10.21	4.20	324.5	0	0	1.54	55	1.0273	
280	11.67	4.46	399.5	0	0	2.03	50	1.0276	
333	13.88	4.90	554.7	0	0	3.14	55	1.0281	
368	15.33	5.20	680.8	0	0	4.14	55	1.0290	Teeth and caudal fin rays appearance.

Noting that ; B.T.LEN :body total length ;VOL :volume ;SWB :swimbladder ;INFLAT : inflation.

On the third day (Figure 4.10 I-J) the larvae, with their eye incompletely pigmented, were aggregating in the top third of the observation tank. On the fourth day of age (Figure 4.10 K-L) when the larvae started to feed externally with completely pigmented eye and opened mouth and anus, a very dense patch of larvae were observed to aggregate in the top 10-20 cm of the observation tank. At this stage they showed a phototactic behaviour with good swimming skills as could be observed in Figure 4.10 at age 83 hours after hatching. The larvae in darkness (Figure 4.10 J and M) were observed to be motionless or very slowly sinking with their bodies in a vertical position and their heads pointing downwards.

On the fifth day of age (Figure 4.10 N and O) the larvae maintain the same movement observed on Day 4 but seem to swim for longer distances with fewer interruptions. The vertical movement of larvae from Day 6 to Day 15 are of a similar nature as shown in Figure 4.10 P-V. Overall, the larvae tend to form dense aggregations near the water surface of the tank during daylight hours and rest motionless with a very slow sinking rate at night.

D. Buoyancy Budget Sheet

The overall buoyancy budget sheets are presented in Tables 4.5 and 4.6. These summarize all of the previously mentioned results and relate them to the buoyancy of larvae or fry either with, or without, a functional swimbladder. The net buoyancy is indicated by the sinking factor value which if <1 means positively buoyant, equal to 1 means neutrally buoyant and >1 means negatively buoyant. This relationship is illustrated in Figure 4.11 for both groups, with and without functional swimbladder. The buoyancy budget sheet for fish with functional swimbladders (Table 4.5) shows that just hatched larvae were positively buoyant until the age of Day 5. Neutral

Table (4.5) Buoyancy related measurements for both larvae and fry of A. cuveiri with a functional swimbladder.

AGE (days)	T.BODY.L (mm)	FISH b.wt. (ug)	FISH DENSITY (g/ml)	FISH VOL. (ul)	YOLK VOL. (ul)	YOLK wt.* (ug)	OIL VOL. (ul)	OIL wt.** (ug)	SWB.VOL. (ul)	TISS.VOL (ul)	TISS.wt. (ug)	CAL.DENSITY (g/ml)	SBW.VOL (%)	SINKING FACT.***
J.HATCH	1.87	204	1.0233	0.200	0.164	166	0.0042	3.8	0	0.0314	35	1.0233	0	0.9970
0.5	2.73	133	1.0252	0.130	0.041	41	0.0038	3.4	0	0.0849	88	1.0252	0	0.9988
1	3.00	138	1.0256	0.135	0.021	21	0.0030	2.7	0	0.1110	115	1.0256	0	0.9993
2	3.20	151	1.0259	0.147	0.008	8	0.0022	1.9	0	0.1372	141	1.0259	0	0.9996
3	3.33	163	1.0261	0.159	0	0	0.0008	0.7	0	0.1581	162	1.0261	0	0.9998
4	3.37	168	1.0262	0.163	0	0	0.0008	0.7	0.0006	0.1621	167	1.0262	0.34	0.9999
5	3.39	170	1.0262	0.166	0	0	0.0004	0.4	0.0006	0.1647	170	1.0262	0.34	0.9999
6	3.44	176	1.0263	0.171	0	0	0.0002	0.2	0.0006	0.1707	176	1.0263	0.35	1.0000
7	3.55	191	1.0265	0.186	0	0	0.0001	0.1	0.0007	0.1853	191	1.0265	0.38	1.0001
8	3.70	215	1.0267	0.209	0	0	0.0001	0.1	0.0009	0.2084	215	1.0267	0.41	1.0003
10	4.31	355	1.0274	0.345	0	0	0	0	0.0017	0.3435	355	1.0274	0.50	1.0010
15	4.83	528	1.0290	0.513	0	0	0	0	0.0029	0.5098	528	1.0290	0.57	1.0026
20	6.15	1189	1.0330	1.151	0	0	0	0	0.0090	1.1418	1189	1.0330	0.78	1.0065
25	6.85	1669	1.0330	1.615	0	0	0	0	0.0149	1.6006	1669	1.0330	0.92	1.0065
35	10.00	8532	1.0330	8.259	0	0	0	0	0.1391	8.1203	8532	1.0330	1.20	1.0065
45	15.00	35030	1.0336	33.892	0	0	0	0	0.4347	33.4574	35030	1.0336	1.30	1.0070
55	20.00	83459	1.0350	80.637	0	0	0	0	1.2814	79.3552	83459	1.0350	1.59	1.0084
65	25.00	169540	1.0361	163.632	0	0	0	0	2.9406	160.6914	169540	1.0361	1.80	1.0095
75	30.00	314494	1.0370	303.271	0	0	0	0	5.6739	297.5975	314494	1.0370	1.87	1.0104
85	35.00	539543	1.0378	519.907	0	0	0	0	9.7426	510.1648	539543	1.0378	1.87	1.0111

Noting that ;T.:total ;L.:length ;b.wt.:body weight ; VOL.:volume ;SBW.:swimbladder ;TISS.:tissue ;FACT.:factor;CAL.:calculated;J.HATCH.:just hatched.

* Assuming that yolk density is 1.0160 g/ml.

** Assuming that oil density is 0.90 g/ml.

*** Rearing water density is 1.02635 g/ml (38 - 40 ppt. at 25 C).

Table (4.6) Buoyancy related measurements for both larvae and fry of A. cuveiri without a functional swimbladder.

AGE (days)	T.BODY.L (mm)	FISH b.wt. (ug)	FISH DENSITY (g/ml)	FISH VOL. (ul)	YOLK VOL. (ul)	YOLK wt.* (ug)	OIL VOL. (ul)	OIL wt.** (ug)	SWB.VOL. (ul)	TISS.VOL (ul)	TISS.wt. (ug)	CAL.DENSITY (g/ml)	SB.VOL (%)	SINKING FACT.***
J.HATCH	1.87	204	1.0233	0.200	0.164	166	0.0042	3.8	0	0.0314	35	1.0233	0	0.9970
0.5	2.73	133	1.0252	0.130	0.041	41	0.0038	3.4	0	0.0849	88	1.0252	0	0.9988
1	3.00	138	1.0256	0.135	0.021	21	0.0030	2.7	0	0.1110	115	1.0256	0	0.9993
2	3.20	151	1.0259	0.147	0.008	8	0.0022	1.9	0	0.1372	141	1.0259	0	0.9996
3	3.33	163	1.0261	0.159	0	0	0.0008	0.7	0	0.1581	162	1.0261	0	0.9998
4	3.37	168	1.0276	0.163	0	0	0.0008	0.7	0	0.1624	167	1.0276	0	1.0012
5	3.39	170	1.0277	0.165	0	0	0.0004	0.4	0	0.1650	170	1.0277	0	1.0013
6	3.44	176	1.0280	0.171	0	0	0.0002	0.2	0	0.1710	176	1.0280	0	1.0016
7	3.55	191	1.0287	0.186	0	0	0.0001	0.1	0	0.1856	191	1.0287	0	1.0022
8	3.60	199	1.0289	0.193	0	0	0.0001	0.1	0	0.1928	198	1.0289	0	1.0025
10	4.31	355	1.0326	0.343	0	0	0	0	0	0.3435	355	1.0326	0	1.0061
15	4.83	528	1.0350	0.510	0	0	0	0	0	0.5098	528	1.0350	0	1.0084
20	6.15	1189	1.0400	1.143	0	0	0	0	0	1.1431	1189	1.0400	0	1.0133
25	6.85	1669	1.0422	1.601	0	0	0	0	0	1.6013	1669	1.0422	0	1.0154
35	10.00	8532	1.0500	8.126	0	0	0	0	0	8.1260	8532	1.0500	0	1.0230
45	15.00	35030	1.0583	33.100	0	0	0	0	0	33.1005	35030	1.0583	0	1.0311
55	20.00	83459	1.0642	78.423	0	0	0	0	0	78.4231	83459	1.0642	0	1.0369
65	25.00	169540	1.0688	158.626	0	0	0	0	0	158.6258	169540	1.0688	0	1.0414
75	30.00	314494	1.0726	293.220	0	0	0	0	0	293.2199	314494	1.0726	0	1.0450
85	35.00	539543	1.0757	501.562	0	0	0	0	0	501.5623	539543	1.0757	0	1.0481

Noting that ;T.:total ;L.:length ;b.wt.:body weight ; VOL.:volume ;SBW.:swimbladder ;TISS.:tissue ;FACT.: factor;CAL.:calculated;J.HATCH.:just hatched.

* Assuming that yolk density is 1.0160 g/ml.

** Assuming that oil density is 0.90 g/ml.

*** Rearing water density is 1.02635 g/ml (38 - 40 ppt. at 25 C).

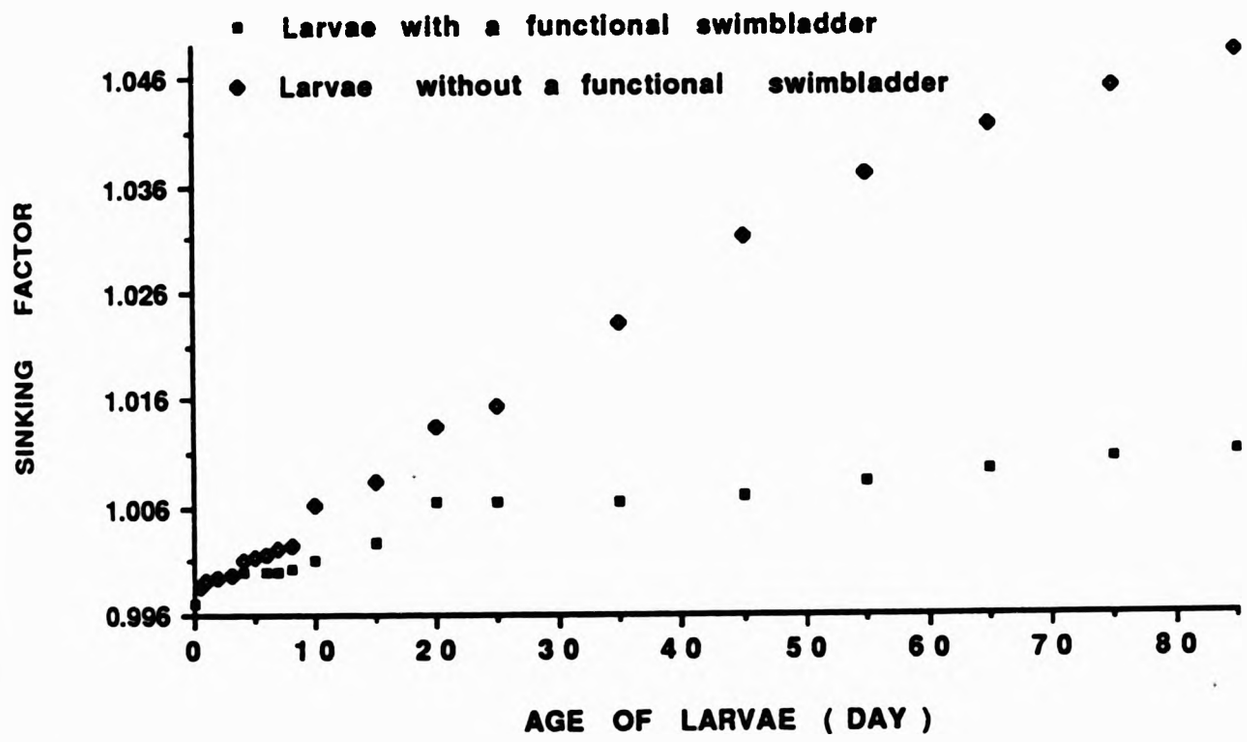


Figure (4.11) A calculated relationship between larval age and the sinking factor

buoyancy was attained on Day 6 when the larval density became similar to that of the rearing water of 1.02635 g/ml. From Day 8 onwards, the larvae became negatively buoyant, even though they have a functional swimbladder from Day 4. This slight negative buoyancy in *A. cuvieri* larvae and fry was consistently observed. However, as *A. cuvieri* is a continuously swimming pelagic fish, this minor sinking could be easily neutralized by fin movements alone.

The buoyancy budget sheet for fish without functional swimbladders is given in Table 4.6. It shows that larvae at 4 days of age without functional swimbladders were slightly negatively buoyant. This shows the early role of the inflated swimbladder as a functional hydrostatic organ, even though its volume does not exceed 0.4% of the total fish body volume. It is also interesting to note the rapid increase in tissue weight of the fish which is mainly additional high density structural muscular and skeletal tissues. From Day 4 onward, the sinking factors are consistently greater than in fish possessing a functional swimbladder.

The column of calculated densities in Table 4.5 and 4.6 shows the same values as density obtained by measurement. This is due to the usage of measured fish density to obtain the fish total volume, which will then be reflected in other calculations leading to the calculated density routines. Nevertheless, the relative contribution of the different components that make up fish density are clearly identificable.

IV. DISCUSSION

Newly-hatched larvae of Blue-Finned Sea Bream (*A. cuvieri*) are about 1.87 ± 0.075 mm in total body length and 204.2 ± 10.5 ug in wet body weight. They are positively buoyant and have a density of 1.0233 g/ml and

a sinking factor of 0.9970. They float at the water surface with their yolk sac facing upward and their tails obliquely pointing downward. The larval density gradually increases as they grow consuming their endogenous nutrients reserves stored in the yolk sac and oil globule. This increase in larval density is possibly due to the conversion of light density nutrient reserves to more heavier constructive materials such as a new larval tissue, including muscle and bone.

Blue-Finned Sea Bream larvae begin to inflate their swimbladder on the fourth day after hatching and this also coincides with the completion of yolk sac resorption and the initiation of external feeding. The total body length of the larvae will then be about 3.37 mm retaining about 11.4% of their oil globule volume. At this age the larvae form dense patches just under the water surface during daylight hours and this behaviour coincides with initial swimbladder inflation.

The tendency of *A. cuvieri* to swim against their bouyancy few hours just after hatching (without visual ability) in the downward direction and on day 3 in the upward direction, which was thought initially to be the optomotor reaction described by Kawamura and Hara (1980) who worked on Milkfish larvae. Kawamura and Hara found that the optomotor reaction is an indication of a complex visual behaviour and it is not fully developed before the metamorphosed larvae or juvenile stage. Therefore, the tendency of *A. cuvieri* larvae to swim in an opposite direction of its buoyancy may not be due to the optimotor reaction.

The newly-hatched larvae grew very fast during the first day after hatching consuming most of their nutrient reserve in the yolk sac, as a result of that, they almost doubled their total body length and lost about

32.3% of their wet body weight. There is a rapid growth of actual body tissue which increased from 35 ug at hatching to 115 ug on Day 1. Thus, the observed drop in total wet body weight was due to the fast utilization of yolk and the rapid rate of building new body tissue within the larvae. A plateau in growth of total body length was observed to occur between Day 2 and Day 5 after hatching. In general, the larval growth during the period of yolk and oil resorption of many marine species is characterized by a growth plateau which has been observed by many workers (Farris, 1959; Kuo *et al.*, 1973; Ellertsen *et al.*, 1980; Hunter and Kimbrell, 1980; Doroshev *et al.*, 1981; Bagarinao, 1986; Chatain, 1986; Kohno *et al.*, 1986 and Avila and Juario, 1987).

Usually, initial swimbladder inflation begins during this growth plateau period of yolk and oil resorption (Doroshev *et al.*, 1981; Chatain, 1986). After yolk resorption, and while the larvae are still learning to feed externally, the oil globule plays an important role in providing energy for tissue maintenance, swimming and possibly in tissue growth. In a more extreme case, Doroshev *et al.* (1981) noted that *M. saxatilis* larvae retain more than 55% of their oil globule throughout the period of swimbladder inflation. Even though, the oil globule provides energy during the static growth phase and during initial swimbladder inflation, it was observed that the highest percent of initial swimbladder inflation occurred in *A. cuvieri* larvae during the accelerated growth phase which begins slightly after the larvae have established external feeding. It was also observed that, at the time of major initial inflation of swimbladder, the larvae were characterized by a slight negative buoyancy, phototactic behaviour and well-established external feeding. The development of a functional swimbladder provides the larvae with a capability for hydrostatic regulation which is important for

pelagic larvae to facilitate searching for food and possibly to a lesser extent in avoiding predators.

Most marine larvae that hatch from pelagic eggs have positive buoyancy which tends to become negative at the end of yolk resorption. This has been observed in ***Gadus morhua*** (Ellertsen *et al.*, 1980), ***Pleuronectes platessa*** (Blaxter and Ehrlich, 1974), and ***Platichthys flesus*** (Yin and Blaxter, 1987). Density measurements of ***A. cuvieri*** larvae show that negative buoyancy occurred on Day 7 after hatching. This apparently conflicts with the vertical movement observations which showed that the larvae start to be negatively buoyant from Day 2 onwards, revealed by the slow sinking observed on many occasions and secondly by the fact that over 95% of the yolk volume which is the main source of the uplift force for the larvae at that age is already utilized. It is, therefore, assumed that this discrepancy is due to underestimation of the density in the gradient column rather than with the data obtained from the vertical movement of larvae. It is possible that the measurements of the density gradient column are not sufficiently accurate to assess these tiny variations, since the difference between the densities of larva of age 2 days and 7 days is about 0.0005 g/ml.

A similar vertical downward movement of larvae was observed with *M. cephalus*, although this was completed by Day 2.5 after hatching (Kuo *et al.*, 1973) and with ***Gadus morhua*** (Ellertsen *et al.*, 1980). An interesting assumption was made by Kuo et.al. (1973) that one of the causes for early larval mortality in culture systems could possibly be attributed to the mechanical damage brought about by the prolonged contact of the larvae with a solid surface (tank bottom) during their vertical downward movements, suggesting that deeper tanks could enhance early survival.

Nielson *et al.* (1986) worked on the the buoyancy of cod larvae (*Gadus morhua*) and found that poorly fed larvae (reared in low prey density) tend to be more buoyant, inhabiting the top of the water column, and thus may be more vulnerable to predation. By contrast, well fed larvae (reared in high prey density) tended to be less buoyant, inhabiting the middle part of the water column and are thus possibly less vulnerable to predation. They concluded that larval density may be a better indicator of larval condition than those indices based on morphometric data. A similar approach to determining the quality of hatched larvae of *A. cuvieri*, or other species, could be to test the ability of the larvae to swim downwards against their positive buoyancy.

Overall, the results presented here have given a strong indication of how the buoyancy of *A. cuvieri* larvae develops and how this coincides with structural development and growth.

In the next chapter, environmental factors that may affect the initial swimbladder inflation and its consequent development will be examined in an attempt to produce proper rearing guidelines for the maximization of the production of fry having functional swimbladders.

CHAPTER 5

THE EFFECTS OF SELECTED ABIOTIC FACTORS ON INITIAL SWIMBLADDER INFLATION AND SURVIVAL

I. INTRODUCTION

The failure of initial swimbladder inflation in some hatchery-reared marine and brackish-water larvae is a major problem since it is linked to survival, growth and quality (Lordosis) of the fry. The known species affected are; the Striped Bass *Morone saxatiles* (Doroshov and Cornacchia, 1979; Bulak and Heidinger, 1980; and Doroshov *et al.,* 1981), the Red Sea Bream *Sparus major* (Kitajima *et al.,* 1981; Iseda, 1982; and Fukusho, 1985), the Gilthead Sea Bream *Sparus aurata* (Paperna, 1978, 1984; Annanomus, 1987; and Chatain, 1987), the European Sea Bass *Dicentrarchus labrax* (Paperna, 1984; Annanomous, 1987; Chatain, 1987), the Blue-finned Sea Bream *Acanthopagus cuvieri* (Al-Abdul-Elah *et al.,* 1982) and others.

The factors affecting initial swimbladder inflation have not all been identified and the published literature on this topic is limited to only a few papers covering a very wide range of factors. Chapman *et al.*, (1988) working on *M. saxatilis* larvae suggested that spawning stock and optimal condition for spawning could have a major effect on initial swimbladder inflation. Hadley *et al.* (1987) also worked on *M. saxatilis* larvae and tested the effects of rearing temperature and salinity on swimbladder inflation in the different progenies of 13 females. They concluded that the rearing temperature and the sibling group had a significant effect. They also suggested the possible effects of other environmental factors, such as photoperiod, and a maternal effect related to the hormonal induction and vitellogenesis which could affect the size and viability of the embryos. Cornacchia (1982) worked on *M. saxatilis* and studied the effect of salinity (0-20ppt) on swimbladder development and inflation. He reported that 5 and 10 ppt were found to be optimum salinities for normal development and resulted in a high rate of inflation (86%). Paperna (1978) suggested a hereditary

origin for the differences in swimbladder inflation between rearing trials in Gilthead Sea Bream larvae. Taniguchi *et al.* (1984) worked on Red Sea Bream and concluded that the physiological condition of eggs and breeders and possibly genetic factors could be the cause of vertebral malformation (Lordosis) which is linked with the absence of functional swimbladder. Fukusho (1985) stated that genetic differences could not be a factor influencing the appearance of Lordosis (absence of functional swimbladder), since the same batch of eggs from the same parent gave different percentages in different rearing tanks.

Iseda (1982) worked on Red Sea Bream and found that, whereas tanks with no aeration and no water current had a low percentage swimbladder inflation, a moderate water current and suitable aeration (50-100 ml/min) increased the swimbladder inflation percent and reduced the frequency of Lordosis. Doroshev and Cornacchia (1979) also reported that strong aeration and turbulent water in rearing containers could enhance normal swimbladder inflation of *M. saxatilis* larvae. Chatain (1982), worked on Red Sea bream, reported the positive effect of water-flow during the first and second weeks of rearing on the initial swimbladder inflation.

Watanabe (1986a) noted that feeding rotifers with a low w3 HUFA content to larval Red Sea Bream resulted in low swimming activity and lack of reflex responses and a consequent failure to initially inflate their swimbladder at the air-water interface layer. Chatain (1982) also reported the importance of the nutritional quality of rotifers as first feed to larvae and its effect on initial swimbladder inflation. She concluded that rotifers enriched with *Chlorella* gave the best survival and swimbladder inflation percent compared to other enrichment media. Watanabe *et al.* (1984c), tested

different broodstock diets on Red Sea Bream and suggested that there is an effect of broodstock diet on initial swimbladder inflation.

From this brief review, it can be seen that the factors influencing initial swimbladder inflation in marine and brackish-water larvae can be generally grouped into two categories namely; abiotic and biotic.

The objective of this chapter is to examine the possible effect of some of the abiotic factors on initial swimbladder inflation, survival and growth, while the next chapter will deal with some of the biotic factors. The environmental factors considered here are rearing temperature, salinity, light intensity, photoperiod, aeration rate and water exchange.

II. MATERIALS AND METHODS.

A. General considerations.

The newly-hatched larvae used in these trials were obtained from natural spawnings at MFD, KISR. The details of broodstock maintenance and spawning, egg collection and incubation, larval estimation and stocking, larval feeding and rearing procedures have already been given in the general materials and methods presented in chapter 2.

The trials were carried out during a period of 21 days in the 1986 spawning season. During the following spawning seasons of 1987 and 1988 they were conducted within a 16 day period. This cut in rearing time was necessary to ensure good egg quality and hence better larvae, and this was achieved by not using the later egg batches. Secondly, a duration of 16 days is enough for the purpose of these tests, since the pneumatic duct becomes occluded between Day 10-12 and therefore no more initial swimbladder inflation will take place after Day 16. Thirdly, as a static water system was used, reducing

the trial duration to 16 days helped minimize any deterioration of water quality.

It was planned that over the three experimental seasons (1986-1988) results obtained would be used to optimize the rearing conditions, thus increasing the efficiency both of initial swimbladder inflation and survival as the trials progressed. The best results from the water temperature and salinity trials carried out in the 1986 season (i.e 25°C and 38-40‰) were implemented during the 1987 season. Similarly, the best results from the light intensity and photoperiod work carried out in the 1987 season (i.e 1000 Lux and continuous light) were implemented in the 1988 season tests. Specific details on experimental design, sampling and measurements for each of the trials will be given individually as follow:

B <u>The effect of rearing temperature on swimbladder inflation and survival</u>

Water temperature may affect swimbladder inflation as it affects the rate of tissue development and hence, the rate of appearance, functioning and atrophy of the pneumatic duct which is vital for initial swimbladder inflation. A secondary effect could be the reduction of surface tension at higher water temperatures.

Swimbladder inflation was monitored at four temperatures namely, 22, 25, 28 and 31°C. Each treatment was replicated four times in 500 liter fiberglass tanks, and these were placed in a randomly selected $10m^3$ concrete tank which served as a water bath for controlling the rearing temperature (see Figure 2.2). Full details of the 500 liter rearing tanks and the $10m^3$ concrete water bath tanks are given in the general materials and methods in chapter (2). The initial water volume was 450 liters allowing 50 liters for the daily addition of *Chlorella* and rotifers. Newly-hatched larvae of *A.*

cuvieri were stocked into the sixteen 500 liter tanks, at an initial stocking density of 60 larvae/liter (24,000/tank).

The environmental parameters were kept constant in all of the four treatments, except for the rearing temperature which varied. The water was static and the aeration rate was 200 ml/min, rearing salinity 38-40 ppt, light intensity of 1000-1200 Lux (at the water surface using a fluorescent day-light), photoperiod regime of 14 hr light and 10 hr dark. The larvae were fed on L-type rotifers at 5 rotifera/ml of rearing water/day initially, and $300\text{-}400 \times 10^3$ *Chlorella* cell/ml of rearing water/day were added as a conditioner.

The initial water temperature of all the experimental tanks was 20-21°C, similar to that of the incubation system and hatching nets. The selected range of temperatures to be tested were gradually approached at a rate of 5°C/12hr. Larvae were sampled daily for 21 days from each tank from different locations using a 250 ml beaker after aeration was stopped for 5 min and all larvae appeared in dense patches near the water surface. Measurements of total body length were made in about 20-30 larvae and the percentage of initial swimbladder inflation was measured in about 50-80 larvae, using a dissecting microscope with eyepiece graticule. Survival of larvae was measured by a final count of all larvae in each of the test tanks on day 21.

C. <u>The effect of salinity on swimbladder inflation and survival</u>

Larvae have different sinking rates when reared in different salinities. It is thus possible that at lower salinities the larvae will be induced to swim upward to fill their swimbladders to attain buoyancy as a reflex to their sinking.

Swimbladder inflation was monitored at five salinities, namely, 10, 20, 30, 40, and 50 ppt. Each treatment was replicated four times with the

exception of the 10 ppt treatment which was replicated only twice. All the work was in 500 liter tanks as in the temperature experiment. The water temperature was fixed at 25°C.

The newly-hatched larvae were stocked directly into the experimental tanks without any previous acclimation to the tested salinities, and water temperature was raised gradually from 20-21°C initially to 25°C on the second day. The salinities of all treatments were adjusted once on the 10^{th} day of rearing as they have slightly increased.

The experiment lasted for 21 days and larvae were sampled on day 5, 8, 12, 15, and 21. The sampling method, sample size and parameters measured were as in the temperature experiment. An additional measurement of swimbladder volume was made by measuring swimbladder length and height. Larval survival was checked by counting all larvae in each replicate on day 21.

D. <u>The effect of Light intensity on swimbladder inflation and survival</u>

This experiment was first conducted in 1986 using larvae from the last batch of eggs spawned which, generally, tends to be of low quality. Because of this, the test was modified and repeated in 1987 using good quality eggs. The objective of these trials were to determine the optimum light intensity at which larvae of *A. cuvieri* best exhibit phototactic behaviour.

D.1. <u>Trial in the 1986 season</u>.

In the 1986 tests, five treatments of light intensities were used namely, dark for the first 4 days and then 500 Lux, 250 Lux, 500 Lux, 1000 Lux and 1750 Lux. The source of illumination used was five feet fluorescent day-

light tubes. The photoperiod in all treatment was 14hr light and 10hr dark. Each treatment was replicated four times in 500 liter fiberglass tanks at 25°C, arranged as described previously. All other rearing parameters were as used in the temperature experiments.

The trials was continued for 21 days, and larvae were sampled on day 5, 10, 15, and 21. The sampling method, sample size and parameters measured were as in the temperature experiment. Larval survival was checked by counting all larvae in each replicate on day 21.

D.2. Trial in the 1987 session

In the 1987 season, earlier egg batches were used and the light intensity treatments used were as follows; 250 Lux, 1000 Lux, 2000 Lux and 5000 Lux. The change of treatments was due to the fact that the previous range of light intensity used (1986 trial) was small and that the treatment were close to each others. And the first 4 days dark treatment was omitted in order to included it in the photoperiod test. The duration of this test was 16 days only, with samplings carried out on day 3, 7 and 16. All other details were as in the 1986 test.

D.3. Larval movements under different light intensities

In an attempt to understand the results of the previous light intensity tests, a simultaneous, but independent, trial was conducted to assess larval movements and aggregations in a 1 meter water column kept under the different light intensities.

Six tanks were constructed from transparent acrylic sheet (100x30x15 cm) for use as the observation columns. One of these tanks was placed under light intensities of 0, 250, 1000, 4000, 6000 and 10000 Lux, the

light intensity being read at the water surface of the observation tank. The larvae used in this test were reared in two 500 litre tanks at density of 60 larvae/l using the rearing procedures described earlier. The columns were filled with fresh seawater and were stocked with larvae on days 3, 4, 5 and 6. After 2 hours from stocking the vertical distribution and aggregation was noted.

E. The effect of photoperiod regime on swimbladder inflation and survival.

Swimbladder inflation was evaluated under four photoperiod regimes, 24hr light, 14hr light and 10hr dark, dark during the first three days and then 14hr light and 10hr dark and finally dark during the first three days and then 24hr light. The idea behind the 24hr light and first 3 days dark treatments was taken from the vertical movement study presented previously in chapter 4. It was considered that the 24hr light treatment could help in keeping the larvae near water surface all the time especially after the larvae attaining their phototactic behaviour which could facilitate better initial swimbladder inflation. By contrast, the first 3 dark days could help in reducing larval vertical movement thus reducing energy expenditure and leading to bigger and possibly stronger larvae at the end of the first 3 days. This larvae fitness could enhance the success of first filling of the swimbladder by enabling breaking of water surface tension and gulping atmospheric air. A single light intensity of 1000 Lux was used in all the treatments trials which were replicated three times in 500 litre fiberglass tanks as described earlier. The water temperature was 25°C.

The trials was continued for 16 days, and larvae were sampled on day 6, 12 and 16. The sampling method, sample size and type of measurements

were similar to those in the temperature test. Again, larval survival was checked by counting all larvae in each replicate on day 16.

F. The effect of aeration rate and air access on swimbladder inflation and survival

This trial focusses upon the mechanical effect of aeration and not the oxygenation effect. The instability of water surface tension and the cleaning effect of aeration to water surface are major mechanical effects of interest which could have an effect on initial swimbladder inflation. In addition it was intended to reconfirm the results obtained by Al-Abdul-Elah *et al.* (1982) that gulping of atmospheric air is essential for swimbladder inflation.

Three aeration rates were compared with rearing in static water, the rates tested were 50-70 ml/min, 200-300 ml/min and 1600-1800 ml/min. In a further treatment, the lowest aeration rate of 50-70 ml/min was used in conjunction with 0.5ml of a silicon-base surfactant (Silicone antifoaming agent 30% w/w silicone, BDH product) added per litre of rearing volume which formed a thin film covering all of the water surface. A trial was carried out to determine this concentration of silicon-base surfactant where 0.1, 0.25, 0.5, 1.0 and 2.0 ml/l were tested. An earlier trial had shown that an air-stone producing very fine air bubbles resulted in an impenetrable mat of foam at water surface. Consequently, in this trial the size of air bubbles produced by the air-stone used were approximately 2-5mm in diameter at the surface. Each treatment was replicated four times in 30 litre fiberglass tanks which were placed in a 330 litre galvanized tank which served as waterbath (see figure 2.2).

The number of newly-hatched larvae stocked in each tank (25 litre as working volume) were 1500 making a stocking density of 60 larvae/litre. The rearing parameters were similar to that of the temperature test except that in this trial the aeration rate is 50-70 ml/min and the photoperiod is continous light.

The trial continued for 16 days, with larvae being sampled on day 6, 12 and 16. The sampling procedure, size and type of measurement were similar to that of previous tests and larval survival was checked by counting all larvae from each replicate on day 16.

G. <u>The effect of water-exchange on swimbladder inflation and survival</u>

The introduction of fresh seawater at the surface of the rearing tank after the third day is a way of ensuring a clean water surface layer, free of any film or debris which may impede air access and hence initial swimbladder inflation.

Three water-exchange regimes were evaluated; static (no exchange), continuous water flow at a rate of 250 ml/min, starting on day 3, and 9hr water flow at a rate of 250ml/min, starting on day 3. Water flows of 500ml and 1000ml/min were tested initially but induced high larval mortality. Each treatment was replicated three times in 100 litre cylindroconical tanks (central bottom drain). All nine tanks were placed in a water bath at 25°C (see figure 2.2), the temperature and salinity of ambient well water being 24.5°C and 39 ppt respectively. The larvae were reared in continuous light (24hr) at 1000-1200 Lux, using an aeration rate of 50-70 ml/min, a stocking density of 60 larvae/litre (6000 newly-hatched larvae/100 litre tank). They were fed on rotifers of L-type at initial feeding rate of 5 rotifers/ml/day, and about 300-400 x 10^3 *Chlorella* cell/ml of rearing water/day.

The trial continued for 16 days, with larvae being sampled on day 6, 12 and 16. The sampling method, sampling size and type of measurement were as in the temperature test and larval survival was checked by counting all larvae from each replicate on day 16.

Samples for water quality monitoring were collected on the early morning of the same days of sampling larvae before opening water inlet for the 9hr water flow treatment. The sample was taken from the center of the tank where the aeration produced maximum mixing. A 500ml bottle was immersed slowly inside a hand net (made from 100µm mesh to prevent larvae from entering into the water sample bottle) which was placed in the center of the experimental rearing tank. In the laboratory all samples were subjected to two steps of filtration, first using a glass microfilter (Whatman, GF/D) then a 0.6µm filter (Selectron, 4.7cm Diam.) to remove all the rotifers and *Chlorella* cells. The water samples then were tested for nitrite, and total ammonia-nitrogen using the procedures described by Grasshoff (1976).

H. Statistical analysis

The data from all the above mentioned tests were calculated, manipulated and prepared using the software programme Lotus 123, Statistical analyses were carried out using one way ANOVA and Duncan's multiple range tests provided in the software programme SPSS/PC.

III. RESULTS

A. The effect of water temperature on swimbladder inflation and survival

The effect of different rearing temperatures on the percent initial swimbladder inflation versus age is shown in Figure 5.1. The initial swimbladder inflation was observed to increase sharply from day 3 to day 7-8 after hatching at both 22°C and 25°C with a plateau appearing after 8 to 12 days at 25°C and 10 to 14 days at 22°C. At both 28°C and 31°C the increase in initial swimbladder inflation was very gradual. It is clear that at 22°C and 25°C most of the initial swimbladder inflation occurred on day 3 to 7. It is most probable that the apparent second stage of inflation occurring on day 14 to 21 is a false one, due to selective mortality of weak and stressed larvae. These weak and stressed larvae are assumed to have a non-functional swimbladder as was found in larvae of *Dicentrarchus labrax* which died first (Chatain, 1986). This assumption is supported by the fact that the pneumatic duct is occluded by day 10 -12 at 25°C and even for slow growers it will be occluded by day 14-16 (chapter 3). This assumption applies equally to all tests of 21 days duration reported in this chapter. Even though their could be a selective mortality after day 14 of rearing which would interfere with the results obtained, this can probably be safely neglected assuming that this selective mortality affects all treatments similarly.

Figure 5.2 shows the percent swimbladder inflation on days 5, 7, 14, and 21 days after hatching. Statistical tests were carried out on the values of swimbladder inflation occurring on these selected days and the results are tabulated and shown in Table 5.1. Temperatures of 22, 25 and 28°C have similar effect on day 14 as the best, but 25°C is best and significantly different from all others on day 21.

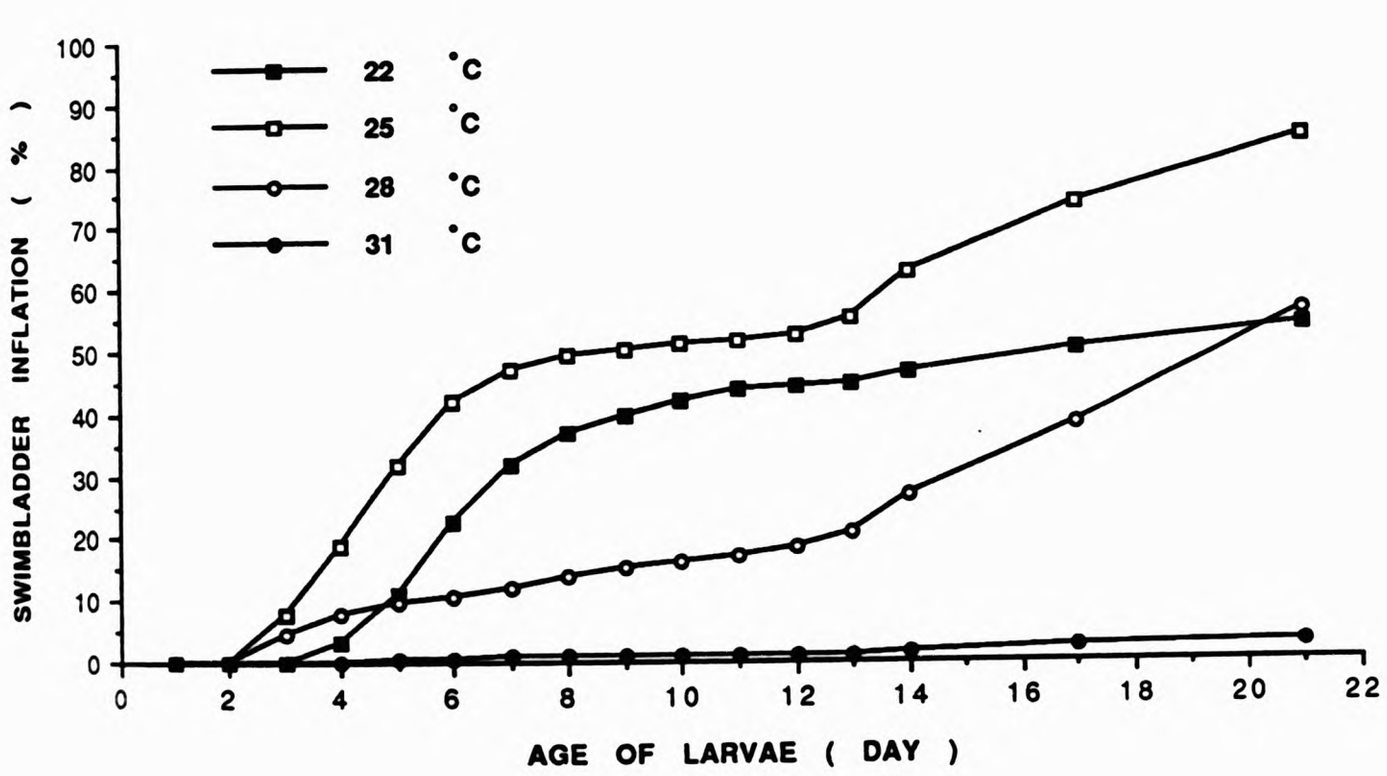


Figure (5.1). Daily percent inflation of swimbladder of *A. cuvieri* larvae reared in four different temperatures.

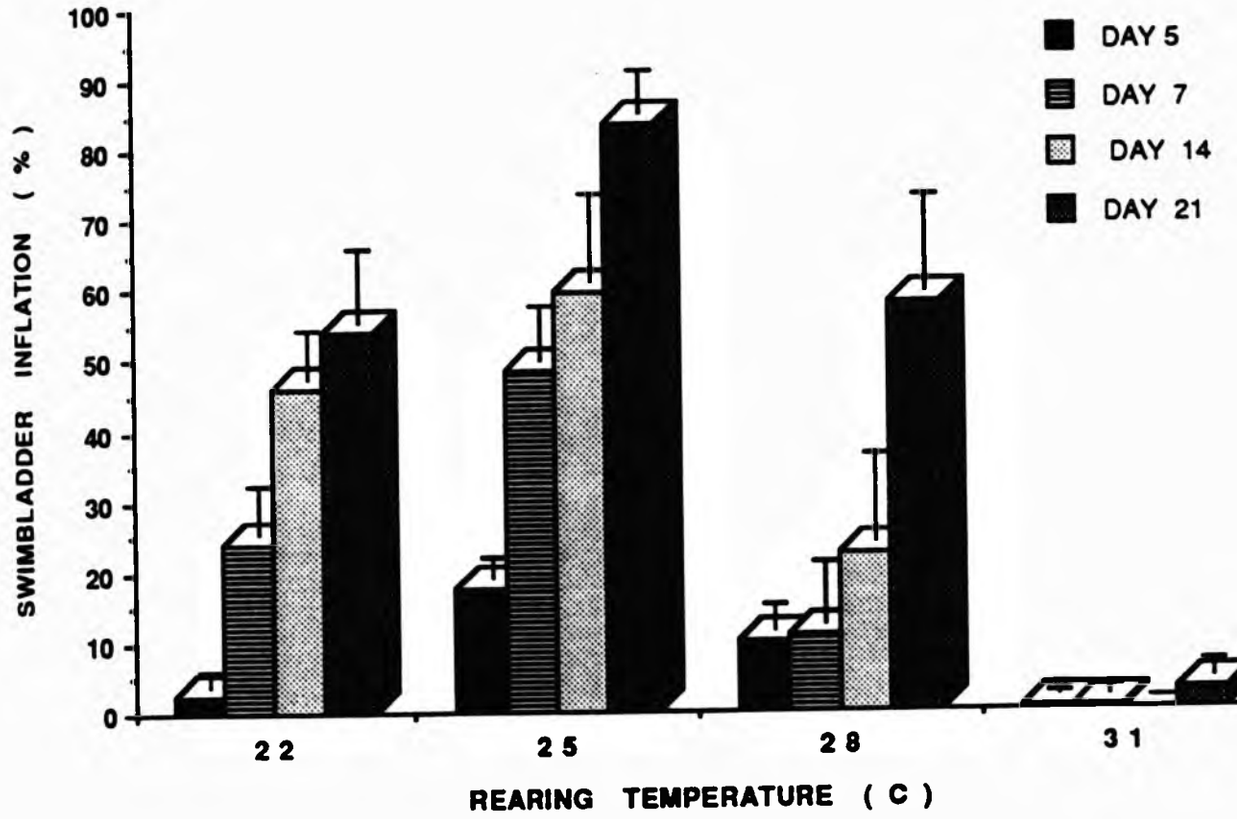


Figure (5.2). The effect of different rearing temperatures on swimbladder inflation of *A. cuvieri* larvae of different ages. Vertical bars represent one standard deviation.

Table (5.1). Results of the statistical analysis carried out on the swimbladder inflation data presented in the above figure.

Statistical tests	Rearing temperatures (°C)			
	22	25	28	31
Day 5				
. One -way ANOVA	Significantly different, P= 0.0001			
. Duncan's test,P<0.05	B	A	B	C
Day 7				
. One-way ANOVA	Significantly different, P= 0.0001			
. Duncan's test, P<0.05	A	A	B	C
Day 14				
. One-way ANOVA	Significantly different, P= 0.0005			
. Duncan's test, P<0.05	A	A	A	C
Day 21				
. One-way ANOVA	Significantly different, P= 0.0001			
. Duncan's test, P<0.05	B	A	B	C

The survival of *A. cuvieri* larvae reared at different water temperatures for 21 days is shown in Figure 5.3 and best results were obtained at 25°C. The effect of rearing temperatures on total survival (survival of larvae with and without a functional swimbladder) and on survival of larvae with a functional swimbladder were found to be statistically different at $P < 0.001$ (Table 5.2).

The growth of larvae in terms of total body length, is shown in Figure 5.4. It appears from the curve that growth is slower at 22°C, and Table 5.3 shows that the total body lengths attained on day 21 were statistically different. The highest larval growth being obtained at 28°C and 31°C.

In summary, it is clear that at 25°C maximum production of normal larvae having a functional swimbladder and a good growth rate was achieved.

B. <u>The effect of salinity on swimbladder inflation and survival</u>.

Percent swimbladder inflation versus age of larvae at different salinities is shown in Figure 5.5. In all treatments, except 40 ppt, a plateau was observed to occur on day 12 to 15. This plateau may represent the time of occlusion of the pneumatic duct. The additional increases in swimbladder inflation observed to occur after day 15 could be due to selective mortality.

The percentage swimbladder inflation measured on day 5, 8, 12, 15 and 21 are shown in Figure 5.6 and the results of the statistical analysis are shown in Table 5.4. No significant difference was detected between the treatments on day 8, 12 and 15, although there was a significant difference at 10 ppt on day 5. The highest % inflation obtained was at 20 ppt on day 21.

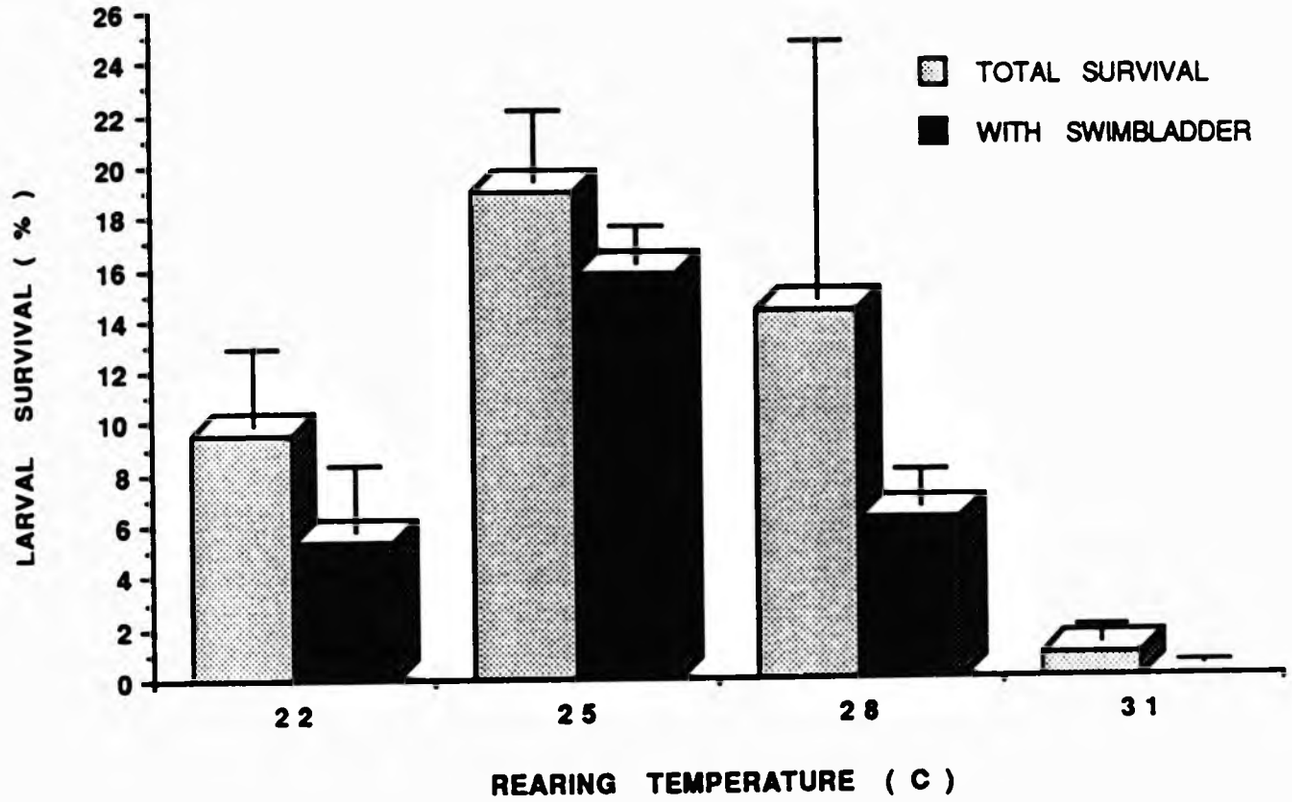


Figure (5.3). The effect of rearing temperature on total larval survival and survival of larvae having swimbladder after 21 days of rearing. Vertical bars represent one standard deviation.

Table (5.2). Results of the statistical analysis carried out for the larval survival presented in the above figure.

Statistical tests	Rearing temperatures (°C)			
	22	25	28	31
Total survival				
. One -way ANOVA	Significantly different, P= 0.0002			
. Duncan's test,P<0.05	B	A	AB	C
With swimbladder				
. One-way ANOVA	Significantly different, P= 0.0001			
. Duncan's test, P<0.05	B	A	B	C

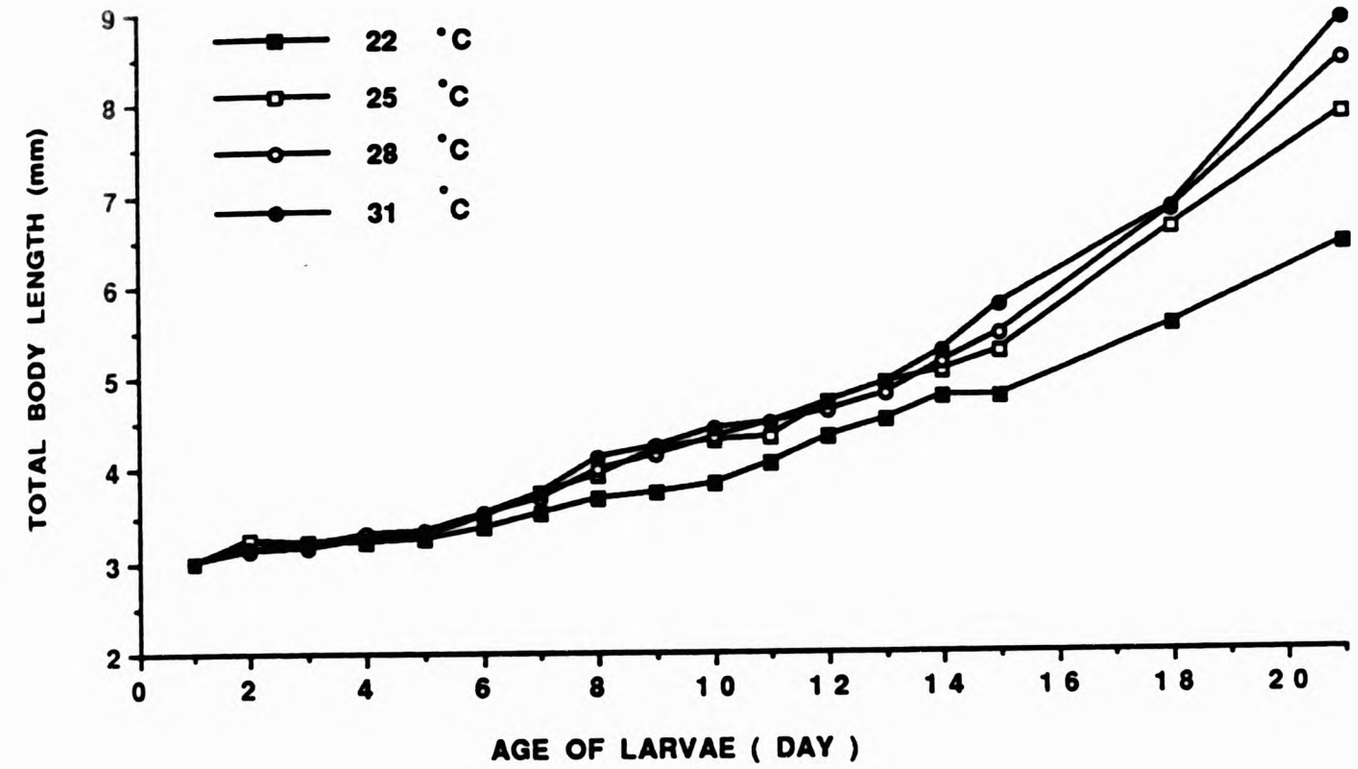


Figure (5.4). Daily growth in total body length of *A. cuvieri* larvae reared in four different temperatures.

Table (5.3). Results of the statistical analysis carried out for the values of total body length at Day 21, presented in the above figure.

Statistical tests	Rearing temperatures (°C)			
	22	25	28	31
. One -way ANOVA	Significantly different, P= 0.0007			
. Duncan's test, P<0.05	C	B	AB	A

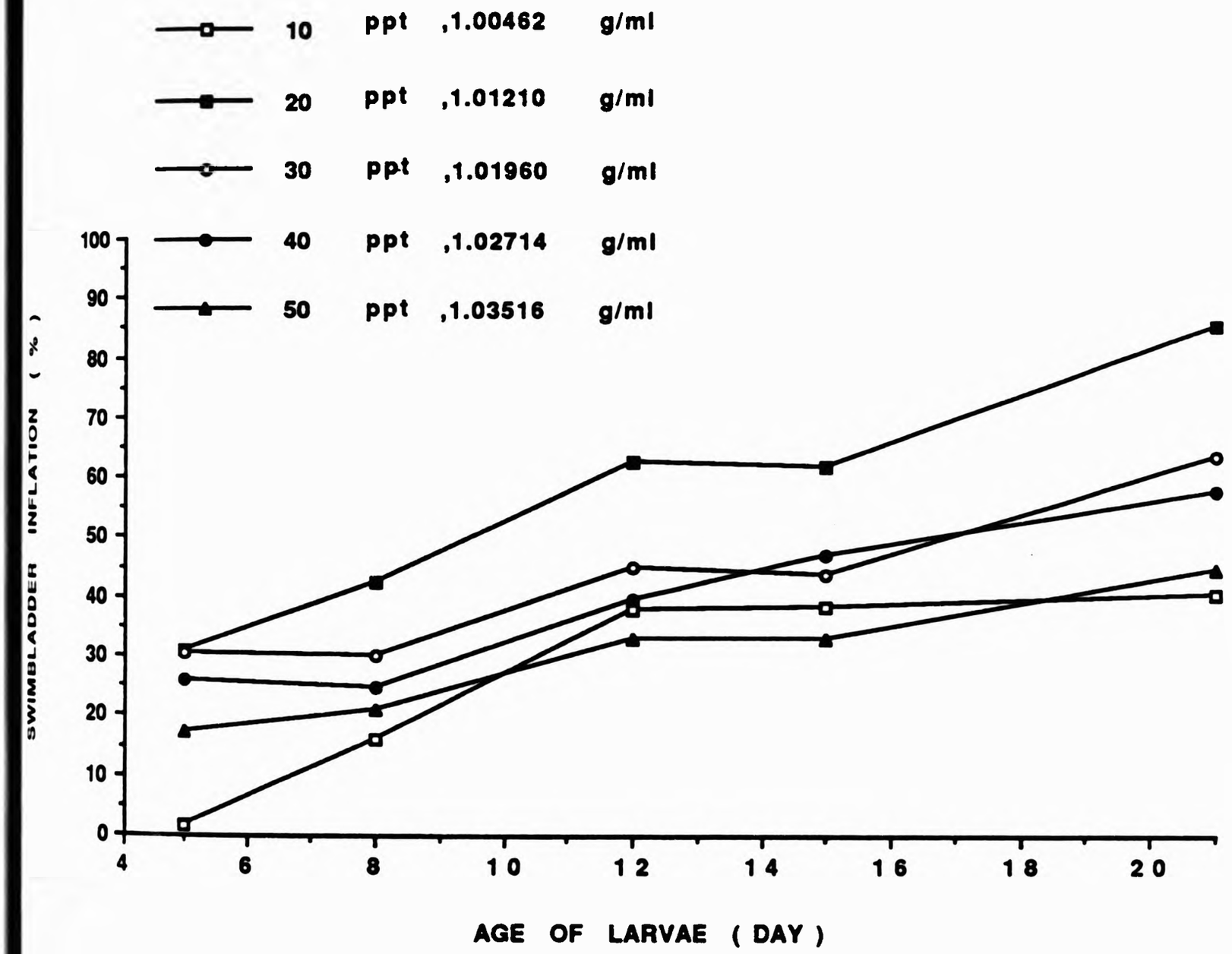


Figure (5.5) Percent swimbladder inflatiCn of *A. cuvieri* larvae reared in different salinities through out the rearing period.

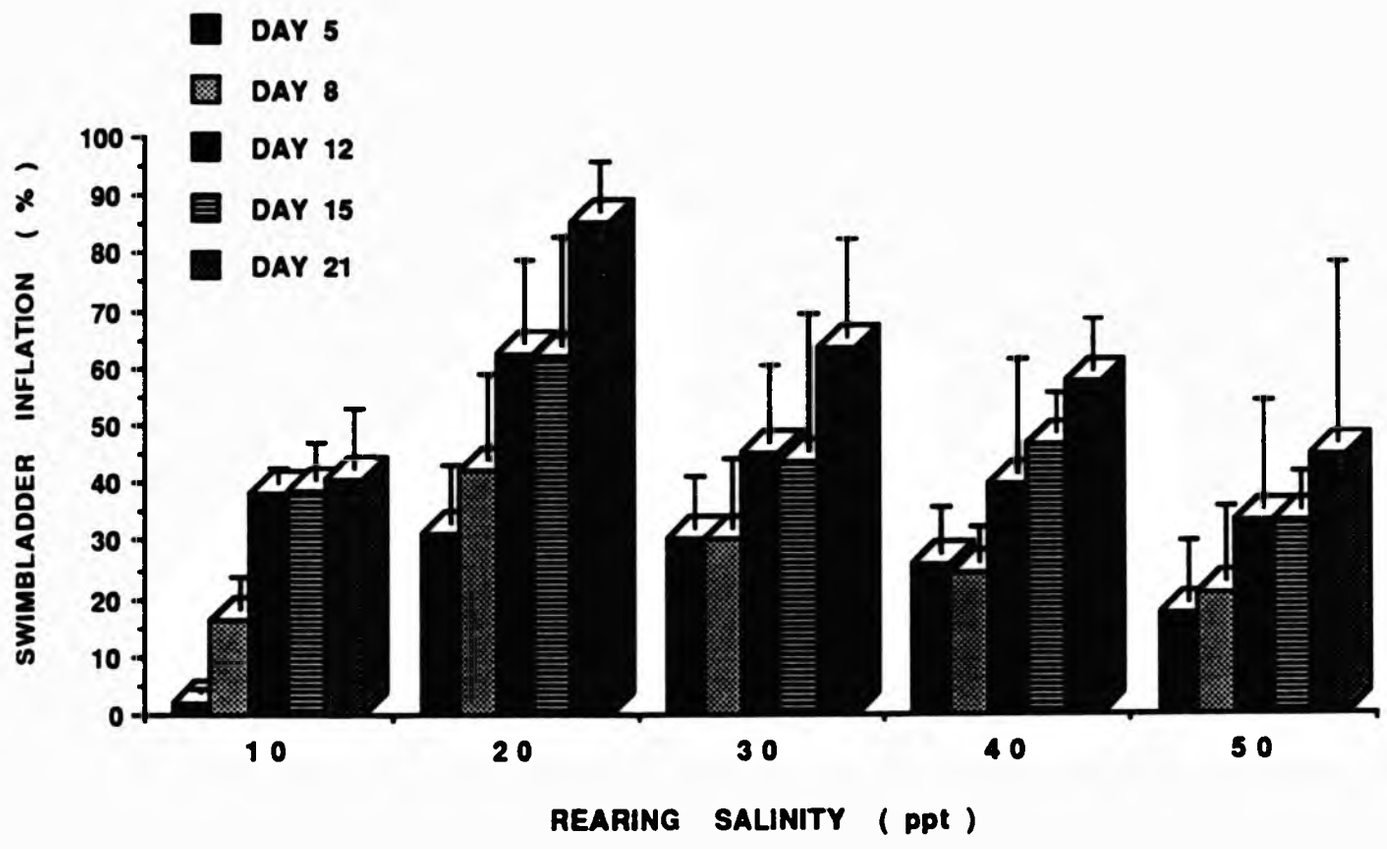


Figure (5.6). The effect of different rearing salinities on swimbladder inflation of *A. cuvieri* larvae at different ages throughout the rearing period. Vertical bars represent one standard deviation.

Table (5.4). Results of the statistical analysis carried out for the data presented in the above figure

Statistical tests	Rearing salinities (ppt)				
	10	20	30	40	50
Day 5					
. One -way ANOVA	Significantly different, P= 0.0024				
. Duncan's test,P<0.05	B	A	A	A	A
Day 8					
. One-way ANOVA	Not statistically different, P>0.05				
Day 12					
. One-way ANOVA	Not statistically different, P>0.05				
Day 15					
. One-way ANOVA	Not statistically different, P>0.05				
Day 21					
. One-way ANOVA	Significantly different, P= 0.021				
. Duncan's test, P<0.05	B	A	B	B	B

The effect of salinity on survival of larvae after 21 days of rearing is shown in Figure 5.7 and analysed statistically in Table 5.5. It can be seen that survival is very poor at 10 ppt and 50 ppt.

The effect of the different salinities on swimbladder volume was examined in two ways, as swimbladder index (swimbladder volume (nl)/ larval body length cubed (mm^3) * 100) at day 21, shown in Figure 5.8 and secondly as swimbladder volume versus total body length of larvae shown in Figure 5.9. Figure 5.8 clearly shows that there is a decrease in swimbladder index at higher salinities, and this is statistically significant (Table 5.6).

The relationship between swimbladder volume at different rearing salinities and total body length is shown in Figure 5.9. It appears that swimbladder volume increases as rearing salinity decreases at any given body length to achieve a buoyancy close to neutral. The slopes of these regression lines were found to be significantly different at $P < 0.05$.

The growth of *A.cuvieri* larvae at different salinities is shown in Figure 5.10. There was a significant difference at $P < 0.05$ between the total body lengths of the larvae measured on day 21 Table 5.7, the highest growth being attained at 20 and 30 ppt.

To summarize, there was no significant difference between swimbladder inflation at 20, 30, 40 ppt and a salinity of 40 ppt is probably the most practical, since it is close to the ambient salinity. 40 ppt also appeared to give a higher total survival, although this was not significantly different from the 20 and 30 ppt due to the high variation between the replicates.

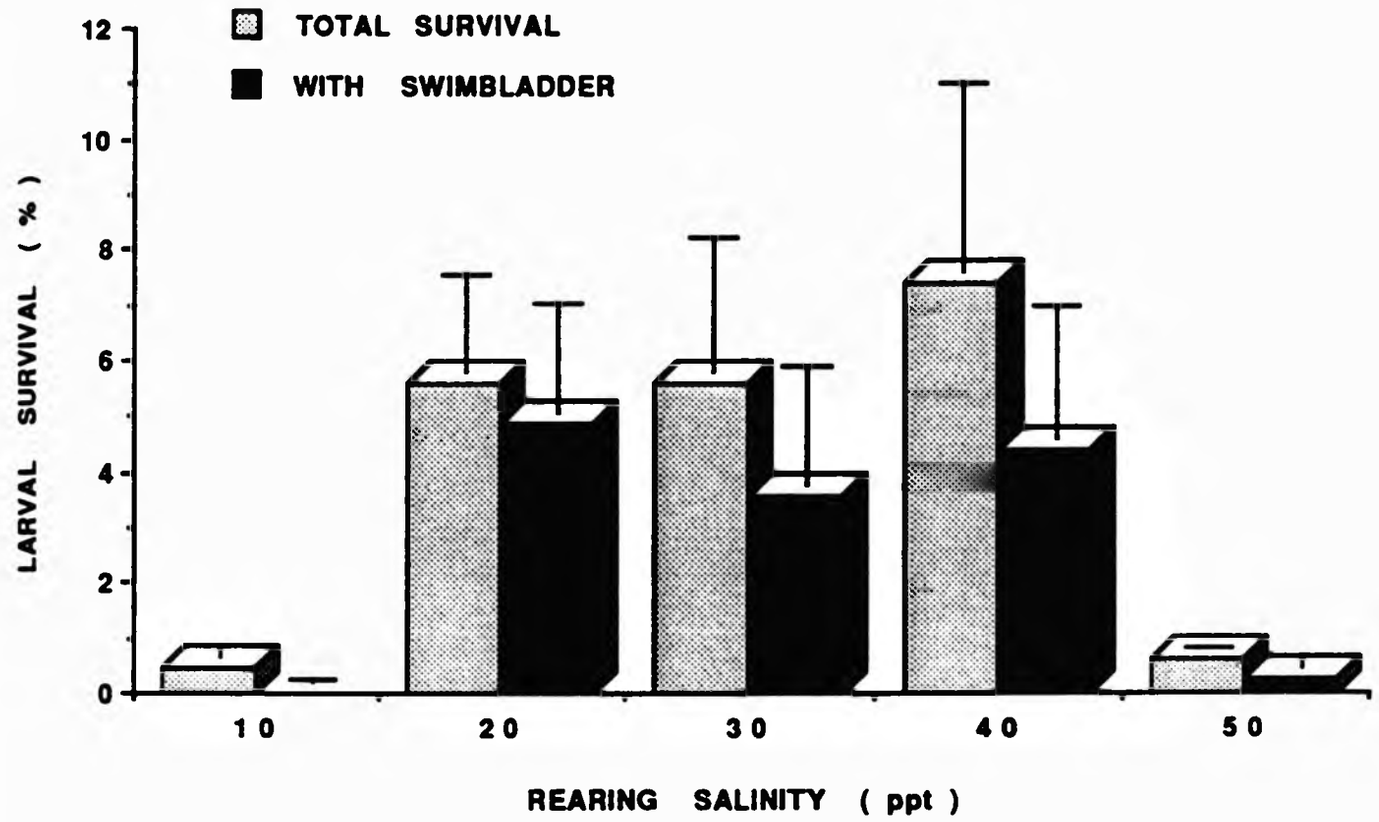


Figure (5.7). The effect of different rearing salinities on larval survival of *A. cuvieri* after 21 days of rearing. Vertical bars represent one standard deviation.

Table (5.5). Results of the statistical analysis carried out for the larval survival data presented in the above figure.

Statistical tests	Rearing salinities (ppt)				
	10	20	30	40	50
Total survival:					
. One-way ANOVA	Significantly different; P=0.001				
. Duncan's test, P<0.05	B	A	A	A	B
With swimbladder:					
. One-way ANOVA	Significantly different; P=0.001				
Duncan's test, P<0.05	B	A	A	A	B

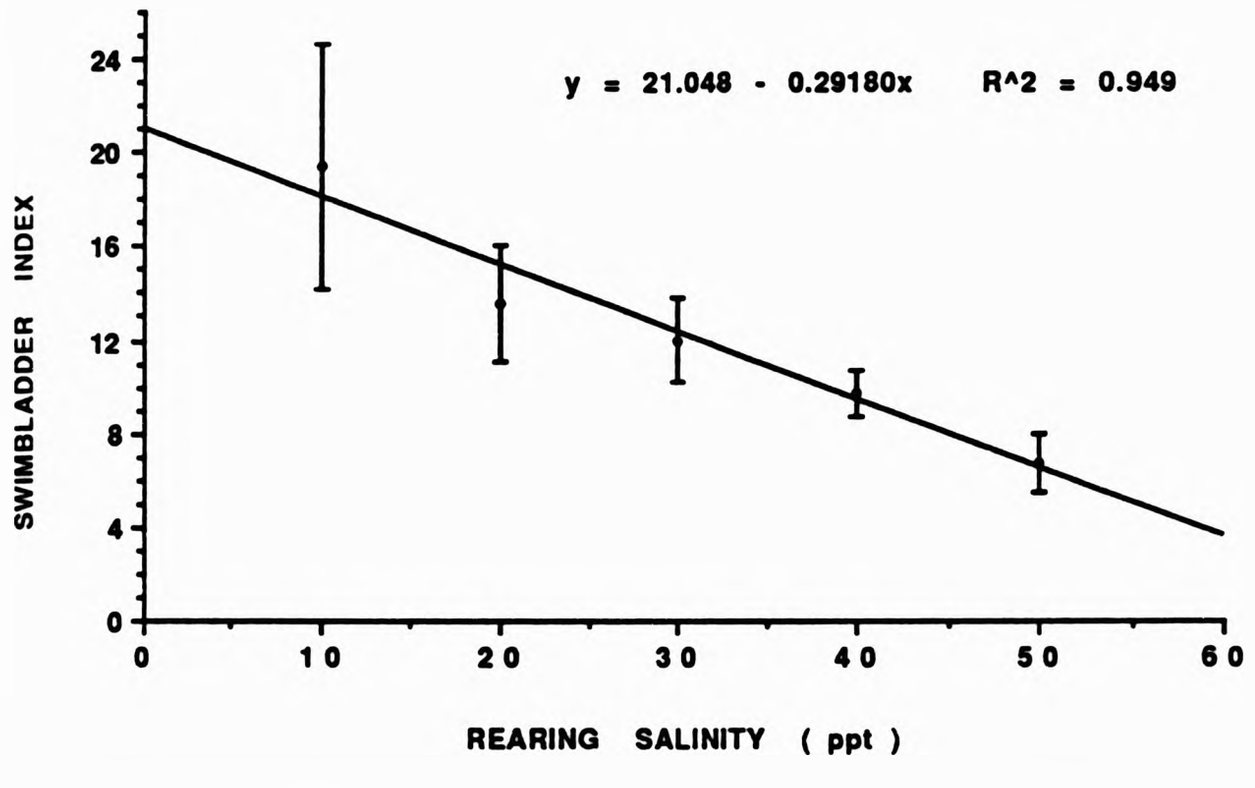


Figure (5.8). The relationship between rearing salinities and swimbladder index of *A. cuvieri* larvae at day 21. Vertical bars represent one standard deviation.

Table (5.6). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Rearing salinities (ppt)				
	10	20	30	40	50
One-way ANOVA	Significantly different; P=0.0012				
Duncan's test, P<0.05	A	B	BC	CD	D

y = - 81.373 + 21.921x R^2 = 0.965 ● sb vol at 10 ppt

y = - 57.841 + 14.241x R^2 = 0.932 ○ sb vol at 20ppt

y = - 53.530 + 12.963x R^2 = 0.912 ■ sb vol at 30 ppt

y = - 43.250 + 10.374x R^2 = 0.917 □ sb vol at 40ppt

y = - 24.383 + 5.8337x R^2 = 0.877 ▲ sb vol at 50 ppt

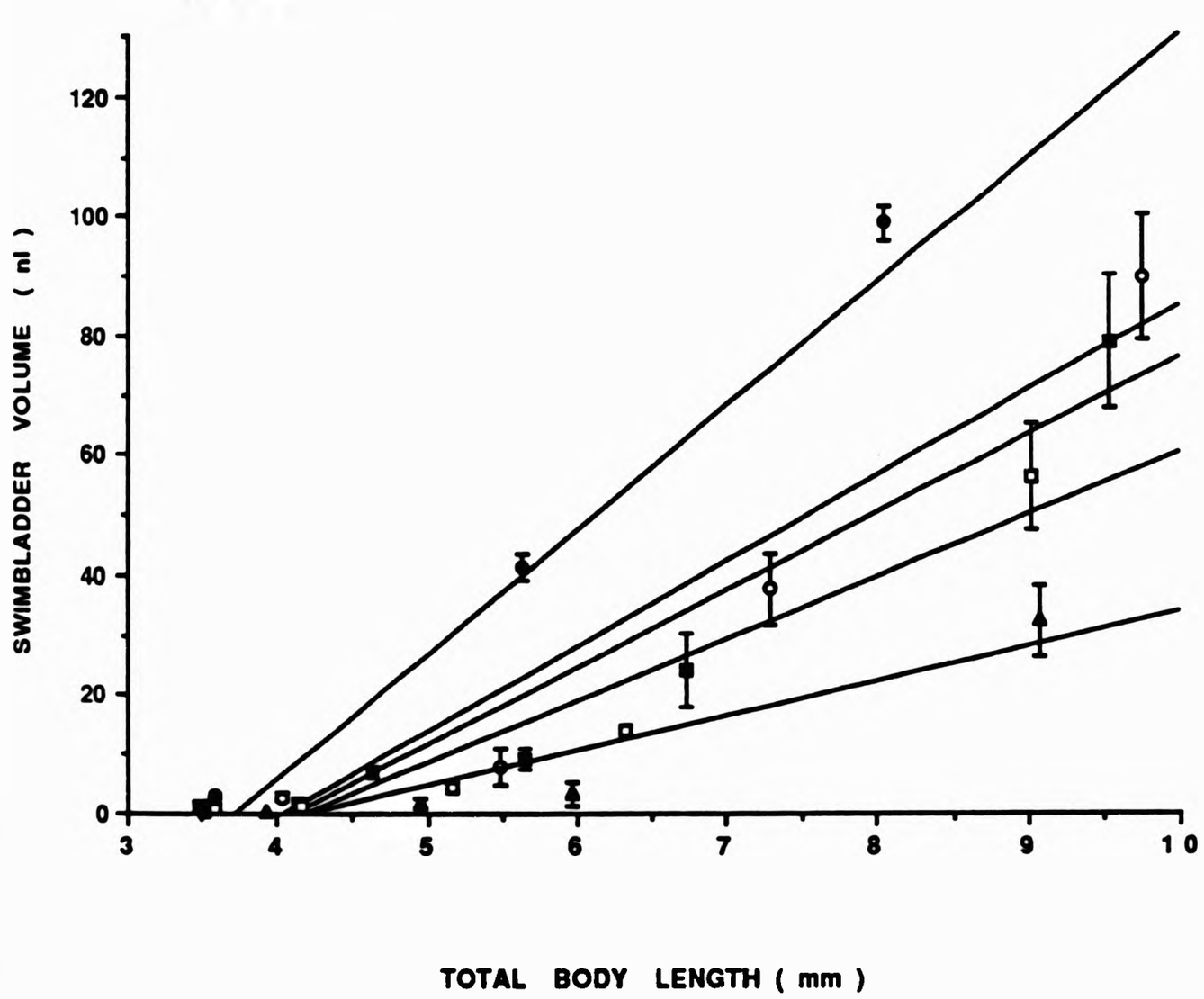


Figure (5.9). The relationships between total body length and swimbladder volume of larvae reared in different salinities.

sb vol = swimbladder volume

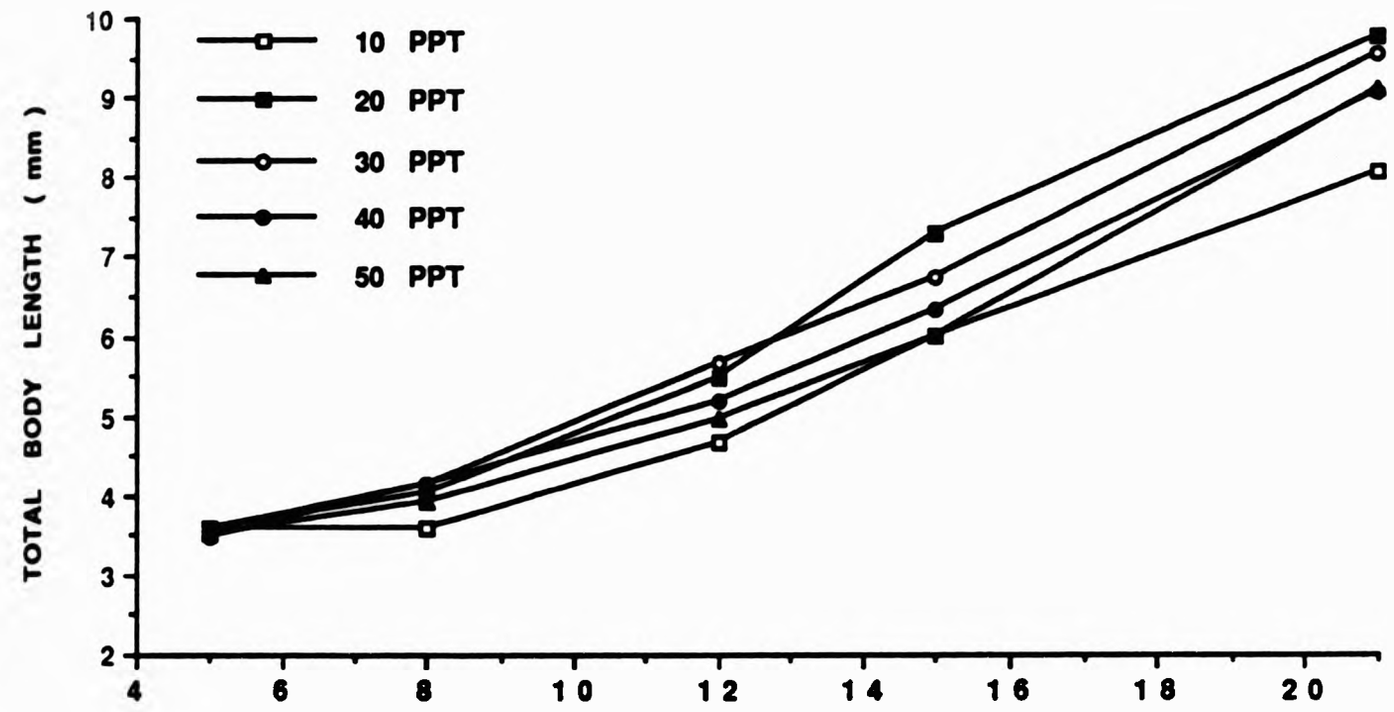


Figure (5.10). Growth in total body length of *A. cuvieri* larvae reared in different salinities over 21 days of culture.

Table (5.7). Results of the statistical analysis carried out for total body length at day 21, presented in the above figure.

Statistical tests	Rearing salinities (ppt)				
	10	20	30	40	50
One-way ANOVA	Significantly different; P=0.030				
Duncan's test, at P<0.05	B	A	A	AB	AB

C. The effect of light intensity on swimbladder inflation and survival.

C.1. Trial in the 1986 season

The effect of the different light intensities used on the percent swimbladder inflation over a period of 21 days is shown in Figure 5.11. An increase in swimbladder inflation was observed at all light intensities up to day 15. After this time it remained stable both at 0-500 Lux and 250 Lux, and showed a slight drop in the other treatments. The highest effect on swimbladder inflation was observed under 250 and 500 Lux. The results of ANOVA presented in Table 5.8 confirms that there is a significant difference ($P < 0.01$) between the tested treatments on day 10, 15, 21.

The effect of the different light intensities on the overall survival of larvae is shown in Figure 5.12. The best survival is at 1750 Lux while at 0-500 Lux it is the lowest in both types of survival namely; total and larvae with swimbladder. Table 5.9 confirms that there is a significant difference between treatments ($P < 0.001$) and that 1750 and 250 Lux give the highest yield of larvae having a functional swimbladder.

The growth of the larvae, plotted against age of larvae is shown in Figure 5.13. Table 5.10 shows that there was no statistical difference between treatments ($P < 0.05$).

C.2. Trial in the 1987 season

This trial differs from that in 1986, in that the larvae used were hatched from egg batches early in the season which are believed to be of a better quality. Another difference is the choice of the light intensities to be tested.

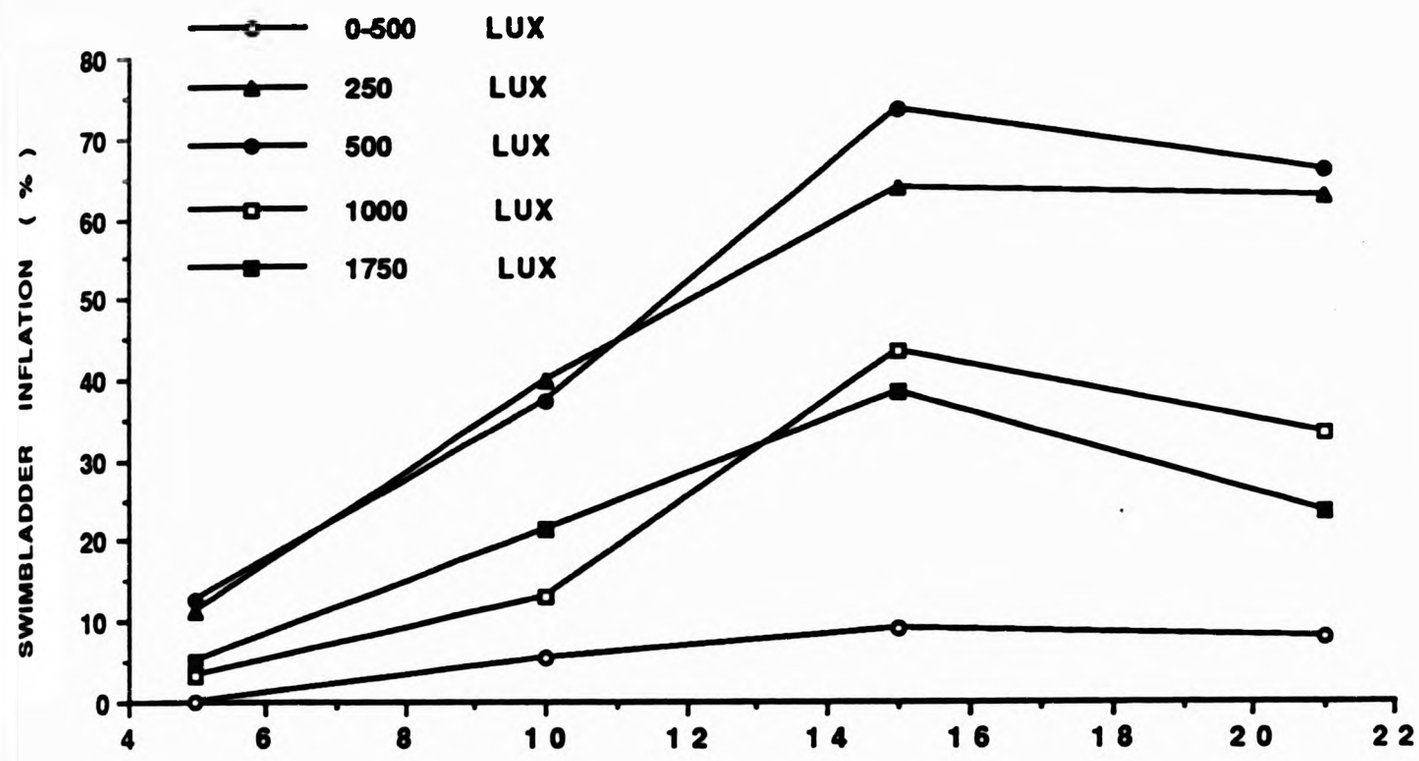


Figure (5.11). Percent swimbladder inflation of *A. cuvieri* larvae reared under different light intensities for 21 days.

Table (5.8). Results of the statistical analysis on the data presented in the above figure.

Statistical tests	Rearing light intensities (lux)				
	0-500	250	500	1000	1750
Day 5					
. One-way ANOVA	No significant difference; P>0.05				
Day 10					
. One-way ANOVA	Significantly different; P=0.0025				
. Duncan's test, P<0.05	C	A	A	BC	AB
Day 15					
. One-way ANOVA	Significantly different; P=0.0001				
. Duncan's test, P<0.05	D	AB	A	BC	C
Day 21					
. One-way ANOVA	Significantly different; P=0.0003				
. Duncan's test, P<0.05	C	A	A	B	BC

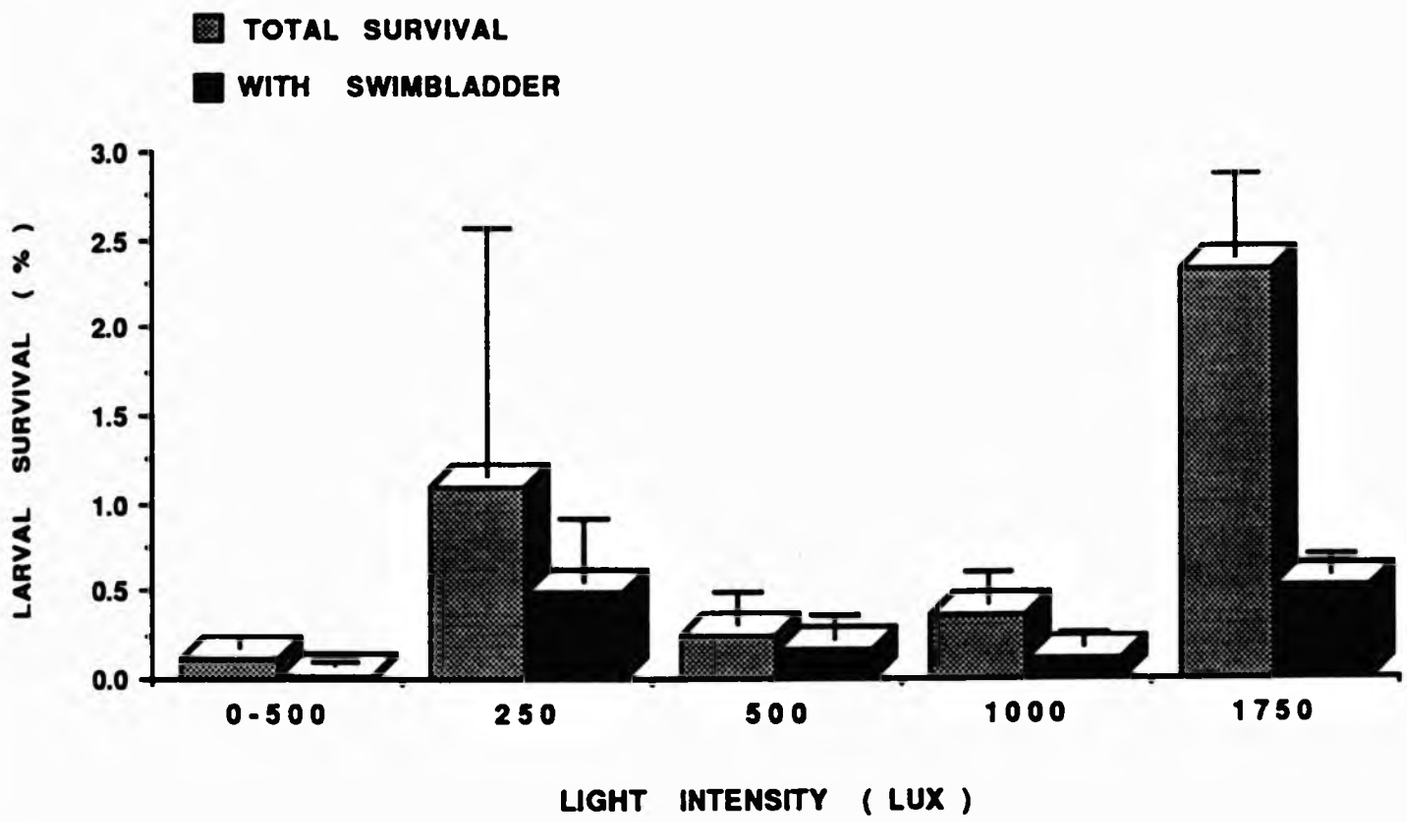


Figure (5.12). The effect of light intensity on larval survival of *A. cuvieri* reared for 21 days. Vertical bars represent one standard deviation.

Table (5.9). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Rearing light intensities (lux)				
	0–500	250	500	1000	1750
Total survival					
. One-way ANOVA	Significantly different, p=0.0003				
. Duncan's test, p<0.05	C	B	C	BC	A
With swimbladder					
. One-way ANOVA	Significantly different, p=0.0002				
. Duncan's test, p<0.05	C	A	B	BC	A

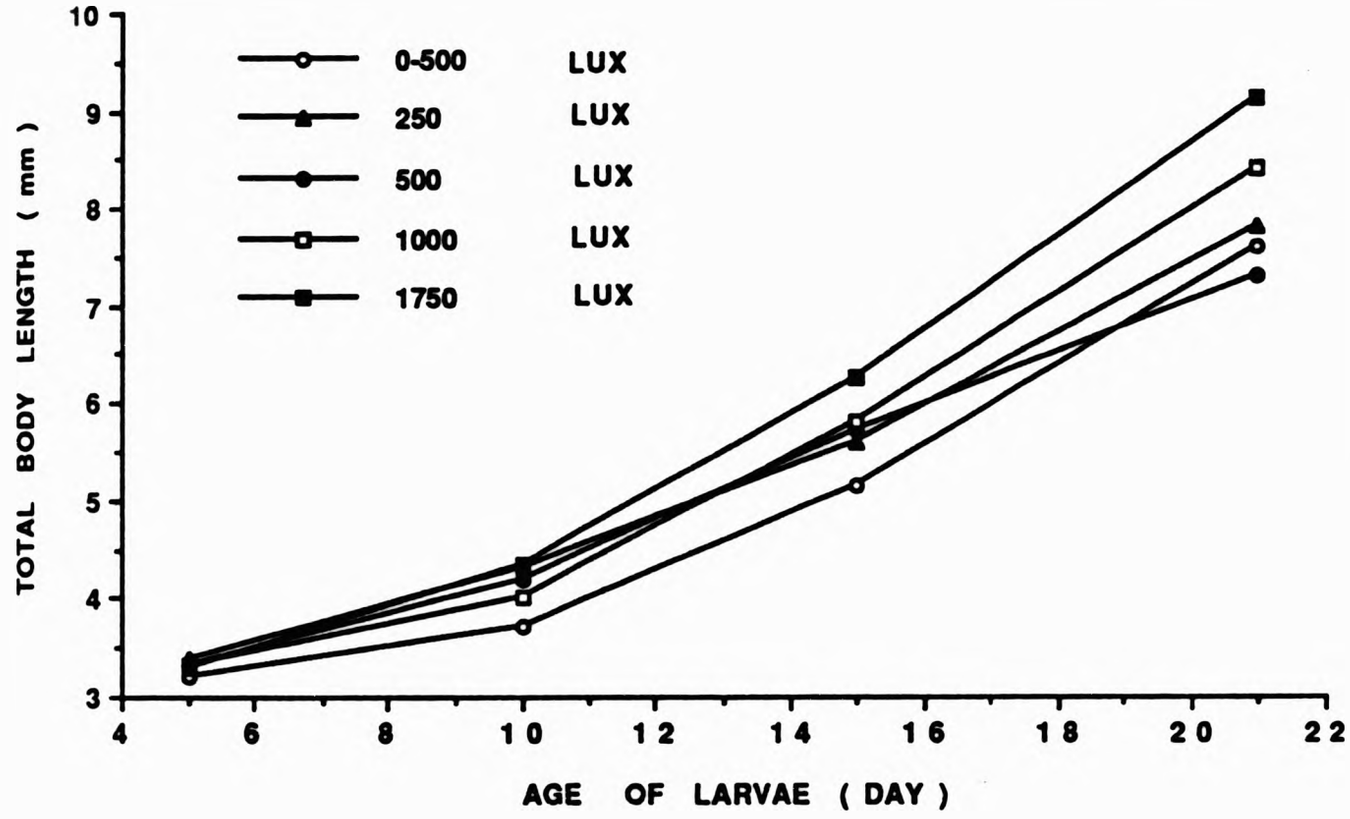


Figure (5.13). Growth in total body length of *A. cuvieri* larvae reared under different light intensities.

Table (5.10). Results of the statistical analysis carried out for data of total body length at Day 21 presented in the above figure.

Statistical test	light intensities (lux)				
	0-500	250	500	1000	1750
One-way ANOVA	Not statistically different; p>0.05				

Percent swimbladder inflation on day 7 and 16 is summarised in Figure 5.14. It is clear that most of the swimbladder inflation occurred before or at the 7th day of age with little further increase to day 16. Overall, swimbladder inflation was greater than in the 1986 season. The swimbladder inflation is clearly inversely related to the increase in light intensity. Table 5.11 confirms that there is a significant difference between the treatments at $P < 0.05$ and that 250 Lux and 1000 Lux have the strongest effect on initial swimbladder inflation.

The effect of light intensity on both type of larval survival is shown in Figure 5.15. Even though the total survival at 5000 Lux appears to be the highest, this could not be confirmed statistically due to the high variation between replicates in treatments at 250 and 1000 Lux (Table 5.12).

The effect of light intensity on the initiation of feeding and inflation of the swimbladder by day 6 is shown in Figure 5.16. The number of feeding larvae that possessed a functional swimbladder were found to be the highest at 250 Lux. This is significantly different from all other results ($P < 0.01$), which are insigficantly different from each other (Table 5.13). Although the number of feeding and non-feeding larvae without a functional swimbladder seems to increase with the increase in light intensity, the statistical analysis (Table 5.13) shows that only the non-feeding larvae are significantly different ($P < 0.05$). The 2000 and 5000 Lux treatments have the highest numbers of larvae without a functional swimbladder.

The growth in total body length of larvae reared under different light intensities is shown in Figure 5.17. The results are not significantly different ($P>0.05$).

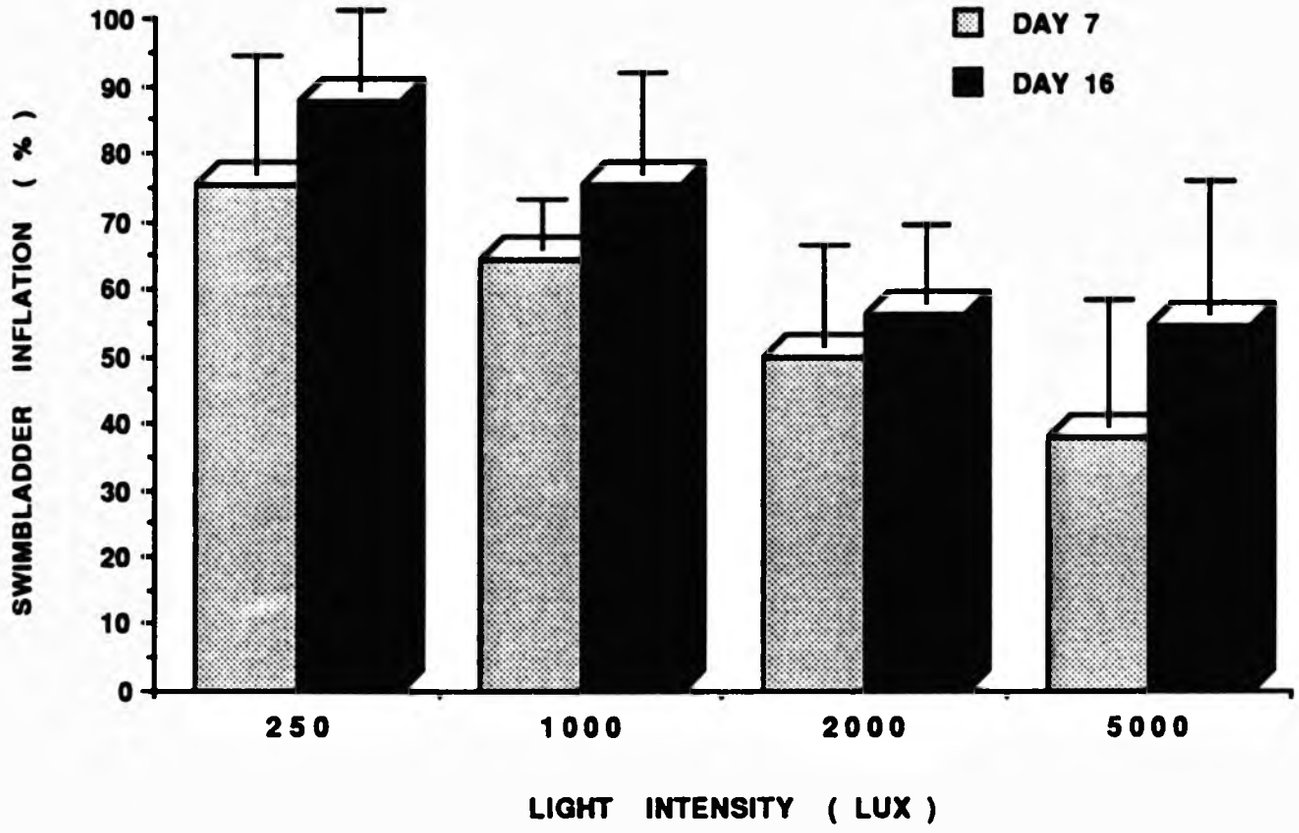


Figure (5.14). Swimbladder inflation of *A. cuvieri* larvae reared at different light intensities for 16 day. Vertical bars represent one standard deviation.

Table (5.11). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Rearing light intensities (lux)			
	250	1000	2000	5000
Day 7				
. One-way ANOVA	Significantly different,			P=0.023
. Duncan's test, at P<0.05	A	AB	C	BC
Day 16				
. One-way ANOVA	Significantly different,			P=0.018
. Duncan's test, at P<0.05	A	AB	B	B

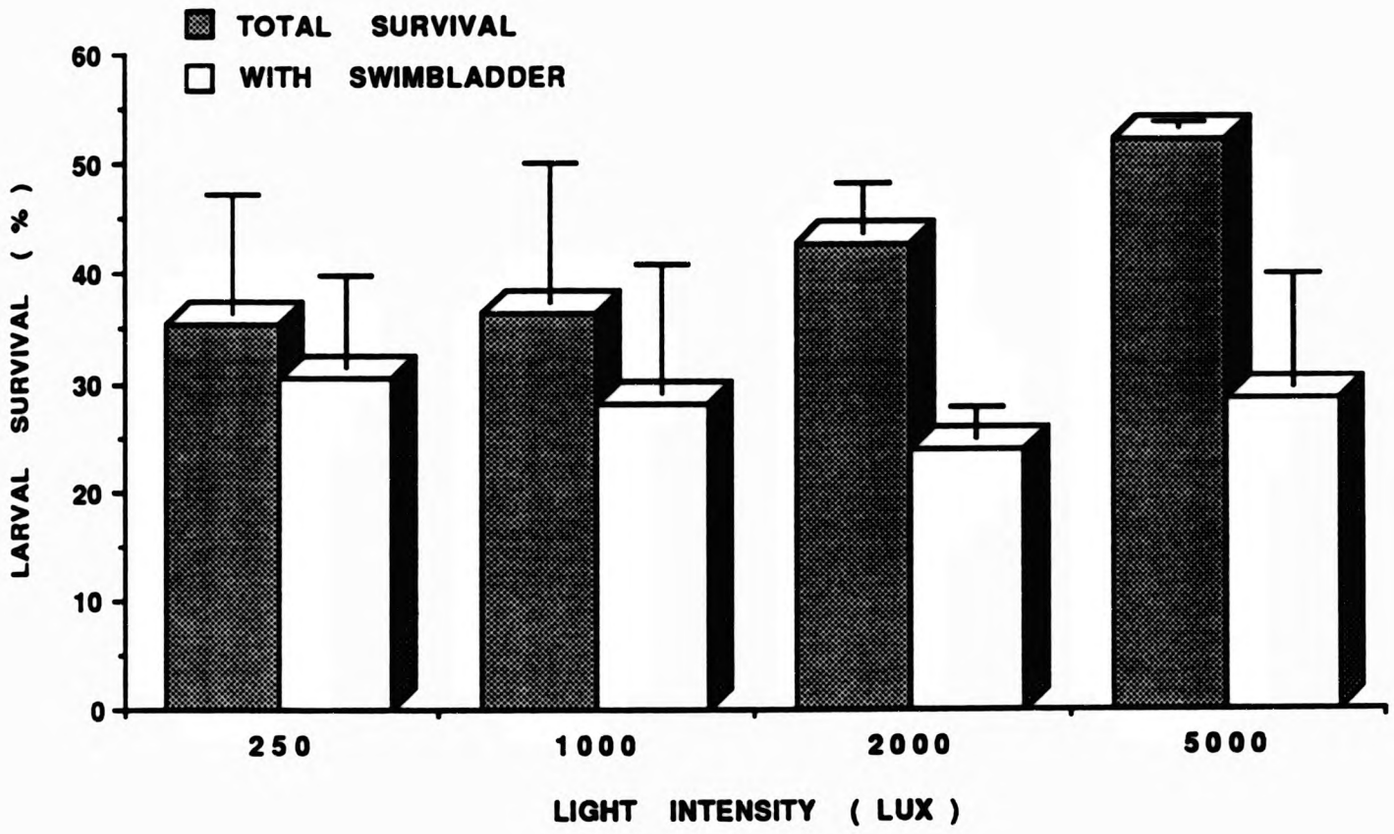


Figure (5.15). The effect of the rearing light intensity on larval survival of *A. cuvieri* larvae reared for 16 days. Vertical bars represent one standard deviation.

Table (5.12). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Rearing light intensities (lux)			
	250	1000	2000	5000
Total survival				
. One-way ANOVA	No statistical difference; P>0.05			
With Swimbladder				
. One-way ANOVA	No statistical difference; P>0.05			

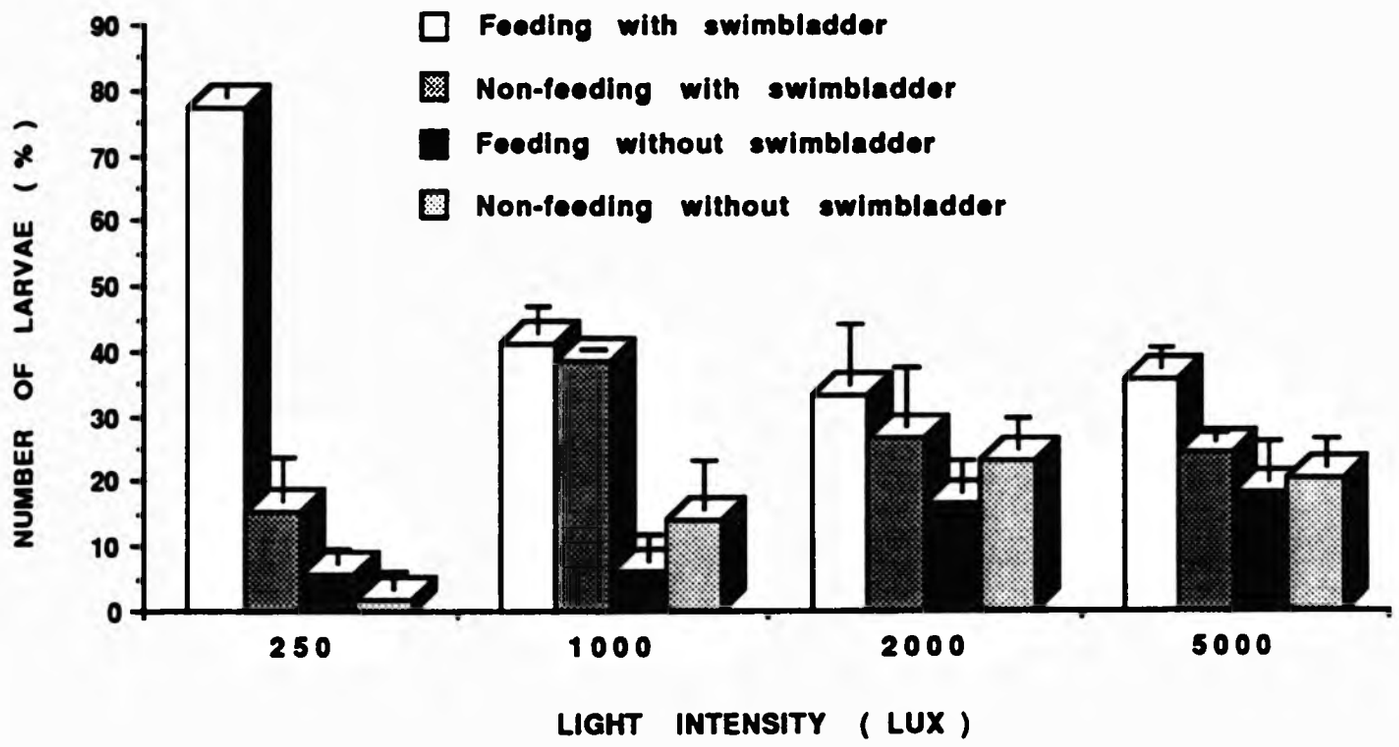


Figure (5.16). The effect of different rearing light intensities on initial feeding and swimbladder inflation of 6 day old *A. cuvieri* larvae. Vertical bars represent one standard deviation.

Table (5.13). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Light intensities (lux)			
	250	1000	2000	5000
Feeding with swimbladder:				
. One-way ANOVA	Significantly different,			P=0.0033
. Duncan's test, at P<0.05	A	B	B	B
Non-feeding with swimbladder:				
. One-way ANOVA	Not significant; P>0.05			
Feeding without swimbladder:				
. One-way ANOVA	Not significant; P>0.05			
Non-feeding and without swimbladder:				
. One-way ANOVA	Significantly different,			P=0.047
. Duncan's test, at P<0.05	B	AB	A	A

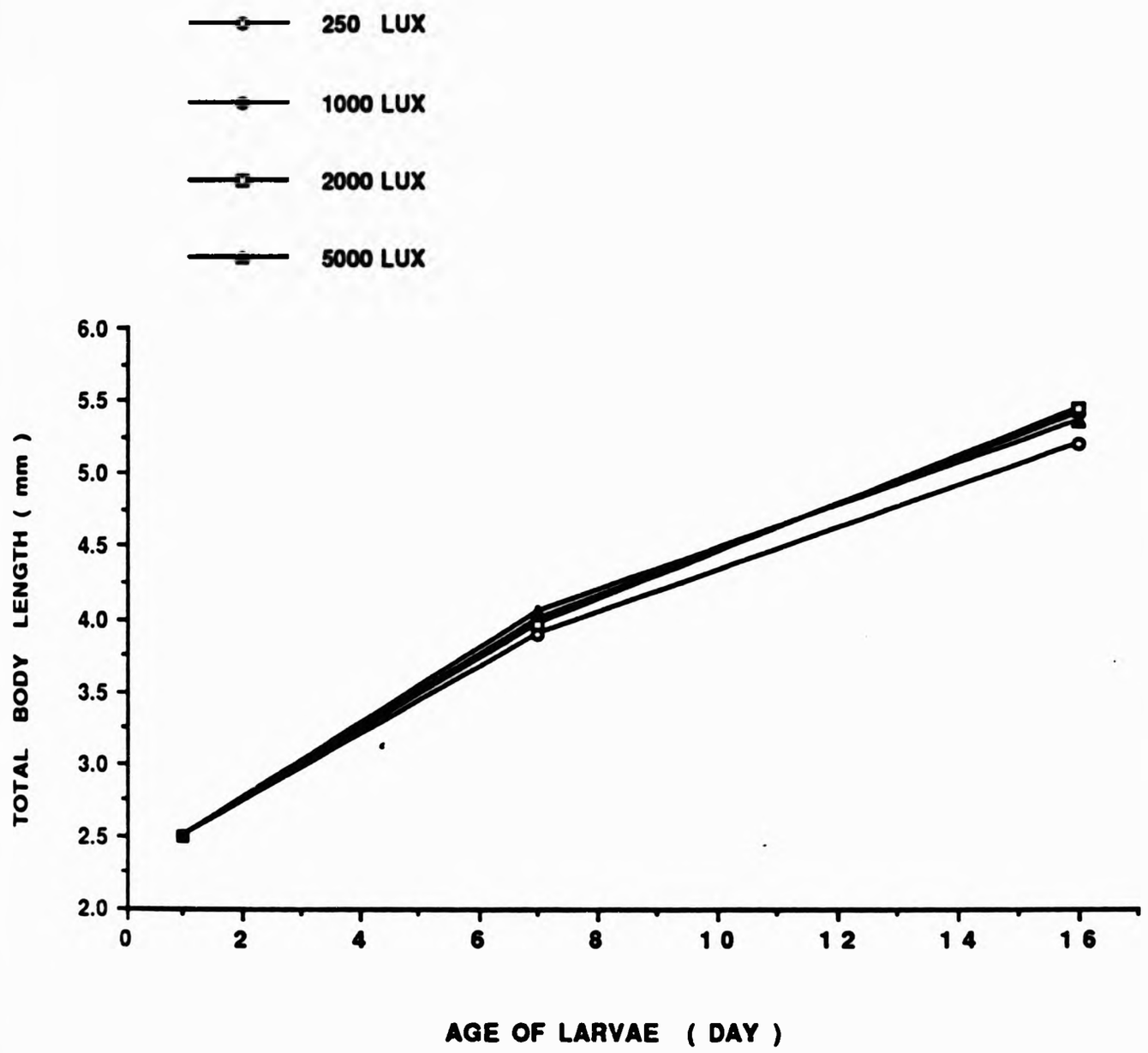


Figure (5.17). Growth in total body length of *A. cuvieri* larvae reared under different light intensities.

In summary, it can be said that a low light intensity gave better swimbladder inflation and that the different quality of larvae used in the 1986 and 1987 season responded differently to the application of the light intensity treatments tested. The low quality larvae seem to be more affected by the higher light intensities used, since the difference between the results is greater in the 1986 trials.

C 3. Larval vertical movements under different light intensities

The effect of light intensity on the vertical movements of larvae is illustrated in Figure 5.18. The dark (0 Lux) treatment (1A and 1B) on day 3 and 4 had similar effect on the larvae as the 250 Lux treatment (2A and 2B) where larvae stay near surface, aggregating in the top layer . On day 5 and 6 (1C and 1D), the larvae kept in darkness showed a night distribution behaviour as was explained in chapter 4. The distribution of the larvae at 250 Lux did not change much from day 3 to 6 (2A-2D), except for a slightly denser aggregation on day 5 (2C).

At 1000 Lux the larvae tend to occupy the top half of the observation column , with denser aggregation of larvae in the top layers on day 4 and 5 (3B and 3C). At higher light intensities, the larvae occupied progressively more of the water column (4, 5 and 6, A-D). At the highest light intensity of 10,000 Lux the larvae cleared the upper 5cm of the water column, suggesting an aversion to very bright light.

D. The effect of photoperiod on swimbladder inflation and survival.

The inflation of the swimbladder in larvae reared under different photoperiod regimes is shown in Figure 5.19. 24hr light gave the highest inflation and this was significantly different ($P < 0.01$) from the other photoperiod regimes Table 5.14.

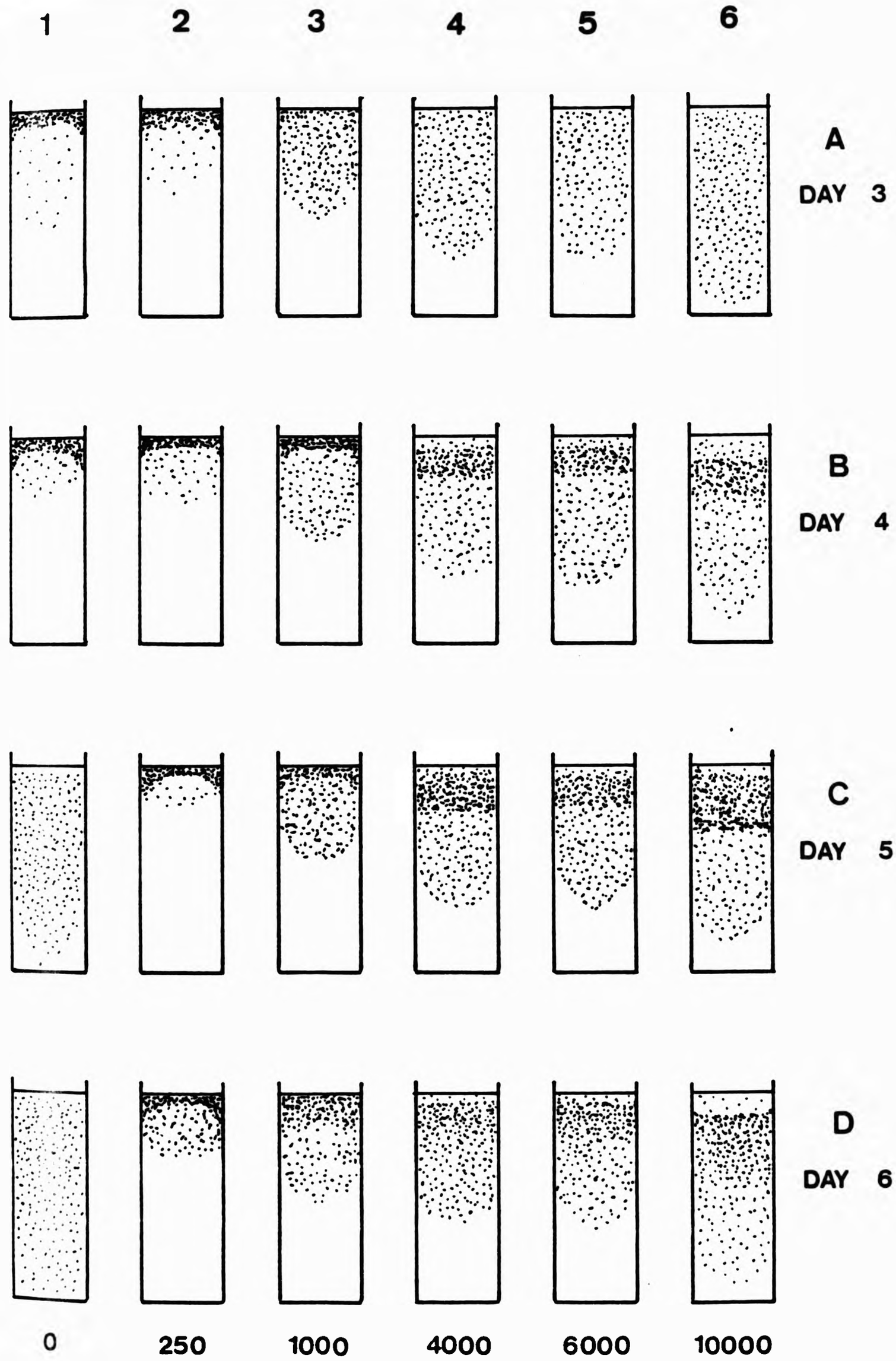


Figure (5.18). Larval migration of *A. cuvieri* under different light intensities (lux) in one meter column of water, from day 3 to 6 after hatching.

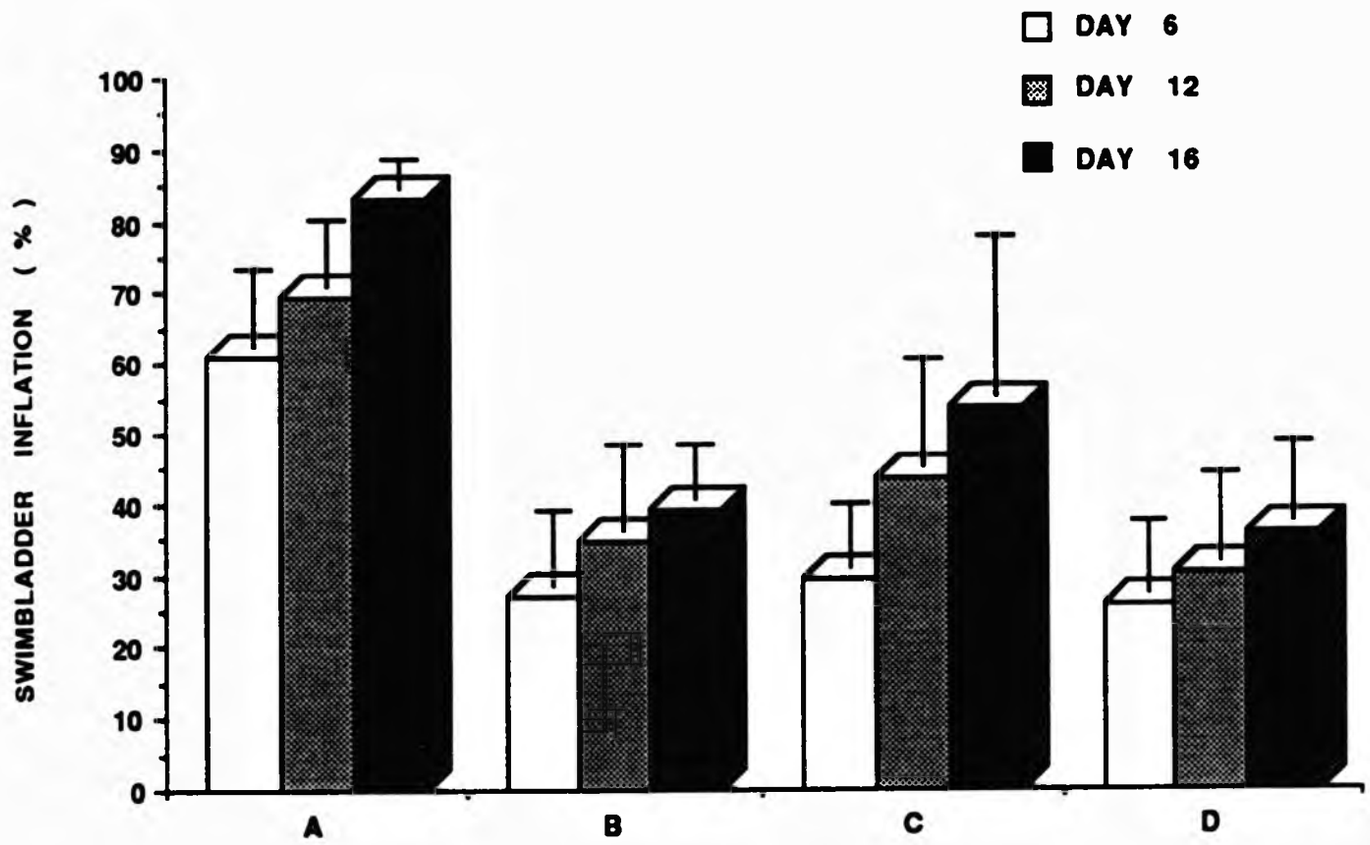


Figure (5.19) The effect of photoperiod regime on percent swimbladder inflation of *A. cuvieri* larvae during a 16 days rearing period.
A; 24hr light.
B; 14hr light and 10hr dark.
C; dark during first three days, then 14hr light, and 10hr dark.
D; dark during first three days, then 24hr light.

Vertical bars represent one standard deviation.

Table (5.14). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Rearing photoperiod regime			
	A	B	C	D
Day 6				
. One-way ANOVA	Significantly different,			P=0.001
. Duncan's test, at P<0.05	E	F	F	F
Day 12				
. One-way ANOVA	Significantly different,			P=0.033
. Duncan's test, at P<0.05	E	F	F	F
Day 16				
. One-way ANOVA	Significantly different,			P=0.0011
. Duncan's test, at P<0.05	E	F	F	F

The survival of larvae under these different photoperiods is summarised in Figure 5.20. Again in both categories of larval survival the 24hr light treatment was found to have the best effect ($P < 0.01$), Table 5.15.

The growth of larvae under the different photoperiod regimes is shown in Figure 5.21. It is clear that more normal photoperiods gave better growth (Table 5.16).

To summarize, it is clear that the 24hr light photoperiod is the one to be selected to achieve best inflation, although growth performance of larvae was slightly poorer.

E. <u>The effect of aeration rate on swimbladder inflation and survival</u>.

The effects of different aeration rates on initial swimbladder inflation are shown in Figure 5.22. The 50-70 ml/min treatment with silicon anti-foaming film resulted in zero inflation of swimbladder in all replicates on all sampling days and showed clearly that access to the air is required. The 200-300 ml/min rate with air access gave a good inflation rate and aeration at 50-70 ml/min with an open water surface gave a slight improvement. The high aeration rate at 1600-1800 ml/min gave a very marked reduction effect on swimbladder inflation. In Figure 5.23, the percent of initial swimbladder inflation is shown on selected days. The effect of the aeration rates tested was found to be significantly different ($P < 0.001$), Table 5.17.

The survival of larvae reared in different aeration rates is shown in Figure 5.24. Table 5.18 confirms that there are significant differences between the different survival percentage attained ($P < 0.001$) with the best total survival, and survival of larvae with a functional swimbladder being

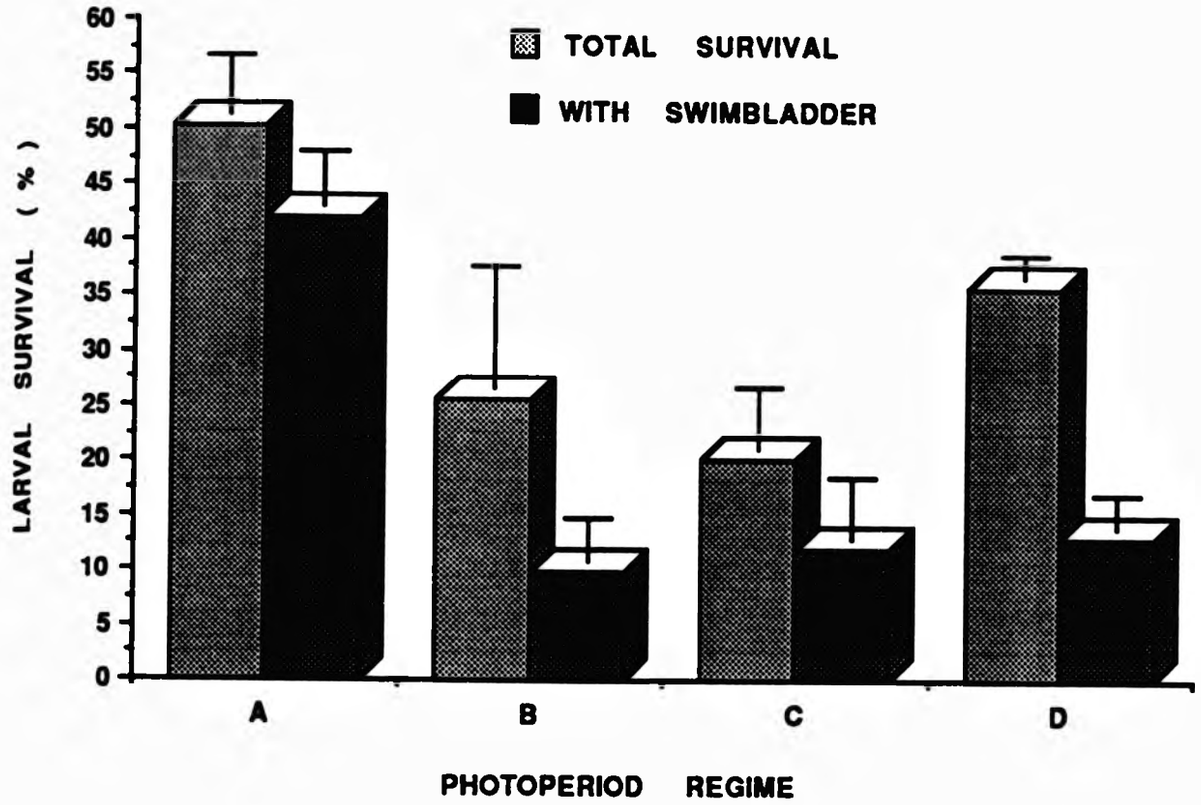


Figure (5.20) The effect of photoperiod regimes on larval survival of *A. cuvieri* reared for a period of 16 days. Vertical bars represent one standard deviation. The given letters for treatments are similar to figure 5.19.

Table (5.15). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Rearing photoperiod regimes			
	A	B	C	D
Total survival				
. One-way ANOVA	Significantly different,			P=0.008
. Duncan's test, at P<0.05	E	FG	G	F
With swimbladder				
. One-way ANOVA	Significantly different,			P=0.0001
. Duncan's test, at P<0.05	E	F	F	F

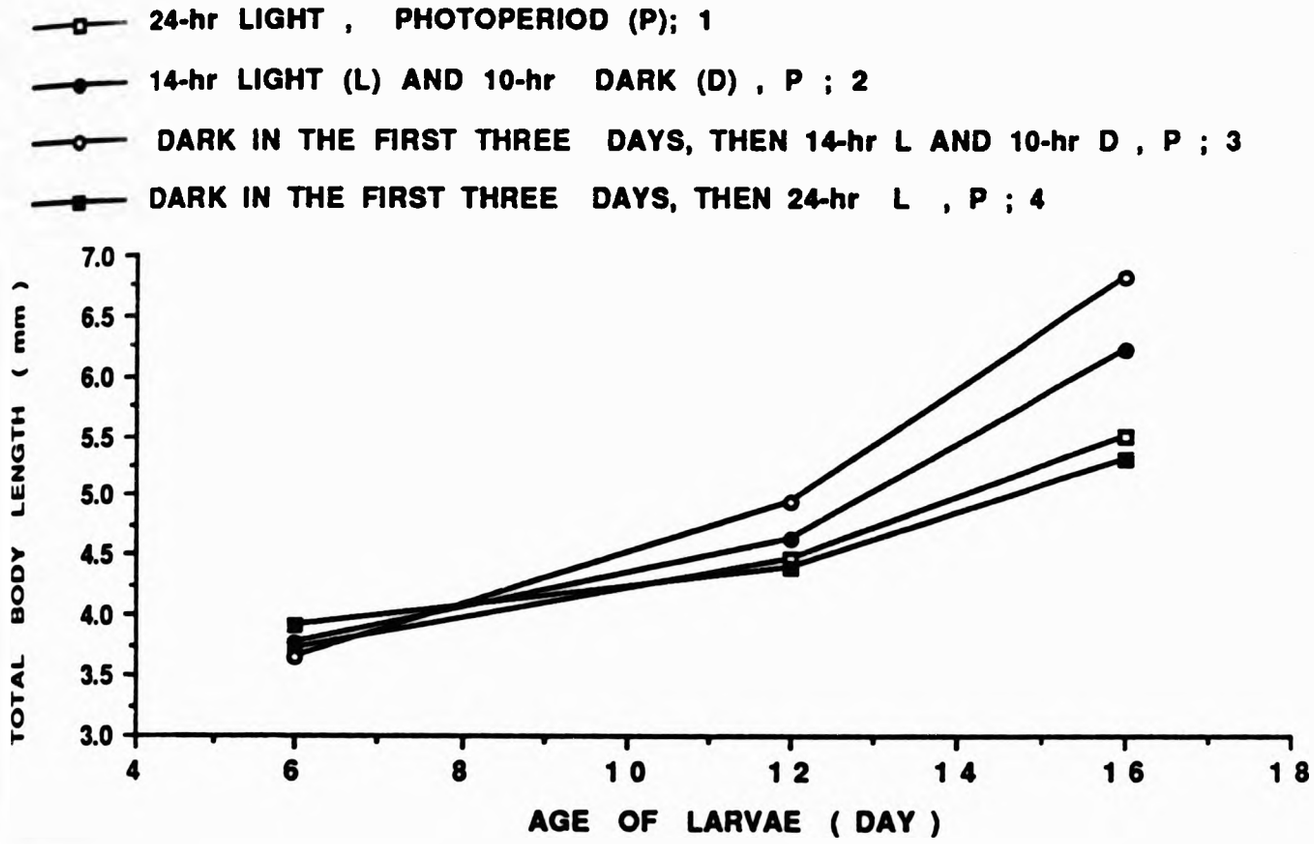


Figure (5.21) Growth in total body length of *A. cuvieri* larvae reared under different photoperiod regime.

Table (5.16). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Rearing photoperiods			
	1	2	3	4
Day 16				
. One-way ANOVA	Significantly different, P=0.0009			
. Duncan's test, at P<0.05	B	A	A	B

—●— 50-70 ml/min, covered with Silicon-based surfactant ;Treatment(T):1

—■— No aeration , open water surface ; T:2

—▲— 50-70 ml/min, open water surface ;T :3

—□— 200-300 ml/min, open water surface ; T:4

—○— 1600-1800 ml/min, open water surface ; T:5

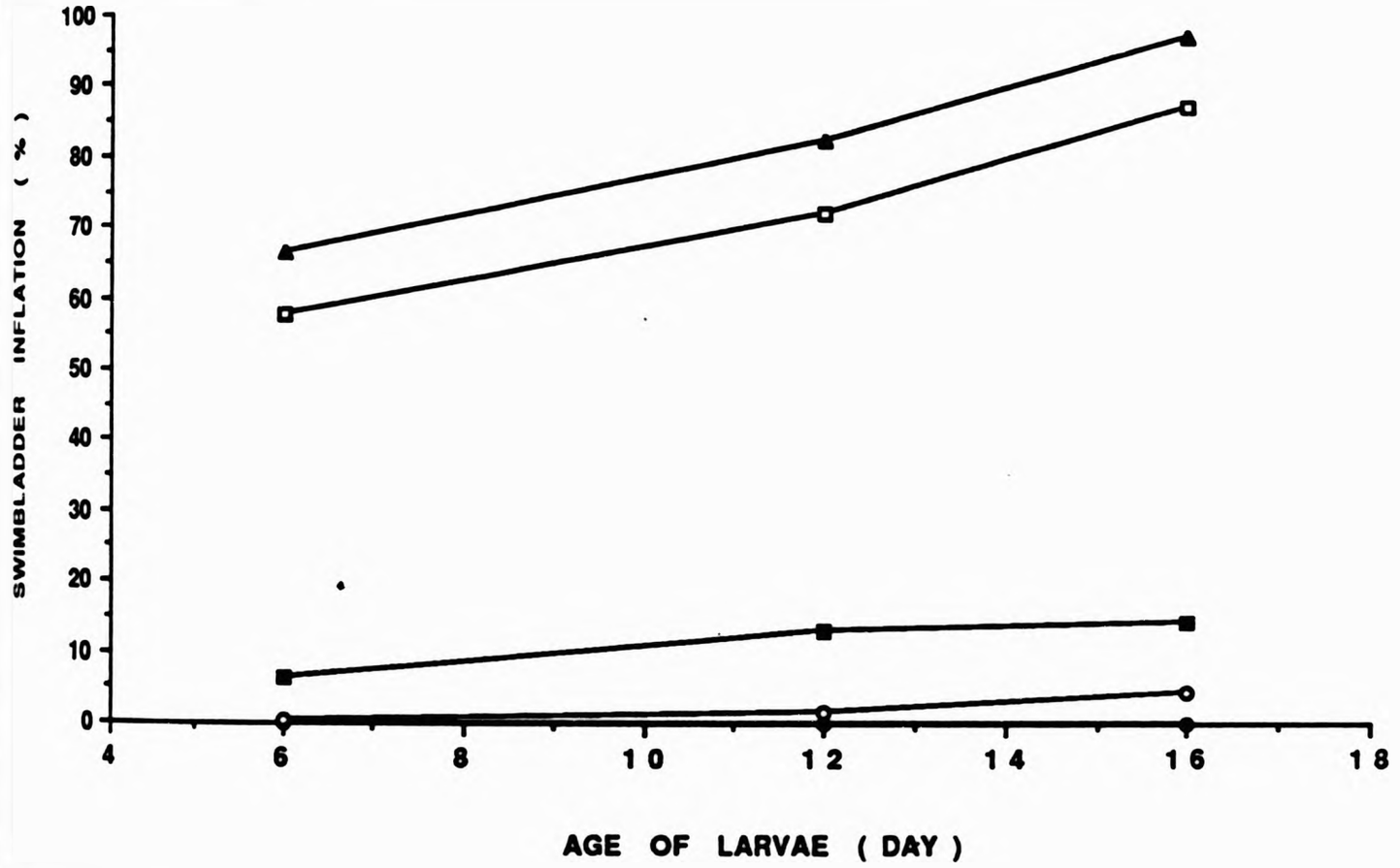


Figure (5.22). Relationship between percent swimbladder inflation and age of *A. cuvieri* larvae reared in different aeration rates.

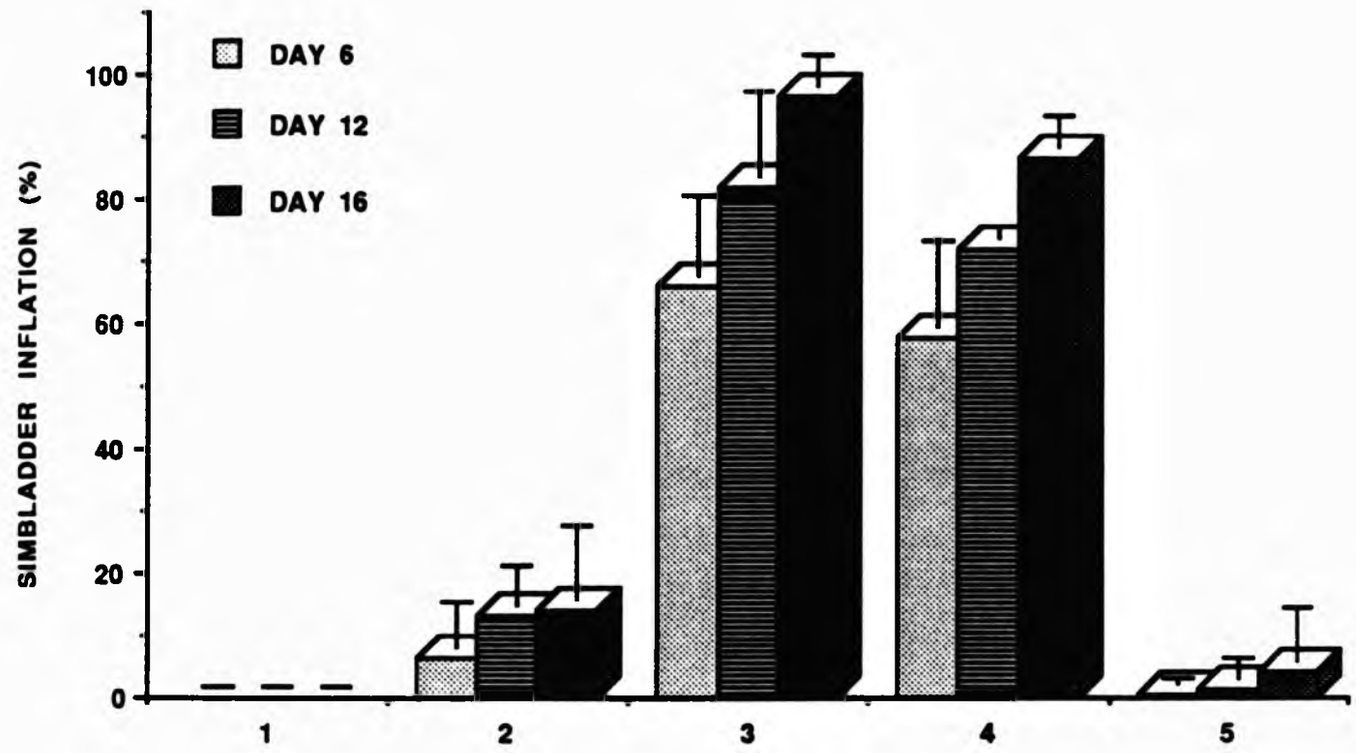


Figure (5.23). Percent swimbladder inflation of *A. cuvieri* larvae reared in different aeration rates. The aeration rate treatments are given in Figur 5.22. Vertical bars represent one standard deviation.

Table (5.17). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Aeration rate treatments				
	1	2	3	4	5
Day 6					
. One -way ANOVA	Significantly different, P= 0.0001				
. Duncan's test,P<0.05	B	B	A	A	B
Day 12					
. One -way ANOVA	Significantly different, P= 0.0001				
. Duncan's test,P<0.05	D	C	A	B	D
Day 16					
. One -way ANOVA	Significantly different, P= 0.0001				
. Duncan's test,P<0.05	C	B	A	A	BC

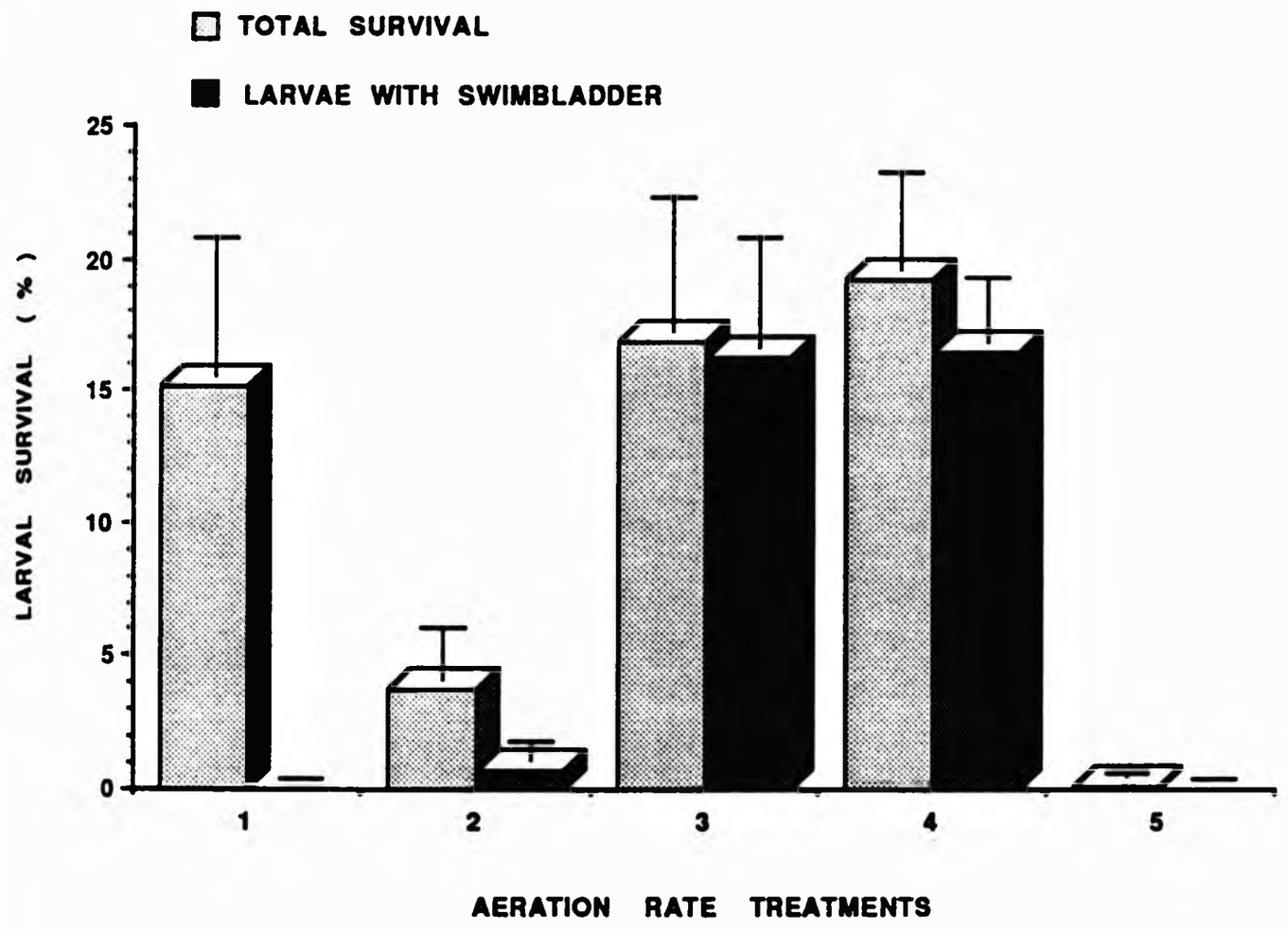


Figure (5.24). Effect of aeration rate on the survival of *A. cuvieri* larvae after 16 days. The aeration rate treatments are given in figure (5.22). Vertical bars represent one standard deviation.

Table (5.18). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Aeration rate treatments				
	1	2	3	4	5
Total survival					
. One-way ANOVA	Significantly different, P= 0.0001				
. Duncan's test,P<0.05	A	B	A	A	B
With swimbladder					
. One-way ANOVA	Significantly different, P= 0.0001				
. Duncan's test,P<0.05	B	B	A	A	B

from the 50-70 ml/min (3) and 200-300 ml/min (4) treatments both with an open water surface.

The dissolved oxygen measured in the different treatment tanks over the 16 days rearing period is shown in Figure 5.25. Three levels of dissolved oxygen can be seen to occur among the treatments, high, medium and low. These three levels of dissolved oxygen are found to be significantly different at $P < 0.001$ and grouped into A,B and C as shown in Table (5.19).

The growth of larvae reared with different aeration rates, shown in Figure 5.26 was not significantly different between treatments.

To conclude, there was a significant difference in the effect of aeration rate on the initial swimbladder inflation and larval survival. The aeration rate producing the maximum number of larvae with a functional swimbladder and acceptable growth rate was found to be the 50-70 ml/min treatment (with a open water surface).

F. <u>The effect of water-exchange on swimbladder inflation and survival</u>

Swimbladder inflation was best when there was some water exchange (Figure 5.27). The static treatment showed a steeper slope than the other treatments after day 6 indicating that the flow-through system enhanced the initial inflation of swimbladders very early in the larvae of age 3 to 6 days after hatching.

The effect of the water exchange on larval survival is summarised in Figure 5.28. All treatments were statistically different (Table 5.20) the best effect on larval survival of both types being obtained from the 8hr flow treatment, while the worst was in the static water regime.

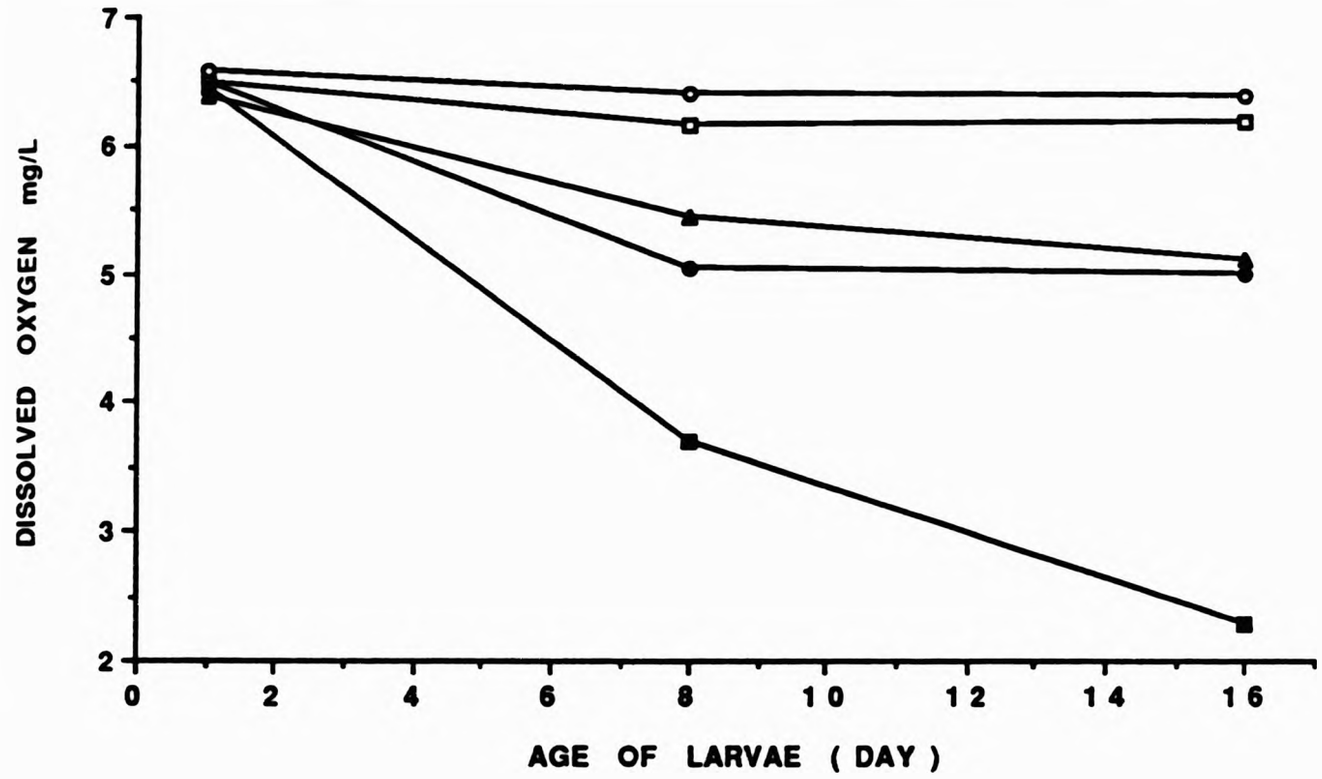


Figure (5.25). Dissolved oxygen measurements during rearing *A. cuvieri* larvae reared in different aeration rates. The aeration treatments are given in Figure 5.22. (100% saturation at 25°C and 39ppt is 6.63 mg/l). Key to symbols are given in Figure 5.22.

Table (5.19). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Aeration rates				
	1	2	3	4	5
Day 8					
. One -way ANOVA	Significantly different, P= 0.0001				
. Duncan's test,P<0.05	B	B	A	A	C
Day 16					
. One -way ANOVA	Significantly different, P= 0.0001				
. Duncan's test,P<0.05	B	B	A	A	C

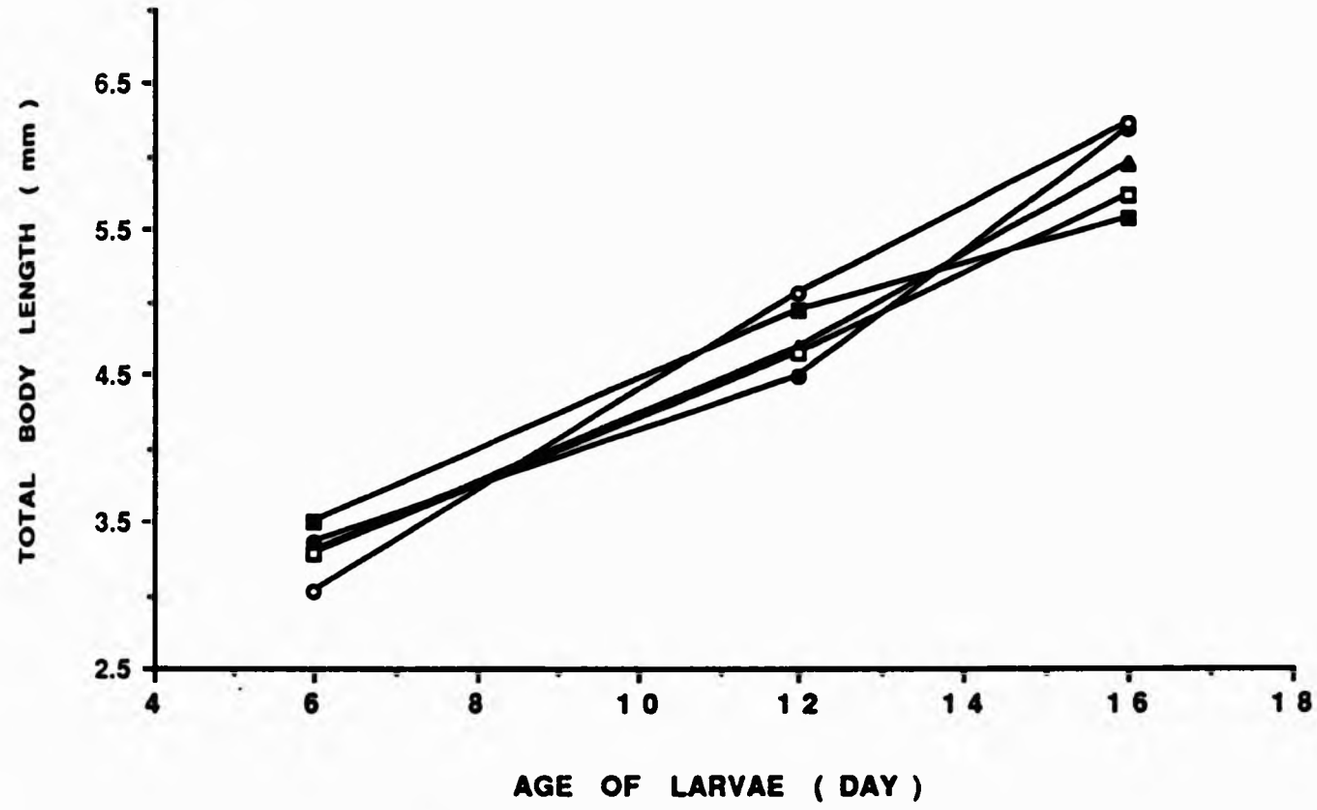


Figure (5.26). Growth in total body length of *A. cuvieri* larvae reared in different aeration rates. The aeration treatments are given in Figure 5.22.

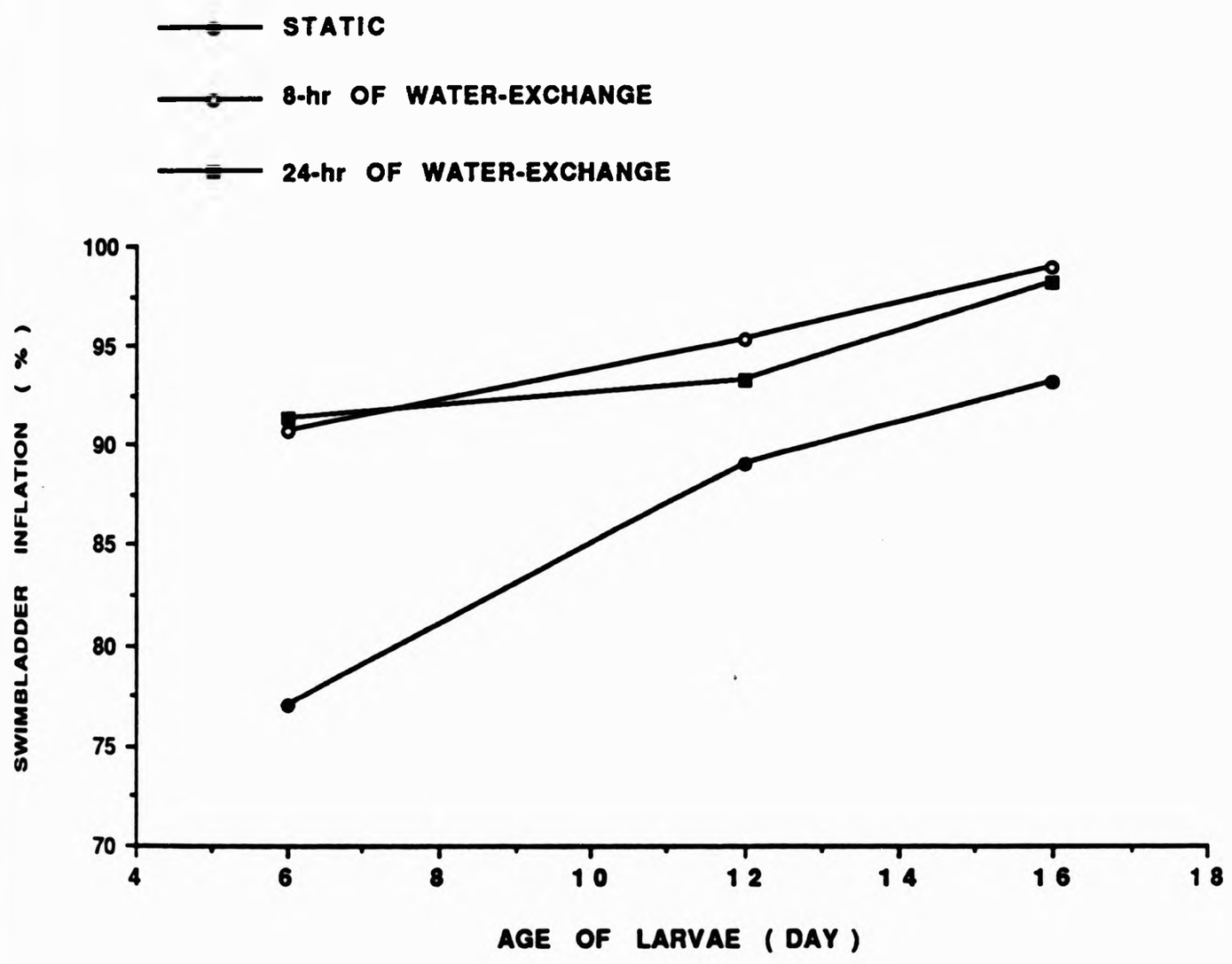


Figure (5.27). The relation between percent swimbladder inflation and age of *A. cuvieri* larvae reared under different water-exchange regime.

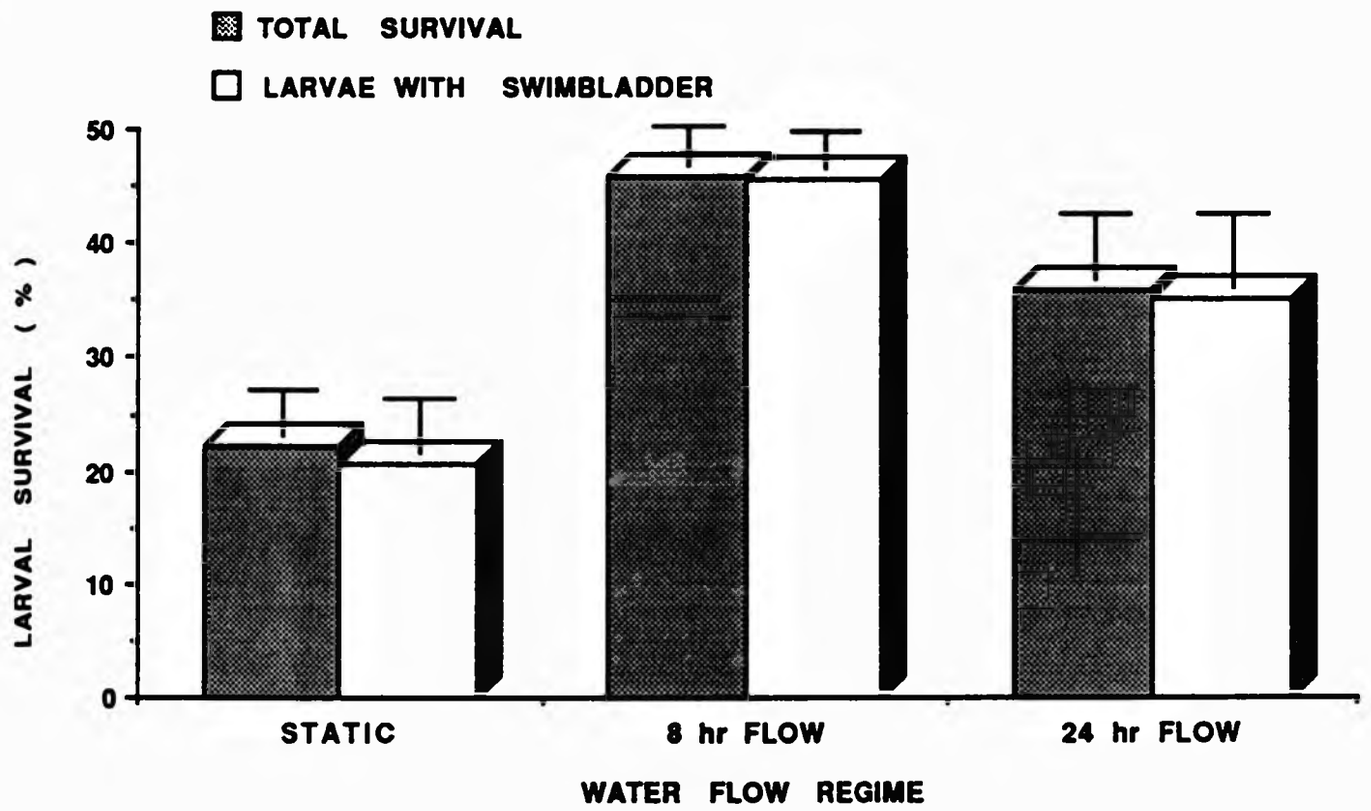


Figure (5.28). The effect of water-exchange regimes on the survival of *A. cuvieri* larvae reared for 16 days. Vertical bars represent one standard deviation.

Table (5.20). Results of the statistical analysis carried out for the data presented in the above figure.

Statistical tests	Water flow regimes		
	static	8hr flow	24hr flow
Total survival			
. One -way ANOVA	Significantly different, P= 0.0016		
. Duncan's test,P<0.05	C	A	B
With swimbladder			
. One -way ANOVA	Significantly different, P= 0.0019		
. Duncan's test,P<0.05	C	A	B

The water quality parameters measured in the different treatment tanks over 16 days of rearing is shown in Figure 5.29. The dissolved oxygen decreased with time, being worst in the 24hr flow (Figure 5.29A) and significantly different ($P < 0.01$). pH showed an increase with time, appearing to plateau at around 8.2 as day 16 was approached (Figure 5.29B). The total ammonia ($NH_4.N$) given in Figure 5.29C showed a steady increase with time and reached 1680 $NH_4.N$ µg/l on day 16. The percent of unionized ammonia ($NH_3.N$) at 25°C, 40 ppt and 8.1-8.2 pH is around 5.32-6.61 (Bower and Bidwell, 1978). The measured values of total ammonia in the different treatments were found to be significantly different on day 5, 11 and 16 ($P < 0.01$). Finally, the level of nitrite ($NO_2.N$) measured on different days of rearing is shown in Figure 5.29D. It appears that the levels of nitrite in the rearing media are inversely related with the level of water exchange. The values of measured nitrite in the different treatments were found to be significantly different ($P < 0.01$) on day 5, 11 and 16.

The growth of larvae, in the different water exchange treatments was not significantly different Figure 5.30.

In summary, the best production of larvae with a functional swimbladder was in the 8hr flow treatment of 250 ml/min starting on the fourth day of rearing. The 24hr flow was only second best.

IV. DISCUSSION

The trials reported here have clearly shown the strong relationship between abiotic rearing factors and the initial inflation of the swimbladder, survival and growth of larval Blue-Finned Sea Bream *A. cuvieri* during the first two critical weeks of rearing. Much of the literature on the abiotic factors in larval rearing is concerned with growth and survival rather

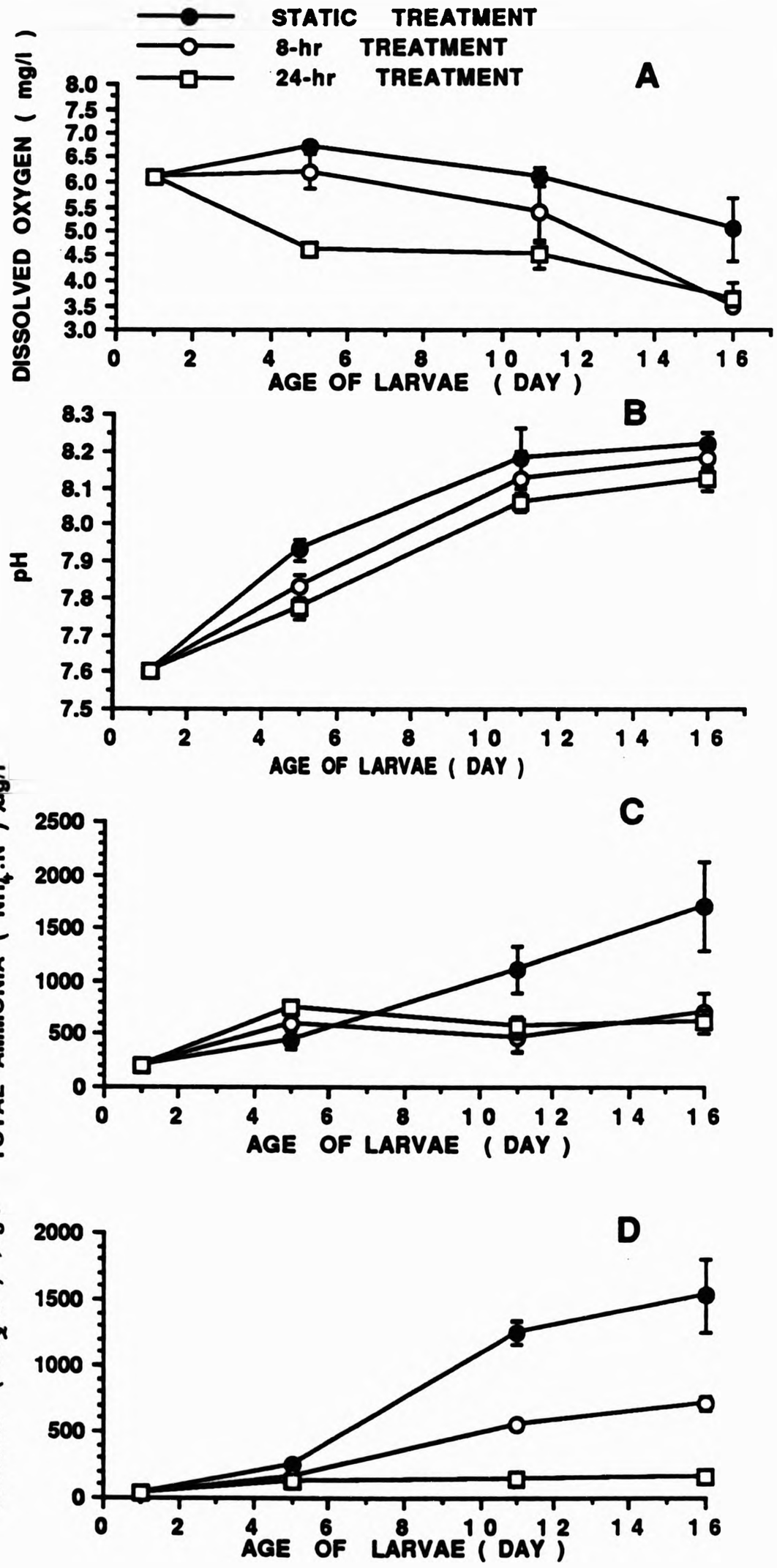


Figure (5.29). The effect of different water-exchange rates on dissolved oxygen (A), pH (B), total ammonia (C) and nitrite (D). Vertical bars represent one standard deviation.

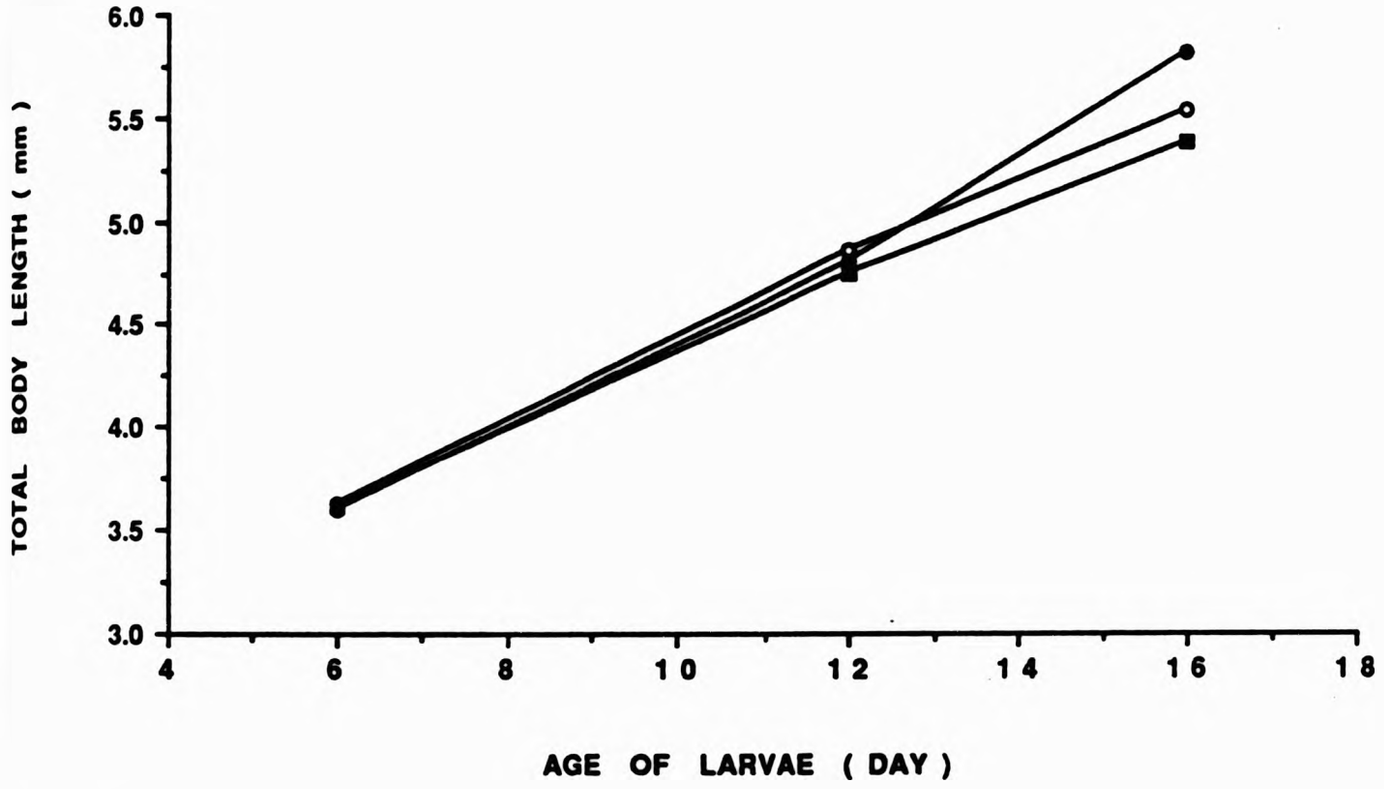


Figure (5.30). Growth in total body length of *A. cuvieri* larvae reared under different water-exchange regimes. The symbols for different treatments are given in Figure 5.29.

than swimbladder inflation. This makes it difficult to compare the results obtained in this study with those of other authors.

Although 25°C gave the best production of usable larvae, the use of 22°C or an even lower temperature (spawning and hatching temperature) during the first 3-4 days of rearing (yolk absorption time) could contribute to a bigger and fitter larva due probably to more efficient utilization of food reserves. The rearing temperature could then be increased to 25°C as the larvae start to feed. Hadley *et al.* (1987) tested water temperatures of 16 and 19°C and salinities of 2.5 and 7.5 ppt on the initial swimbladder inflation success using *Morone saxatilis* larvae, and found greater inflation success at the lower temperature, even though, 19°C is considered close to optimal for larval Striped Bass. The low temperature was also correlated with higher survival, larger body size and larger residual oil globule size. They also found that at 16°C larger larvae (as body area, mm^2) tend to have higher percent swimbladder inflation and this led them to conclude that faster-growing, or perhaps more efficiently developing progeny inflate their swimbladder better.

It is also well known that rearing temperature directly affects organogenesis and metabolism of larvae. Thus, swimbladder development is affected by temperatures, especially the development and atrophy of the pneumatic duct. Consequently, it is possible that at 22°C the presence of a functional pneumatic duct is extended. By contrast, growth and development at 25°C will be faster thus reducing time for additional swimbladder inflation. This may be helpful in hatchery management by enabling a quicker decision to be made as to whether to restock those tanks with a low percent swimbladder inflation. Secondly, the larval cropping rate of rotifers will be higher which may also be helpful in tank management by ensuring that only

high quality rotifers are being consumed, the freshly added ones. It was observed in some of the later trials of water exchange and aeration rate carried out at 25°C that by 6-7 days after stocking a 100% swimbladder inflation was secured. This suggests that, if the abiotic and biotic factors are optimised, there is no need to have a prolonged period of pneumatic duct patency. The effect of higher but tolerable rearing temperature on the reduction of surface tension of sea water is also a possible contribution to facilitate air gulping.

The survival at 22°C was almost half that at 25°C. It is thought that the main reason for this may be the slow rate of cropping of rotifers at 22°C which is confirmed by the daily checking of remaining rotifers. This will cause a decrease in their nutritional quality, since a static system was used and the old rotifers are remained with no new rotifers being added. The *Chlorella* density in the rearing tank (2-3 x 10^5 cell/ml) compared to that used to feed or enrich the rotifers (2.5 x 10^7 cell/ml) showed that the rotifers probably benefitted little. Therefore, performance is probably better at 22°C in a flow-through system, because the old rotifers are continously flushed out and the larvae will thus always get new fresh rotifers.

It seems quite probable that the sinking of larvae on days 3 and 4 after hatching acts as a trigger to drive the larvae to swim upward and eventually to hit the air-water interface to gulp air. The clear phototactic behaviour is a response to that trigger (chapter 4). It is then possible to explain the results obtained from the salinity trials. It seems that 20, 30 and 40 ppt constitute the tolerable range for *A. cuvieri* larvae for acceptable survival in hatchery rearing. The sinking action triggering the larvae to swim upward exist at all three salinities, thus, no difference of their effect was observed on the initial swimbladder inflation at day 8, 12 and 15. Since

the sinking rate is higher at 20 ppt than at 40 ppt due to a difference in rearing water densities, the swimbladder inflation percent on day 21 was highest in the 20 ppt treatment. It appears that the salinity effect on initial swimbladder inflation is through the interaction between the rearing water density and larval density.

Hadley *et al.* (1987) worked on *M. saxatilis* larvae and reported that their was no effect of the two salinities they used (2.5 and 7.5 ppt) on initial swimbladder inflation. Chapman *et al.* (1988) worked on the same species and found that a gradual increase in salinity from 0 to 10 ppt during the larval rearing period did not increase the incidence of swimbladder inflation. Cornacchia (1982) studied the same species in a wide range of salinities (0, 5, 10, 15 and 20 ppt) and found that 5 and 10 ppt gave the highest inflation of about 87% and attributed the low inflation at higher salinities to the possibility that the larvae can delay their swimbladder inflation to regulate their buoyancy due to the high density of the rearing media.

The high rate of rotifer multiplication in the 20 and 30 ppt treatments was a problem encountered in the salinity test. The nutritional quality of the rotifers bred in the larval rearing tank is most probably quite low due to improper feeding, since the *Chlorella* provided was at a very low density. This could effect larval survival which was found not to be different between 20, 30 and 40 ppt ($P > 0.05$), even though, the means appear different (Figure 5.7).

The survival of larvae reared under different light intensities was most affected by the different quality of larvae used, as was shown in both the 1986 and 1987 trials. The percentage survival range increased from

0.2-2.5 in 1986 (late stocking) to 35-53 in 1987 (early stocking). While, the initial swimbladder inflation was less affected, ranging from 10-70 to 60-90% respectively. The general trend observed in both tests was that survival and light intensity were positively related, even though, this was not really clear in the 1987 trials, while, a negative relationship was observed between light intensity and initial swimbladder inflation. This negative relationship is almost certainly related to the vertical migration patterns of larvae under different light intensities, where larvae avoid, and aggregate away from a bright illumination source. This could indicate that larvae in nature inflate their swimbladder either at sunrise or sunset.

The application of this finding may lie in the use of two different light intensities for larval rearing. A high light intensity would be used to enhance survival where the larvae tend to distribute throughout the water column and a lower one to secure acceptable swimbladder inflation where the larvae tend to be aggregating near the water surface. Since a 24-hr light photoperiod was found to be best for both survival and initial swimbladder inflation, the high light intensity could be used during the day and the low one at night. The other possibility is to use low light intensity during the first week of rearing and a higher one during the second week.

The literature available on light intensity and its effect on marine or brackish-water larvae is concerned with survival and growth and not with initial swimbladder inflation. It has been generalized by many researchers that high light intensities (within a tolerable range) lead to better survival. Tandler and Helps (1985) worked with Gilthead Sea Bream larvae and demonstrated that maximum survival was attained with high intensities of 1370-3430 Lux and a 24-hr photoperiod. Kiyono and Hirano (1981) worked on Black porgy larvae (*Mylio macrocephalus*). They tested a

wide range of light intensities of 0, 100, 1000, 3000 and 10000 Lux and found that best survival was achieved at 3000 Lux with second best at 10000 Lux. Barahona-Fernandes (1979) working with European Sea Bass larvae found that poor survival was achieved at 3500 Lux and high survival at 300-700 Lux. It seem that the optimum levels of light intensity for larval feeding and hence survival are species dependant. The use of *Chlorella* in larval rearing procedures requires a high light intensity in order to promote algal growth and to prevent degradation of the environment. Thus, in this case it becomes necessary to use the highest light intensity possible without affecting survival, initial swimbladder inflation, and larval growth.

The reason for using a treatment with no light in the first three days in 1986 was based on the idea that larvae may not move vertically in darkness and thus may grow bigger since most of the yolk reserves go into growth rather than movement. A bigger and possibly fitter larva would have better success in breaking the water surface to air-gulp at the end of the 3 dark days. This idea was not supported by the results and the larvae obtained at the end of the 3 dark days seemed to be weak.

The positive effect of the 24-hr light photoperiod treatment on initial swimbladder inflation is probably because *A. cuvieri* larvae are phototactic during their first 15 days, starting from day 3. This brings them up near the air-water interface. Wales (1984) worked on Herring larvae and found that the vertical migration pattern may be driven continuously by repeated light cycles, and that they do not exhibit a clear circadian rhythm under constant light or darkness.

The maximum survival and poorest growth obtained from the 24-hr treatment with *A. cuvieri* in this study is supported by the findings of

Barahona-Fernandes (1979) who worked on *D. labrax*. Many researchers have similarly shown that survival is maximized under a 24-hr photoperiod (Houde and Palko, 1970; Marliave, 1977; Tandler and Mason, 1983; Tandler and Helps, 1985; El-Zahr *et al.*, 1986 and Duray and Kohno 1988). It is possible that the reason for the increased survival of these small marine larvae that have limited food reserves in the 24-hr photoperiod regime is the maximization of training time for the larvae to learn and feed. This is assisted by the continuous illumination at the time the mouth becomes functional which could not otherwise happen in darkness in a natural photoperiod.

The achievement of high initial swimbladder inflation and survival with an aeration treatment of 50-70 ml/min and 200-300 ml/min can be attributed to several effects. Firstly, the continuous cleaning effect of aeration cleans the water surface of any floating item and pushes it to the side of the rearing tank. Secondly, aeration probably reduces the water surface tension through the continuous disturbance making the air-water interface more accessible. Survival enhancement was probably due to a more even distribution of live-food, break down and prevention of local micro-environments, reduction of bacteria counts (Barahona-Fernandes, 1978) and more equal distribution of the larvae.

The lower level of dissolved oxygen in the second week of rearing in the 50-70 ml/min treatment (Figure 5.25), could be corrected by increasing the aeration rate, to say, 100-200 ml/min. This is supported by the fact that a rate of 200-300 ml/min also gave good results.

The fact that tanks covered with the silicone agent film gave a survival similar to the best achieved, suggests that the lack of a functional swimbladder does not cause any massive initial mortality. Several authors

have indicated that mortalities could happen later as an indirect effect (Chatain, 1987; Chapman *et al.*, 1988; Chatain and Dewavrin 1989). This should not be confused with the massive mortality of larvae due to hypertrophic swimbladder (Johnson and Katavic, 1984).

Doroshev and Cornacchia (1979), working on larval Striped Bass, reported that strong aeration and turbulent water in the rearing tanks gave an enhanced initial swimbladder inflation. Chatain (1982) reported that an aeration rate of 50 ml/min gave higher swimbladder inflation than a rate of 1000 ml/min when Red Sea Bream larvae were fed on rotifers previously enriched with *Chlorella*. Fukusho (1985) stated that the percentage of Red Sea Bream larvae with an inflated swimbladder was lower in non-aerated tanks, while, 50-100 ml/min aeration reduced the incidence of lordosis by increasing the percentage of swimbladder inflation.

The common notation used by most researchers to describe the aeration rate is to express air volume per unit time. This notation is not a precise description of aeration, especially its mechanical effect, since other factors such as water volume, tank shape and depth and size of air bubbles are very important. Therefore, it is suggested that another notation system is used, where the air volume rate (ml/min) is divided by the rearing volume (l). Even though this notation does not give a full description of the actual effect, it is an attempt to improve the simple aeration flow rate.

The high and sudden swimbladder inflation observed at day 6 in the water exchange treatments of 24-hr flow and 8-hr flow (Figure 5.27) is a good indication of optimal condition for initial inflation of swimbladder in *A. cuvieri*. By contrast, a slow and a gradual inflation is probably a sign of sub-optimal conditions. It is believed that the relatively high swimbladder

inflation observed with all three treatments of the water-exchange test is due to the fact that this trial implemented all of the best results from previous tests to improve the initial swimbladder inflation, i.e. 25°C, 40ppt, 50-70 ml/min, 24-hr photoperiod, and 1000 Lux. It seems that the effect of these combined abiotic factors on the initial swimbladder inflation is stronger than the effect of the water-exchange. This is shown clearly in the static water treatment which, overall, had similar percentage of swimbladder inflation to treatments with water exchange. One important additional reason for this is that the rotifers used in this test were enriched with *Chlorella* only and no oil-based enrichment was used. This helped to keep the water surface clean and allows free access to the air-water interface, thus de-emphasising the diluting effect of the water flow. However, final survival was affected significantly and its reduction in the static water treatment was undoubtedly due to the low water quality.

The low oxygen level observed in the 8-hr and 24-hr flow of about 54% saturation on day 16 was caused by the low aeration rate of 50-70 ml/min and the low dissolved oxygen content in the incoming well water (3.5 ppm) which provided about one change every 6-7 hr. Pre-aerating the incoming water, or doubling the aeration rate, would have prevented such conditions. The difference in survival between the 24-hr flow and 8-hr flow of 10% is significantly different and could be attributed either to the lower oxygen content in the 24-hr flow (see Figure 5.29A) or to the treatment itself, since small marine larvae are very sensitive to water exchange during their first week. Alessio (1975) reported a negative relationship between survival of 6 day old larvae of *Sparus aurata* and the rate of water exchange which he attributed to the physical perturbation associated with water-exchange. This has also been observed in KISR hatchery, where a complete mortality occured

when water exchange was started on the first day of stocking. Tandler and Helps (1985) reported that the lack of water exchange had a positive effect on growth for the first 10 days after hatching. On the other hand, Blaxter (1981) stated that marine larvae are likely to withstand a drop in oxygen to 40% of the air saturation volume (about 2.5 ml/l at 10°C in seawater) for long period. Another possible reasons for the difference in survival between the 24hr flow and 8hr flow could be the loss of rotifers and *Chlorella* through the drain mesh (200µm).

The low survival of 22% after 16 days of rearing under static water system could be attributed to the high levels of unionized ammonia (NH_3.N) and nitrite (NO_2.N) reaching 111 and 1540 µg/l respectively. The effect of water exchange was very clear in the 8 and 24-hr flow treatments, where much lower level of these metabolic wastes were found.

Brownell (1980) who worked on several species of marine fish larvae, reported that nitrite is non-toxic at levels likely to be encountered in practical marine fish culture. He measured the concentration of NO_2-N that reduced the incidence of first feeding by 10%, and values ranged from 60,000 to 280,000 µg/l. By contrast, the unionized ammonia is considered to be a potential hazard in the rearing tank. He reported that the 24-hr LC_{50} range from 360-460 µg/l of NH_3-N and the concentration at which first feeding incidences were reduced by 50% after 24 hr exposure (24-hr EC_{50}) was about 110-220 µg/l of NH_3-N.

There are various recommendations for maximum allowable levels of ammonia that have been suggested by Poxton and Allouse (1982), quoting EIFAC (1970), they recommend a safe concentration of ammonia for aquatic organisms to be estimated by using the 96 hr LC_{50} x 0.05. Wickins (1981) suggested that

100 µg/l (NH_3-N) is the maximum for long-term tolerable concentration, which very close to the maximum concentration measured in the static treatment of this study (111 NH_3-N µg/l).

It is thought that the results from some of the trials reported here would be less variable between replicates if a flow-through system were used. This would make the rearing medium more similar in terms of microbial population, water quality and rotifer counts.

Overall, the optimum values of the abiotic factors considered and which contribute most to the production of normal larvae having a functional swimbladder are summarized in Table 5.21. These optimum values were combined in the trials on water exchange and showed very good results. The possible modifications mentioned in Table 5.21 remain to be tested.

Table (5.21). A summary of the findings obtained obtained from the seven tests, previously mentioned, on the effect ofthe abiotic factors on initial swimbladder inflation.

Tested abiotic factor	Selected results	Possible modifications
Water temperature	25°C	22°C during first 3 days then increase to 25°C
Salinity	40 ppt	None
Light intensity	1000 Lux*	1000 Lux during day hours and 250 Lux during night hours
Photoperiod	24 hr	None
Aeration rate	50-70 ml/min**	50-70 ml/min in first week then increase to 200-300 ml/min
Water exchange	8 hr at 250 ml/min	8 hr during first week and then increase to 24 hr

* Based on 1987 trial
** Rearing volume tested was 25 litre and 25 cm deep.

CHAPTER 6

THE EFFECTS OF SELECTED BIOTIC FACTORS ON INITIAL SWIMBLADDER INFLATION AND SURVIVAL

I. INTRODUCTION

There are numerous factors which may have an effect on initial swimbladder inflation ranging from the quality of the broodstock and eggs to larval rearing procedures. Much of the published literature stresses the importance of the environmental factors (abiotic) in influencing initial swimbladder inflation, neglecting the possible role of the biotic ones (Doroshev and Cornacchia, 1979; Doroshev *et al.*, 1981). Other researchers have included both factors in their considerations, for example, Fukusho (1985); Hadley *et al.* (1987); and Chapman *et al.* (1988). Those biotic factors which have been shown to be important for larval survival were discussed earlier (Chapter 1). The same factors are probably also influential on initial swimbladder inflation.

Chapman *et al.* (1988) worked on the Striped Bass (*Morone saxatilis*) and believed that variation in swimbladder inflation between trials could be attributed to the sub-optimal condition of the broodstock they used for spawning. Hadley *et al.* (1987) compared the performance of progeny from 13 different females of Striped Bass and found that there is a significant parental effect on initial swimbladder inflation.

The nutritional quality of the first feed (rotifers) is also considered one of the most important biotic factors within the larval rearing stage (see Chapter 1). This fact is frequently stressed by Japanese researchers (Watanabe *et al.* 1983, Fujita and Kitajima, 1978; Fukusho, 1985).

In this chapter the effect of certain biotic factors on initial swimbladder inflation and larval survival is examined. The initial condition of broodstock, and hence egg quality, was compared by using egg batches at different times during the spawning season. The feeding of larvae was also

examined, comparing different larva: rotifer ratio and also different rotifer types (S or L).

II. MATERIALS AND METHODS

A. The effect of rotifer: Larva ratio on swimbladder inflation and survival

This trial was designed to test the hypothesis that, as more food becomes available to the larvae, their well being and swimming ability increase, thus increasing the likelihood of them successfully breaking the water surface in order to fill their swimbladders. A range of ratios of rotifer: larva ranging from 5 to 1000 were made by combining several different larval and rotifer densities as shown in Table 6.1. These nine combinations were replicated three times, using 30 litre fibreglass tanks containing 25 litre of seawater which were placed in a rectangular galvanized steel tank of about 330 litre which acted as a water bath (see Figure 2.2).

Table (6.1). The different combinations of larval and live-food (rotifers) densities made for test A.

Treatment	Live-food density (number per litre)	Larval density (number per litre)	Live-food: larva ratio (rotifers/larva)
A	1000	200	5
B	1000	50	20
C	5000	200	25
D	10000	200	50
E	5000	50	100**
F	1000	10	100
G	10000	50	200
H	5000	10	500
I	10000	10	1000

** Control treatment

The rearing conditions used were: static water, 50-70ml of air/min, 25°C, 38-40ppt, L-type rotifers, 14hr day/10hr night photoperiod, 1000 lux of fluorescent day-light at the water surface, and 300-400 x 10^3 *Chlorella*/ml.

The duration of this trial was 15 days with two samplings at day 7 and 15. The sampling procedure used and parameters measured were similar to that described for the temperature trial (Chapter 5). On day 5, about 30 larvae were sampled from each replicate of the treatments A, C and D which had similar larval density but different live-food levels. These larvae were checked under the dissecting microscope for swimbladder inflation and were then squashed on a microscope slide to check whether or not they were feeding. Larval survival was checked by counting all swimming larvae from each replicate on day 15.

B. <u>The effect of rotifer type on swimbladder inflation and survival</u>.

This trial was carried out using the S-type rotifer ***Brachionus plicatilis rotundiformis*** and the L-type rotifer ***Brachionus plicatilis typicus*** (James *et al.*, 1989). The main objective was to compare the effect of the rotifer body size and nutritional quality of the two types on initial swimbladder inflation. It is probable that larvae establish their initial feeding pattern faster when fed on the smaller rotifers and that larvae fed on a higher level of W3HUFA are more active.

The two types of rotifers were used as first food for *A. cuvieri* larvae to test their effect on initial swimbladder inflation and survival after a rearing period of 15 days. A third treatment was added where no food was supplied. The three treatments were replicated three times using 30 litre

fibreglass tanks, as described in the previous experiment. The larvae were sampled on day 7 and 15 using procedures and measurements similar to those used in the temperature trial (Chapter 5). Both types of rotifers were conditioned with *Chlorella* prior to feeding to the larvae, as described in Chapter 2. The rotifers were given to the larvae at a rate of 5 rotifer/ml/day during the first week and this was adjusted twice during the second week to 7-8 rotifers/ml. The fatty-acid composition in the S and L type rotifers was analyzed using standard methods as described by James *et al.* (1988) at the Central Analytical Laboratory, KISR.

C. <u>The effect of egg quality on swimbladder inflation and survival</u>

There is some evidence that larvae hatched from early batches of eggs are superior in terms of swimbladder inflation and survival to those larvae hatched from late batches of eggs from the same broodstock. It is widely considered that this difference is due to early season eggs being of better quality than late season eggs.

During the 1986 spawning season three experiments were conducted to examine the effect of water temperature, salinity and light intensity on initial swimbladder inflation and survival of *A. cuvieri* larvae. The larvae used in these three tests came from the same broodstock but at different times namely; early-(January), mid-(February) and late-(March) season. The spawning season usually lasts for 7-8 weeks. The rearing facilities, sampling procedures and measurements were as described for the temperature trials (Chapter 5). The rearing conditions of the controls of the three experiments were identical. The controls from each of the three tests were extracted and compiled to form a set of data which could be used to test the effect of season, and hence probable egg quality, on the initial swimbladder inflation and survival.

III. RESULTS

A. The effect of rotifer: larva ratio on swimbladder inflation and survival

The results of this trial are summarized in Table 6.2. The effects of rotifer:larva ratio on initial swimbladder inflation are shown in Figure 6.1a[A] and 6.1b[B]. Figure 6.1a[A] shows that the swimbladder inflation attained a stable rate after day 7 with a clear difference between treatment A, the lowest rotifer:larva ratio, and the others. Figure 6.1b[B] clearly shows a trend of a positive relationship between live-food:larva ratio and swimbladder inflation, which levelled at about 100 rotifer/larva. The effects of rotifer:larva ratio on larval survival are shown in figure 6.1b[A] where it levelled at about 200 rotifer/larva with an unexplained low survival at 500 rotifer/larva. Figure 6.1a[B] showed the larval growth to be lowest at rotifer/larva ratios of 5 and 50, other ratios being significantly different.

In treatments B, C, E and F a different tank surface area: larva ratio was used; 0.91. 0.22, 0.91 and 4.54 cm^2 per larva respectively. This had no apparent effect on swimbladder inflation (Table 6.2).

The number of feeding and non-feeding larvae on day 5 and its relationship to swimbladder inflation are summarised in Table 6.3. There is a strong positive correlation (correlation coefficient = 0.78) between percent of feeding larvae and swimbladder inflation.

In summary, it seems that the number of rotifers available for one larva per litre should be within the range of 100-200 in order to achieve maximum production of larvae with a functional swimbladder.

Table (6.2) Summary of the effect of rotifer: larva ratio on swimbladder inflation, survival and growth of *A.cuvieri* larvae.

Treatment	Number of rotifers: larva (per litre)	Swimbladder inflation (%) ± SD **		Survival (%) ± SD **		Growth Total body length(mm) ±SD	
		Day 7	Day 15	total	with swim-bladder	Day 7	Day 15
A	5	34.3±7.4	40.3±2.4	4.0± 1.7	1.6± 1.1	3.6±0.3	5.7±1.3
B	20	64.3±0.4	75.3± 6.7	13.0± 2.1	9.7± 1.0	3.9±0.4	5.5±0.5
C	25	58.0±3.0	84.3± 4.7	11.0± 2.6	9.1± 2.3	3.7±0.2	5.0±0.3
D	50	63.7±5.6	80.3±2.5	17.2± 1.0	13.8± 2.8	3.9±0.3	5.3±0.2
E	100*	85.3± 3.2	92.7± 4.9	30.4±0.3	28.2±0.4	4.3±0.5	6.6±0.5
F	100	76.3±7.6	79.1±6.7	29.0± 6.0	22.3± 7.8	4.2±0.5	7.2±0.2
G	200	80.4± 8.3	87.3± 2.5	41.8±4.9	36.9±3.0	4.5±0.7	6.7±0.9
H	500	76.3±7.6	82.0± 9.8	18.5± 3.1	15.3± 4.7	4.4±0.3	7.3±0.4
I	1000	68.8±8.4	94.0± 1.41	43.2± 9.6	40.4± 8.5	4.6±0.5	7.4±0.8

* Control
** SD; standard deviation

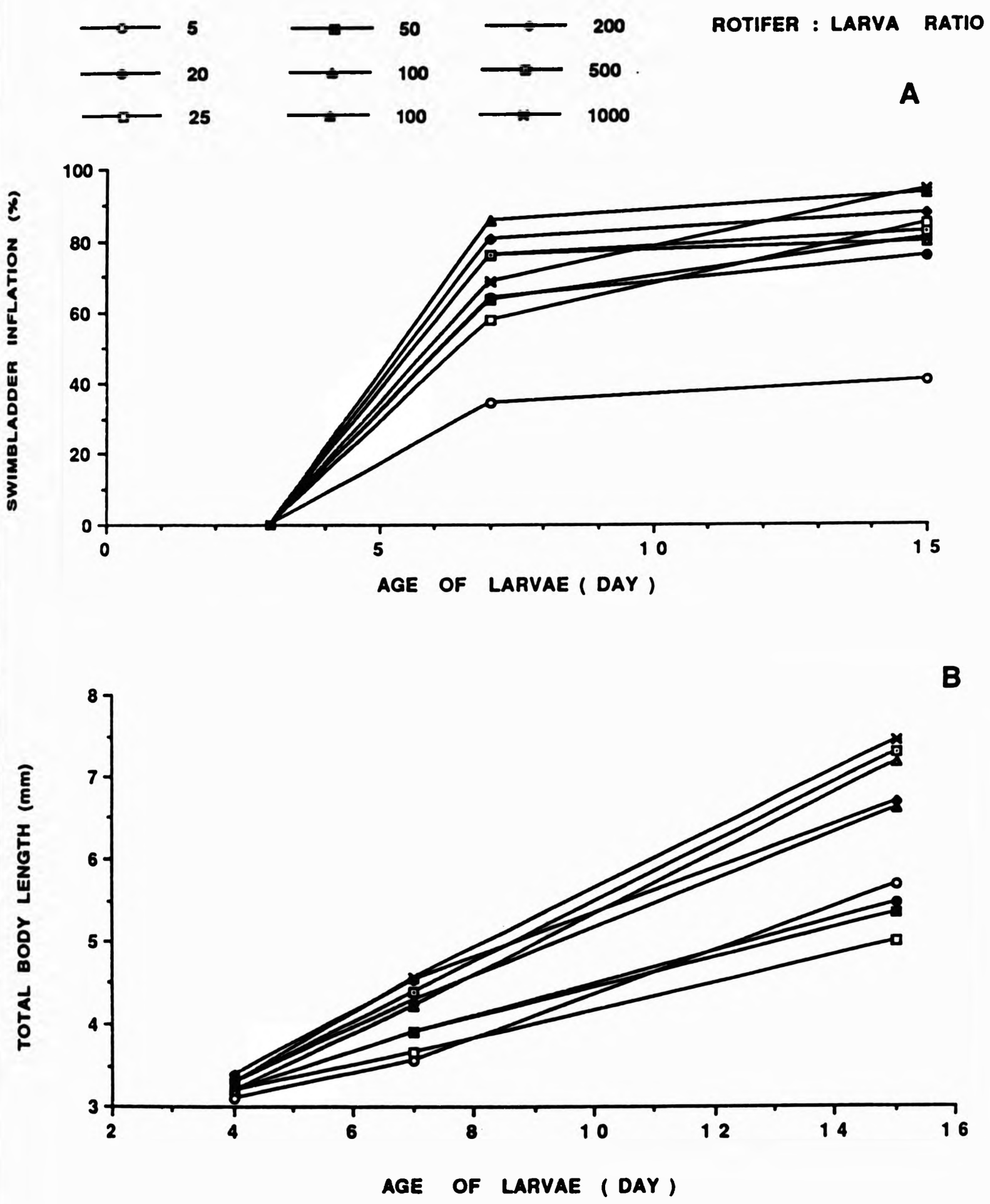


Figure (6.1a) Graphical illustration of the results given in Table 6.2, swimbladder inflation and growth of larvae.

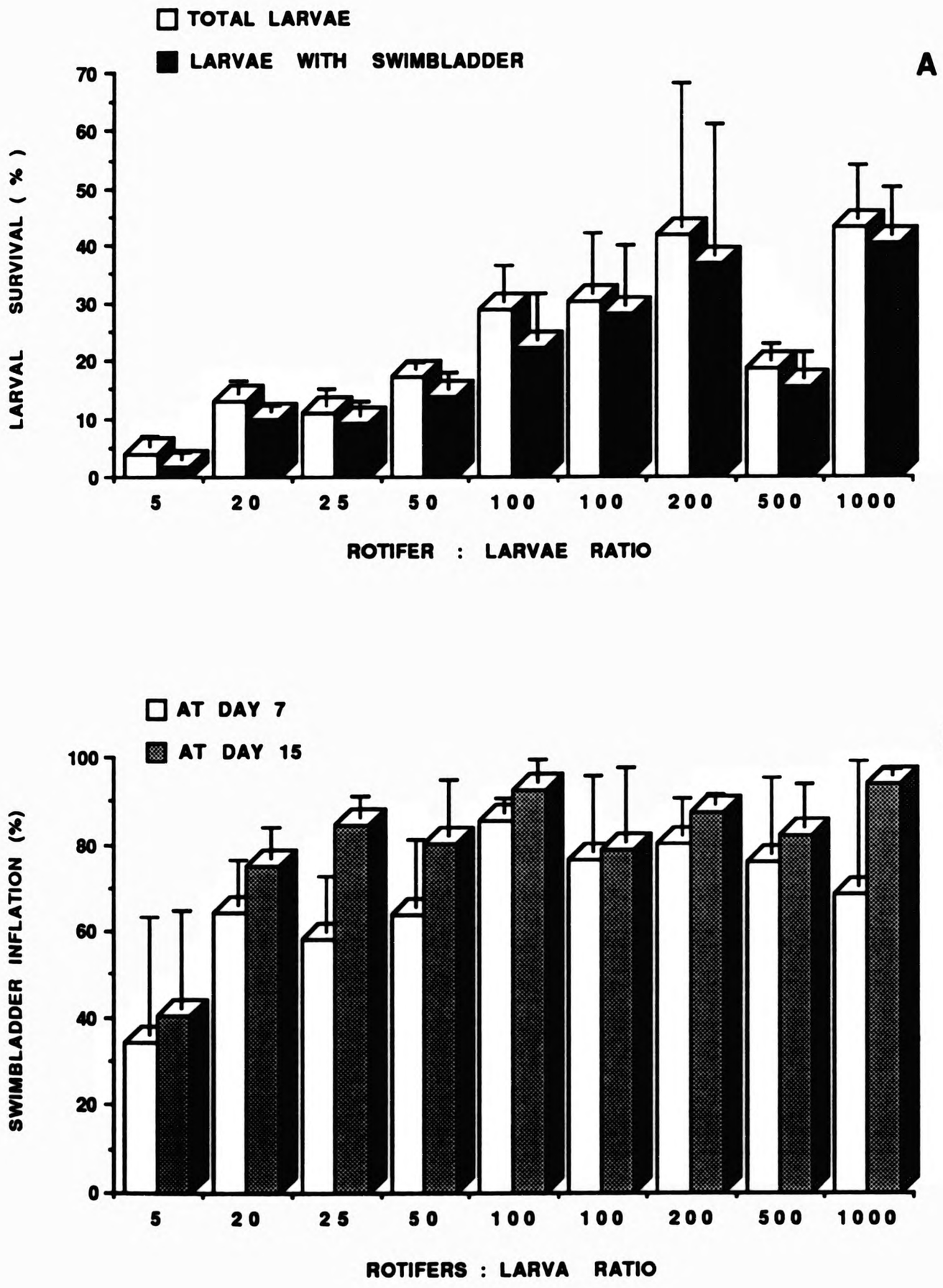


Figure (6.1b) Graphical illustration of the results given in Table 6.2, swimbladder inflation and survival of larvae.

Table (6.3) Relation between percent of feeding larvae and their swimbladder inflation at different feeding levels on day 5.

Treatment rotifers: larva/litre	Percent of feeding larvae at day 5	Percent of larvae with inflated swimbladder at day 15
5	27	60
(1000 rotifer/200	10	16
larvae/litre)	15	45
mean±SD	$17.33\pm8.7^{*}_{a}$	$40.3\pm22.4^{**}_{a}$
25	40	79
(5000 rotifers/200	50	88
larvae/litre)	42	86
mean±SD	$44.0\pm5.3^{*}_{b}$	$84.3\pm4.7^{**}_{b}$
50	64	66
(10,000 rotifers/200	70	86
larvae/litre)	73	89
mean±SD	$69.0\pm4.6^{*}_{c}$	$80.3\pm12.5^{**}_{b}$

* significantly different at P<0.001.
** significantly different at P<0.05.
Defferent subscripts are significantly different, P<0.05

B. The effect of rotifer type on swimbladder inflation and survival

The principal differences between the L-type and S-type rotifers, both in size and nutritional quality are given in Table 6.4. These differences are significant ($P<0.05$).

The percent swimbladder inflation estimated at day 15 after feeding on L-type and S-type rotifers were found to be very similar and both differed significantly from the control treatment in which no food was offered ($P<0.05$) as shown in Figure 6.2 and Table 6.5.

The larval survival results are shown in Figure 6.3. A very clear difference was observed between both estimates of survival and L-type of rotifers were significantly better ($P<0.05$) (Table 6.6).

Larval growth in term of total body length is shown in Figure 6.4. At day 15, no significant difference was found between the tested treatments ($P>0.05$).

In summary, the L-type rotifers seems to be superior to the S-type in achieving maximum production of larvae with a functional swimbladder at day 15, although no difference was found in growth performance.

C. The effect of stocking date on swimbladder inflation and survival

The effect of stocking date on swimbladder inflation monitored on day 5, 10 and 21 is shown in Figure 6.5. It is very clear that early stocking gave better swimbladder inflation, and this was confirmed statistically as shown in Table 6.7 ($P<0.001$).

Table (6.4) Differences between L and S-type rotifers used in feeding *A. cuvieri* larvae.

Rotifer type	Lorica length * (µm)	W3HUFA (area %)		
		C20:5W3	C22:6W5	W3
L-type	236.8	11.8	3.4	19.5
	238.1	11.6	3.3	19.1
	210.3	-	-	-
mean±SD	228.3±15.7	11.7±0.1	3.3±0.1	19.3±0.3
S-type	139.7	2.1	0.3	2.7
	140.5	1.9	0.2	3.2
	145.0	-	-	-
mean±SD	141.7±2.9	2.0±0.1	0.25±0.1	2.9±0.3

* L-type range 143.6 - 287.2 µm

S-type range 97.4 - 179.5 µm

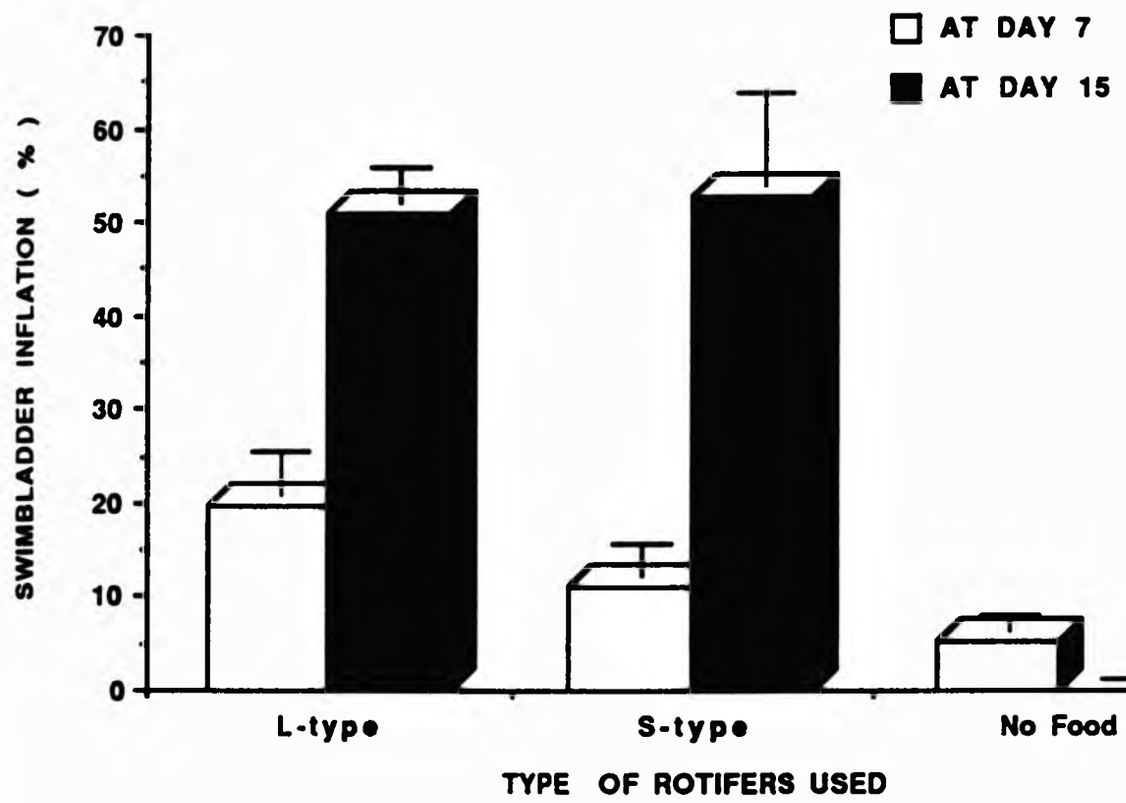


Figure (6.2) The effect of rotifer types on swimbladder inflation of *A. cuvieri* larvae. Vertical bars represent one standard deviation.

Table (6.5) Statistical analysis results, carried out for the data presented in the above figure.

Statistical test	Rotifer types		
	L-type	S-type	No food
Day 7			
. One way ANOVA	Significantly different,		P=0.01
. Duncan's test, P<0.05	A	B	C
Day 15			
. One way ANOVA	Significantly different,		P=0.01
. Duncan's test, P<0.05	A	A	B

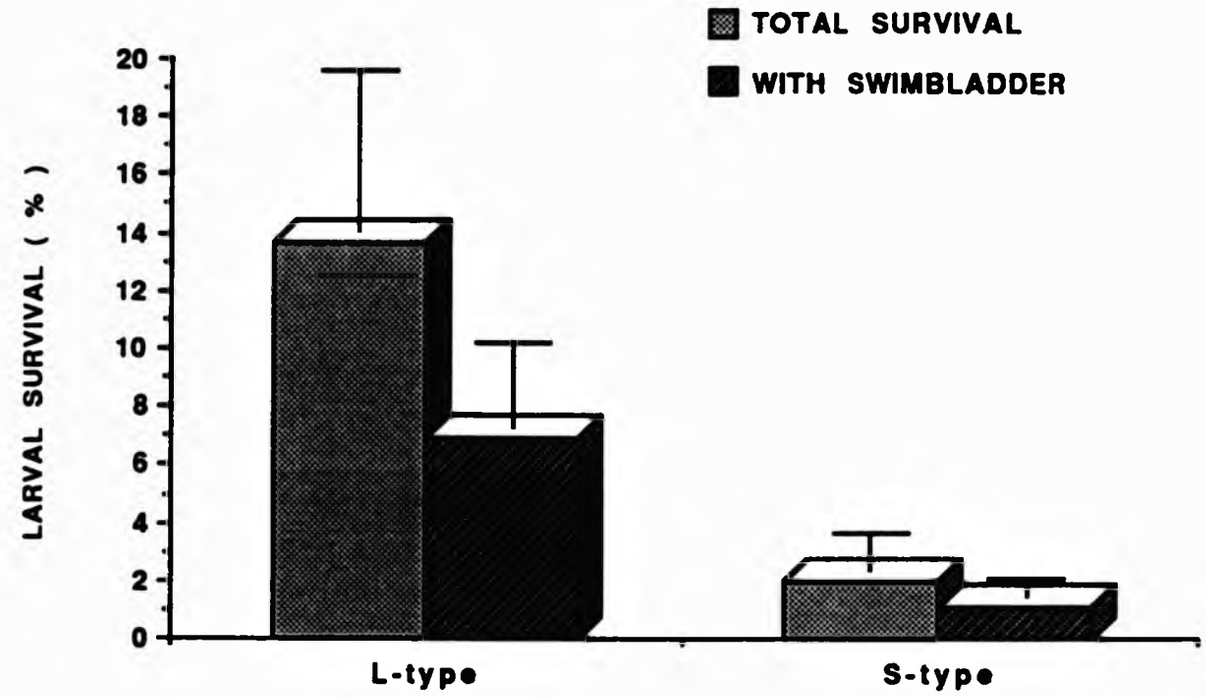


Figure (6.3) The effect of rotifer types used on larval survival of *A. cuvieri* larvae. Vertical bars represent one standard deviation.

Table (6.6) Statistical analysis results, carried out for the data presented in above figure.

Statistical tests	Rotifer types	
	L-type	No food
Total survival		
. One way ANOVA	Significantly different,	P=0.011
. Duncan's test, P<0.05	A	B
With swimbladder		
. One way ANOVA	Significantly different,	P=0.012
. Duncan's test, P<0.05	A	B

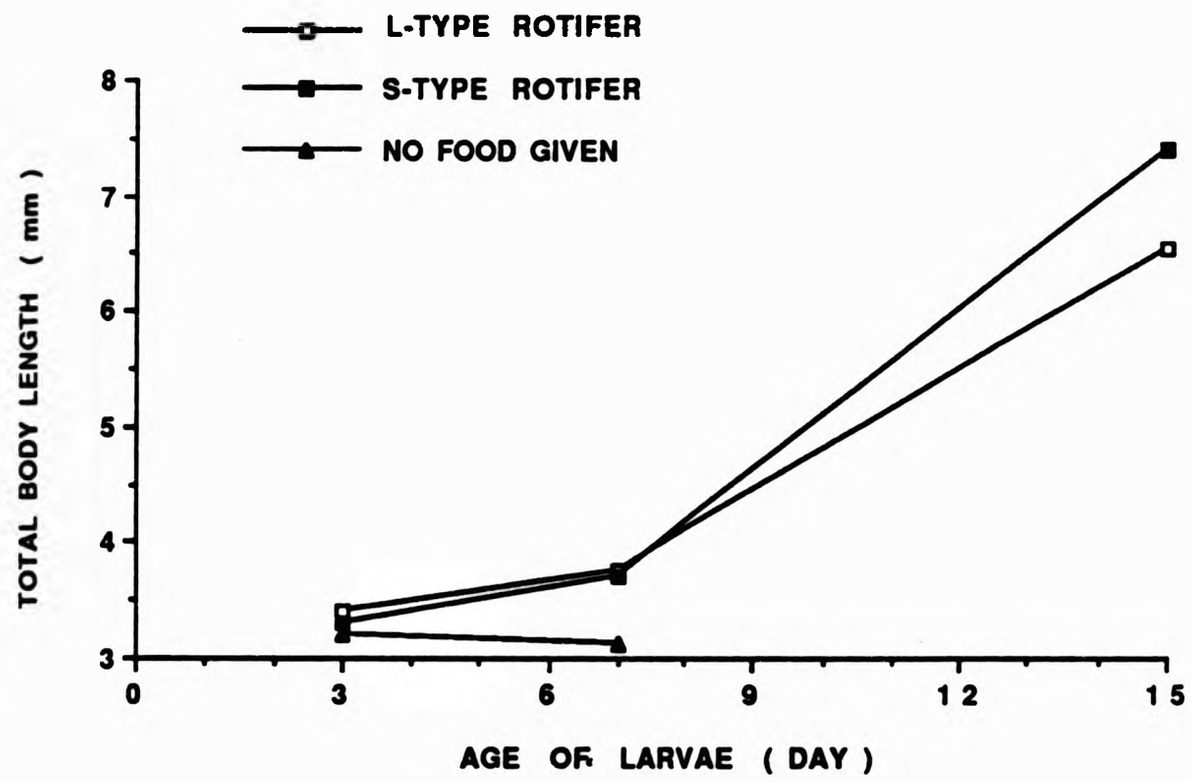


Figure (6.4) The growth in total body length of *A. cuvieri* larvae fed on different types of rotifer.

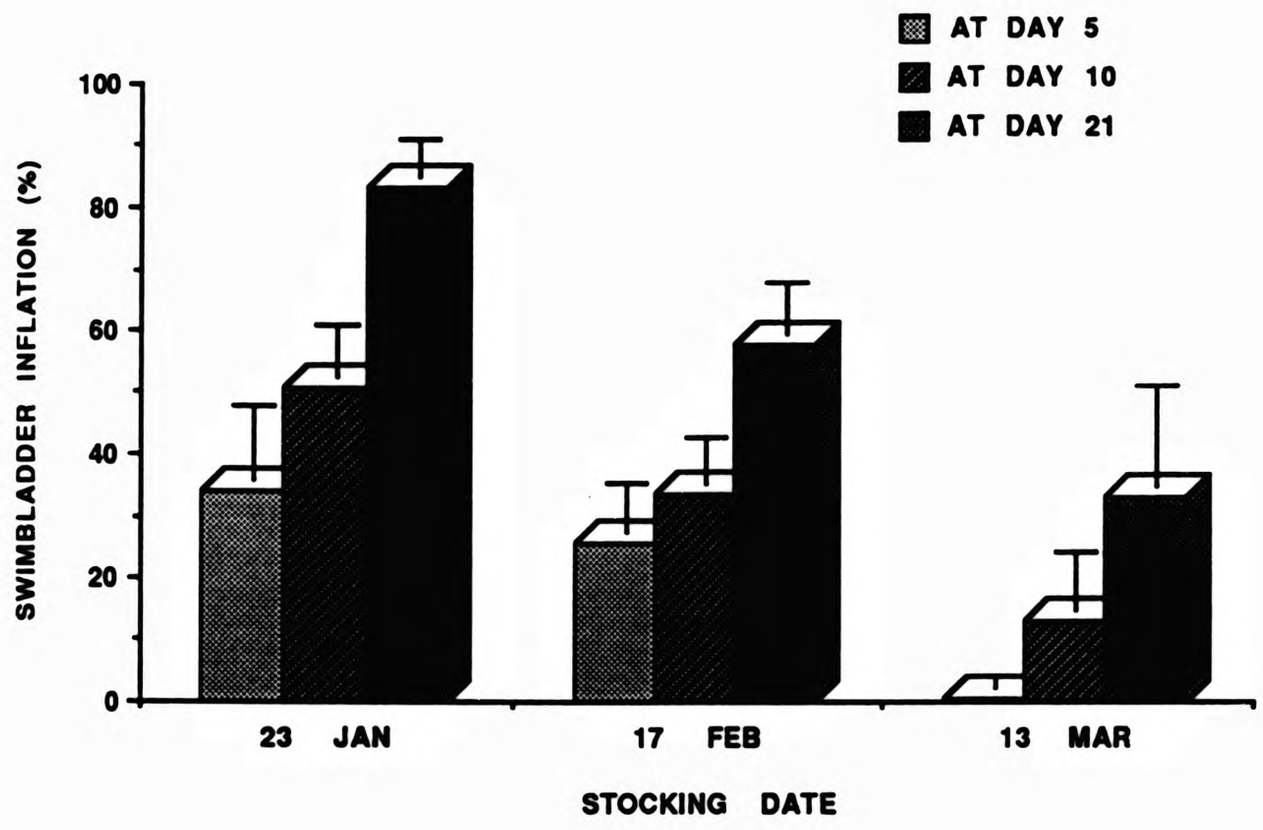


Figure (6.5) The effect of stocking date on swimbladder inflation of *A. cuvieri* larvae. Vertical bars represent one standard deviation.

Table (6.7) Statistical analysis results, carried out for the data presented in above figure.

Statistical test	Stocking date		
	23rd Jan	17th Feb	13th Mar
Day 21			
. One way ANOVA	Significantly different, P=0.0005		
. Duncan's test, P<0.05	A	B	C

The early stocking also gave the best survival, as shown in Figure 6.6. Table 6.8 shows that the survival results from the three stocking dates were also significantly different ($P<0.001$).

Overall larval growth, however, was not significantly affected as the season progressed, Figure 6.7.

In summary, larvae from early egg batches were found to be superior to mid or late egg batches in terms of both initial swimbladder inflation and larval survival.

IV. DISCUSSION

The description of initial swimbladder inflation mechanisms in Chapter 3 and the data on yolk reserve, oil globule and larval buoyancy presented in Chapter 4, suggest that at the time of inflation, the main source of energy for the larvae is obtained externally. The only internal energy source at this stage is a very small residual oil globule which completely disappears by day 7-8. A possible additional function of the oil globule is to act as a linking energy source between the end of the yolk reserve and during the training period of external feeding. The very poor swimbladder inflation obtained when no food was given (Figure 6.2) clearly confirms the idea that external food in some way assists with initial swimbladder inflation. The need for external feeding is also clear from Table 6.2 and Figure 6.1A, where swimbladder inflation increased as more food is given. The results from Table 6.3, again, support the same idea. Therefore food in the right quantity is obviously very important in enhancing initial swimbladder inflation. This can be achieved by giving an equal chance to all the larvae to select and feed on the given live-food which is maintained at a certain

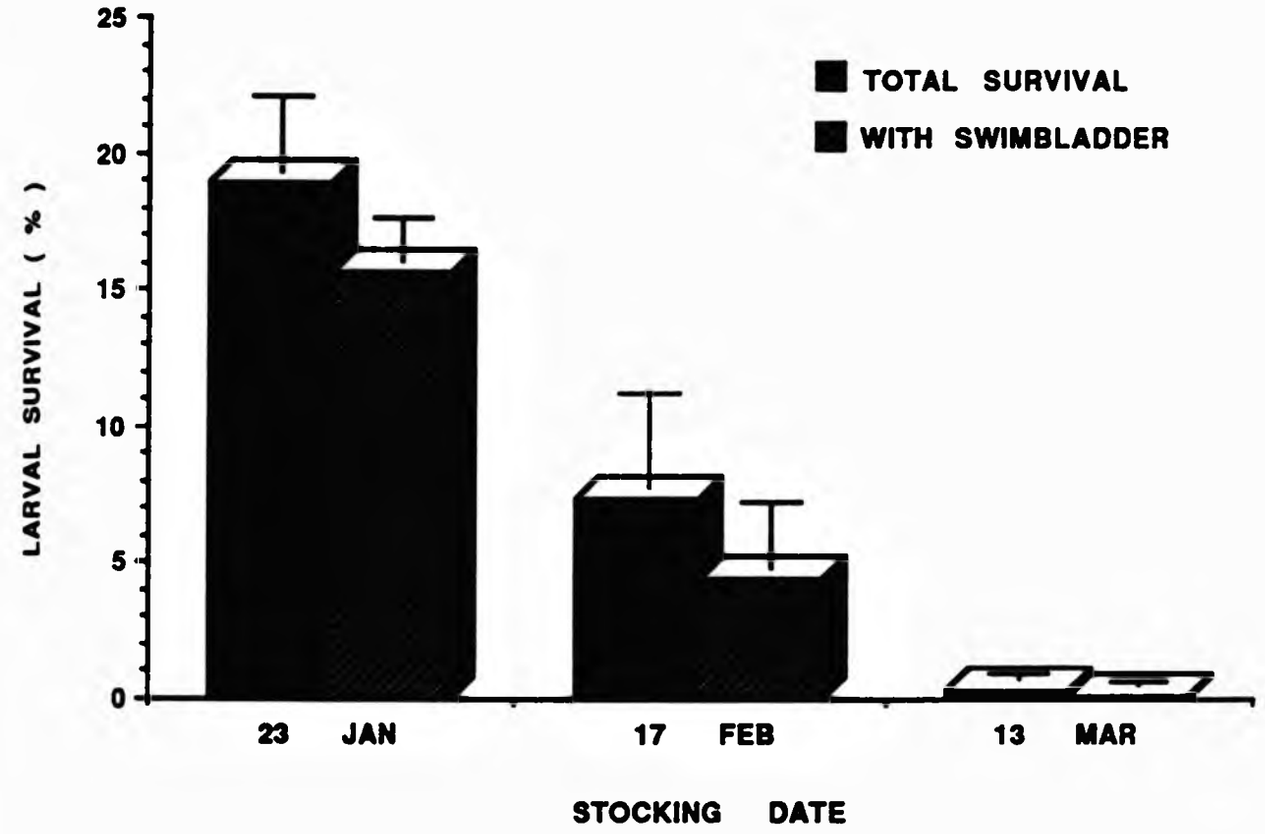


Figure (6.6) The effect of stocking date on larval survival of *A. cuvieri* larvae. Vertical bars represent one standard deviation.

Table (6.8) Statistical analysis results, carried out for the data presented in above figure.

Statistical test	Stocking date		
	23rd Jan	17th Feb	13th Mar
Total survival			
. One way ANOVA	Significantly different, P=0.0001		
. Duncan's test P<0.05	A	B	C
With swimbladder			
. One way ANOVA	Significantly different, P=0.0001		
. Duncan's test P<0.05	A	B	C

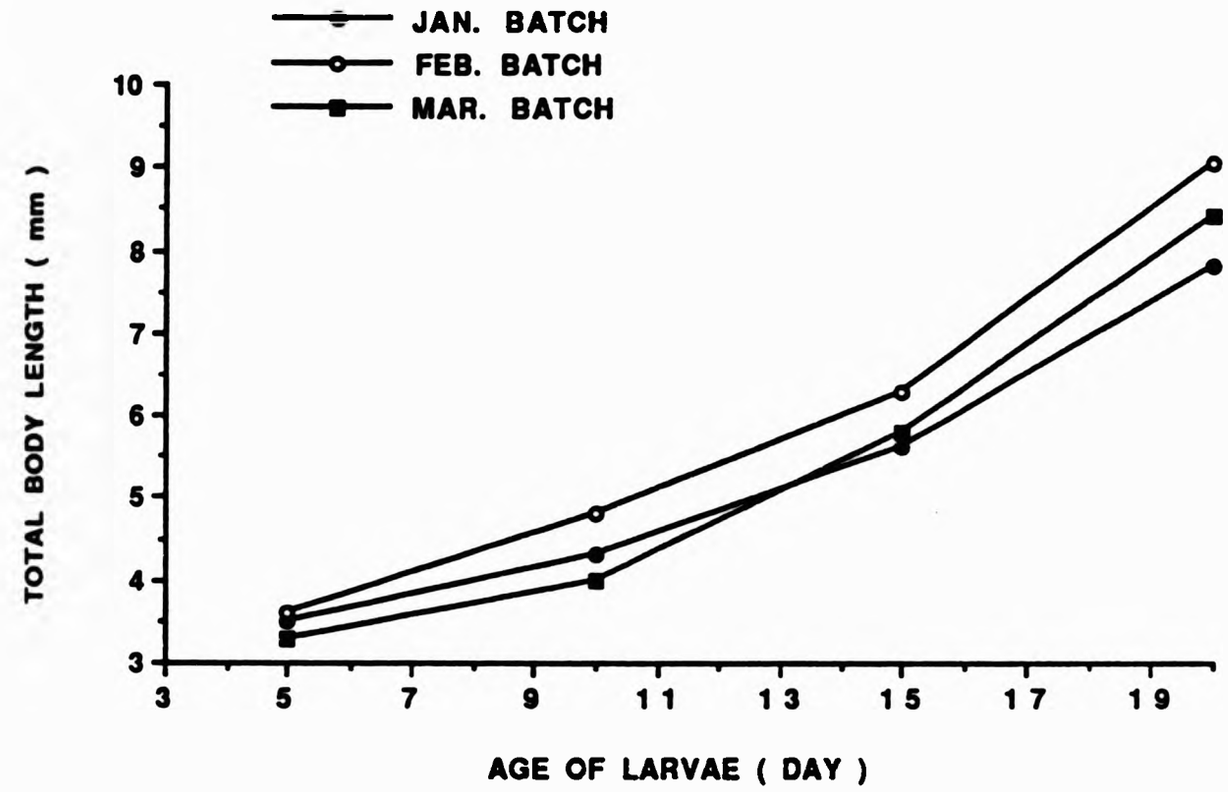


Figure (6.7). The growth in total body length of *A. cuvieri* larvae from different egg batches.

density to assure that chance. Thus, well-feed larvae will be more able to swim upward to break the surface tension and successfully gulp air.

Swimbladder inflation, survival and growth all improved with the increase of rotifer: larva ratio. Houde (1975) working on Sea Bream larvae (*Archosargus rhomboidalis*) found that better growth and survival was attained with higher live-food densities in most of the larval densities he tested. Tandler and Mason (1984) worked on *Sparus aurata* larvae and they also found a direct relationship between rotifer concentration and the ingestion rate. They recommended a concentration of 40 rotifers/ml to achieve the maximum ingestion rate and this rate was calculated to be 82 and 168 rotifer/larva/day at the age of 9 and 14 days, respectively. Fujita (1977) estimated that the daily consumption of rotifers by Red Sea Bream larvae is about 22, 58, 156, and 285 for the total body length of 3.0, 3.9, 5.1, and 6.0mm respectively.

The 1000 rotifer : larva treatment (I, Table 6.2) gave almost double the survival of the control (100 rotifer:larva, treatment E) thus, producing double the number of larvae with a functional swimbladder since both treatments had a similar effect on swimbladder inflation . It should be noted that treatments I and E have different overall larval densities. This suggests that the control treatment (E) which is standard practice at MFD hatchery, KISR, could be revised.

It is probable that more reliable results could be obtained by repeating this work using a single larval density but different rotifer concentrations covering a wide range.

The larger L-type rotifers cultured at MFD, KISR are used normally to feed *A. cuvieri* larvae with satisfactory results. The idea of using a smaller size rotifer (S-type) was to enhance the early ingestion rate which

may lead to faster and more efficient feeding. Larvae which establish feeding early are more fit and have a better chance of breaking the water surface tension for first filling of the swimbladder. Hunter (1984) stated that at the onset of first feeding a small prey item seems to be preferable as larvae are still in the training period. The results obtained in this study did not confirm this idea but this may possibly be due to the low w3 HUFA in S-type rotifers. It is also possible that the effect of the nutritional value of rotifers (w3 HUFA) which is more in the large rotifer was stronger than the use of small size rotifers on larval survival.

It is also possible that, basically, more food was available in the large rotifer treatments (L-type) than in the small rotifers treatments (S-type). This would mean that the L and S-type rotifer trial should not be repeated based not on number of rotifers/ml but on equal biomass of the two rotifers used.

The nutritional quality of the initial live-food has been frequently stressed by Japanese workers as one of the major causes of failure of initial swimbladder inflation. Fujita and Kitajima (1978) reported that a group of Red Sea Bream larvae fed on Bread yeast-fed rotifers showed a high percentage of uninflated swimbladders, whereas, *Chlorella* fed rotifers or w-yeast fed rotifers group had a low percentage of uninflated swimbladders. Watanabe (1986a) stated that feeding Red Sea Bream larvae on rotifers with a low content of w3 HUFA resulted in low swimming activities, lack of endurance and reflex response, consequently producing difficulty in gulping air at the water surface. The percentage swimbladder inflation in the work reported here was not affected by the relatively low content of w3 HUFA in S-type rotifer at day 15, whereas, survival was affected. James and Abu-rezeq (1989), found no differences in w3 HUFA for L and S-type rotifers produced

by an intensive chemostat culture system, which were fed mainly on the microalgae *Nannochloropsis* sp supplemented with Bakers' yeast. However the production system, used to produce both rotifers types in this study was large concrete tanks 7-10 m^3 adopting the batch-culture method using mainly Bankers' yeast supplemented with *Chlorella* as food the rotifers.

The progressively lower swimbladder inflation and larval survival observed as the spawning season advanced could not be attributed to factors other than the egg batch from the different spawning days. The issue of egg quality and its characterisation is not yet a clear matter. Kjorsvik and Lonning (1983) outlined some criteria for good egg quality in Cod. A common way used by hatchery workers is in assume a relationship between larval performance and change occurring in some of the egg characteristics such as egg volume, oil globule volume, hatching rate and percent of floating eggs. EL-Zahar (unpublished data, MFD, KISR,) worked on the Brown-Spotted Grouper, *Epinephalus tauvina* and found that the number of floating eggs decreased as the spawning season progressed. The measurements of egg diameter, yolk sac and oil globule volume of just-hatched larvae were found to have highest values at about the middle of the 65-day spawning season, while the lowest values were found at the very beginning and very end. Watanabe *et al.* (1985b) found that the egg diameter of Red Sea Bream gradually decreased as spawning progressed in all tested groups of breeders fed on different diets and feeding schedules. Kerby *et al.* (1983) stated in a review paper on Striped Bass that low egg quality (low in fertilization rate and incomplete embryo development) is often observed to occur in the early and late parts of the spawning season. And finally, workers in CENMAR, Yugoslavia, observed that low survival and percent swimbladder inflation occurred in late batches of eggs of the European Sea Bass (personal comm., Darko, L., January 1988).

In summary, the biotic factors considered in this chapter were found to influence initial swimbladder inflation and survival of *A. cuvieri* larvae. The treatments that gave the best results were; 100-200 rotifer: larva, L-type rotifer and early egg batches.

CHAPTER 7

THE EFFECTS OF ABSENCE OF A FUNCTIONAL SWIMBLADDER ON GROWTH, SURVIVAL, FOOD CONVERSION RATIO AND SPINAL COLUMN

I. INTRODUCTION

Fish abnormalities are generally due either to genetic causes or to an event occurring during larval development. In either case, the malformation may develop and become apparent later in the fry or juvenile phase (see Chapter 1). A lordotic spinal malformation characterized by a V-shaped deformation close to the swimbladder location has been observed in several marine species. This malformation is linked with the absence of a functional swimbladder in the larval phase as shown in Plate 7.1. These fish are; the Red Sea Bream *P. major* (Kitajima *et al.*, 1977; 1981; Chatain, 1982; Fukusho, 1985) and the Gilthead Sea Bream *S. aurata* (Paperna, 1978; 1984). Paperna (1978) gave a detailed description of some types of these skeletal deformities in hatchery-bred Gilthead Sea Bream.

In addition to causing a general deformity of the body, swimbladder disfunction impairs swimming performance, reduces fish growth and survival, and increases the susceptibility to parasitic and microbial infection (Paperna, 1978; 1983; Chatain, 1987; Chatain and Dewavrin, 1989). The low survival is very pronounced in young fry of 30-50 days old and occur principally during the weaning period rather than in older fish (Teng *et al.*, 1982; Chatain and Dewavrin, 1989) . Growth reduction is commonly observed at all ages in those fish surviving without a swimbladder.

In this chapter, growth, survival, and food conversion ratio of two different sizes of fish with and without a functional swimbladder is examined. The incidence of spinal lordosis in fish without a functional swimbladder is also considered and the types of the spinal deformities linked to the swimbladder absence are examined and discussed.

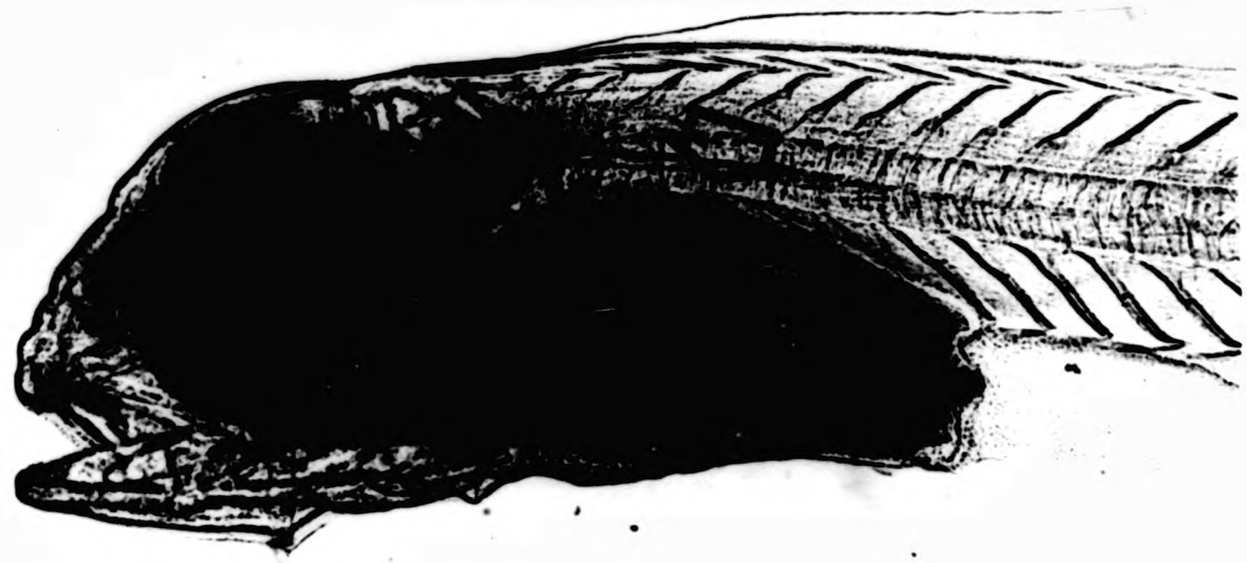


Plate (7.1A). 14-day-old. *A. cuvieri* larvae with inflated swimbladder, located beneath the anterior end of the notochord.

Plate (7.1B). 14-day-old. *A. cuvieri* larvae with inflated swimbladder.

II. MATERIALS AND METHODS

A. The effect of the absence of a functional swimbladder on growth, survival and food conversion ratio in *A. cuvieri*.

Experiment 1

In this trial, two groups each of 700 *A. cuvieri* fingerlings were taken from the hatchery stock at MFD, KISR. One group had a functional swimbladder and an average body weight of about 0.6g, the other group had no functional swimbladder and an average body weight of about 0.9g.

The two groups were separated simply by flotation in high salinity water. Natural sea salt was added to sea water to obtain a 60 ppt solution which was then mixed with Quanildine to make a concentration of 5 ppm. The fish were first graded and then starved for 15 hrs (overnight) before being placed in the saline water for separation. This technique has been previously tested at MFD, KISR and found to have an error of only about 0.0025% and a survival rate after handling of 100% for normal fish and 95% for abnormal fish in large operations. The floating and sinking fish were then removed to their respective experimental tanks.

Three experimental groups were used, namely, 100% normal fish, 100% abnormal fish and 50% normal mixed with 50% abnormal. Each treatment was replicated three times and 130 fish were used per replicate. The fish were separated by saline water a second time prior to the initial measurements to assure 100% purity.

500 litre circular fibreglass tanks (96 cm in diameter and 70 cm depth) were used, and each tank had a bottom central drain connected to an outer stand pipe to control water level and a single aeration stone. The

tanks were covered with black netting and only 75% of the tank volume was utilized as *A. cuvieri* are quite sensitive and easily excited to jump.

The nine 500 litre tanks were supplied with well-aerated water flowing at 10 litre/min. The water was injected obliquely to create a small peripheral current of about 19 cm/sec. The current was simply measured by the use of an aluminium drogue and stop watch. The water temperature, salinity, and pH were 25°C, 39-40 ppt and 7.7 respectively. Except for salinity, these water quality parameters were monitored twice weekly.

The fish were fed four times daily during the first month and this was then reduced to three times per day at 8.00, 12.00 and 16.00hr. The fish were fed to satiation using Aqualim crumbles and 2 mm pellets made for Gilthead Sea Bream.

The initial measurements for each tank were made three day after stocking by lowering the water level to the 50 litre mark and adding Quanildine to make a 1-2 ppm concentration. The fish then were transferred to the saline separation bath, bulk weighed and finally counted back into their tanks. The same procedure was repeated three time at 25 day intervals, the total experiment duration being 76 days. From these measurements, survival, daily growth rate and specific growth rate were calculated. Final checks were made for any secondary inflation that may have occurred among the abnormal fish. Daily growth rate and specific growth rate were calculated as follows;

$$\text{Daily growth rate} = \frac{\text{Total biomass gained (g)/No of fish}}{\text{Duration of experiment (Days)}}$$

$$\text{Specific growth rate} = \frac{(\text{Ln final wt}) - (\text{Ln initial wt})}{\text{Duration of experiment (Days)}} \times 100$$

where wt = mean body weight

Ln = natural logarithm

Experiment 2

Over 9000 fish ranging from 1 to 8 g were removed from stock, graded and each size group was then separated by the saline-water technique. About 1500 fish of similar size (4-5g) were selected for this trial of which, 750 floated and were probably normal and 750 did not float and probably had no swimbladder.

The experimental design and measurements were similar to that described above except that 150 fish were stocked into a 1000 litre fibreglass tank with a water-flow of 15 litre/min which created a peripheral current of about 26 cm/sec. The duration of this test was 94 days with weighings at 30 day intervals. The fish were fed three times per day to satiation using 2mm pellets. The daily amount of food consumed was recorded and used to calculate the food conversion ration (FCR) using the equation;

$$\text{Food conversion ratio} = \frac{\text{Amount of food eaten (g)}}{\text{Biomass gained (g)}}$$

B. <u>The types of spinal deformity induced by the absence of a functional swimbladder in *A. cuvieri* fingerlings and juveniles</u>.

The types of spinal deformity were checked by two methods, namely, radiography and dissection. A sample of fish was obtained from experiment 1 at termination and radiographs were prepared in the Institute of Aquaculture at the University of Stirling.

The dissections were carried out on three size groups, namely, 5-8, 9-12 and 15-21 cm in total body length in an attempt to monitor the development of spinal deformity in fingerling without a functional swimbladder. These fish were obtained from the production line of MFD, KISR at different times and from different tanks. The fish were subjected to the saline-water separation technique and only those without a functional swimbladder were used in this study. The fish were measured for total length and body weight and were then cut laterally with a sharp scalpel to expose the vertebral column, the remaining flesh being removed by scraping. A simple sketch of each fish was made indicating the vertebra number at which the bending of the column occurred. The angle of bending was measured approximately from the sketch.

III. RESULTS

A. The effect of the absence of a functional swimbladder on growth, survival and food conversion ration in *A. cuvieri*

The survival, daily growth rate and specific growth rate of *A. cuvieri* fingerlings with and without a functional swimbladder are given for experiment 1 in Table 7.1. The final survival after 76 days of rearing was found to be significantly different ($P<0.01$), and, perhaps surprisingly, was highest in the groups with no swimbladder. This can, however, be explained by the fact that the mortalities among the normal fish were due to jumping and being caught in the cover net. The daily and specific growth were also found to be significantly different ($P<0.01$ and $P<0.001$ respectively) between the treatments, those fish possessing a swimbladder performing best.

Later saline-water separations carried out with the sinking fish (treatment B) showed that there was no new swimbladder inflation as fish grew from 0.94 to 8.4 g.

Table (7.1) Growth and survival of *A. cuvieri* fingerling with and without a functional swimbladder, experiment 1.

Treatment	Replicate	Body wt. (g) initial	Body wt. (g) final *	Daily growth rate g/fish/day **	Specific growth rate ***	Survival (%) ****
A						
Fish with	1	0.62	9.3	0.112	3.36	85.6
swimbladders	2	0.63	9.1	0.111	3.51	90.4
	3	0.65	9.4	0.114	3.51	86.4
mean ± SD		0.63±0.02	9.26^{a}±0.15	0.112^{a}±0.001	3.46^{a}±0.08	87.5^{b}±2.6
B						
Fish without	1	0.97	8.8	0.103	2.91	93.6
swimbladder	2	0.93	8.5	0.099	2.91	94.4
	3	0.91	8.1	0.093	2.87	92.8
mean ± SD		0.94±0.03	8.47^{b}±0.35	0.098^{b}±0.005	2.89^{b}±0.02	93.6^{a}±0.8
C_1						
Fish with	1	0.67	9.2	0.111	3.46	87.3
swimbladder	2	0.64	9.8	0.118	3.59	78.0
(mixed)	3	0.63	9.3	0.112	3.54	86.0
mean ± SD		0.64±0.02	9.43^{a}±0.32	0.114^{a}±0.004	3.53^{a}±0.06	83.7^{b}±5.0
C_2						
Fish without	1	0.98	8.6	0.100	2.86	98.4
swimbladder	2	0.96	8.9	0.103	2.94	93.6
(mixed)	3	0.97	9.0	0.105	2.93	95.2
mean ± SD		0.97±0.01	8.83^{ab}±0.21	0.103^{b}±0.003	2.91^{b}±0.04	95.7^{a}±2.4

* Superscript with different letters are significantly different (P<0.05)
** Superscript with different letters are significantly different (P<0.01)
*** Superscript with different letters are significantly different (P<0.001)
**** Superscript with different letters are significantly different (P<0.01)

The survival, growth and food conversion ratio of fish with and without a functional swimbladder are given for experiment 2 in Table 7.2. The survival of fish after 94 days of rearing in all treatments was found not to be significantly different ($P>0.05$). However, the daily growth and food conversion ration were found to be significantly different ($P<0.05$) and fish possessing swimbladders performed best.

Again, the late-stage saline water separations carried out on group B fish showed that there was no new swimbladder inflation as fish grew from 4.7 to 35.4 g.

B. <u>The types of spinal deformity induced by the absence of a functional swimbladder in *A. cuvieri* fingerlings and juveniles</u>

Radiographs of *A. cuvieri* fingerlings obtained from experiment 1 are shown in Plate 7.2. It can be seen that the floating fish (control, with a functional swimbladder) grew normally whereas the sinking fish (without a functional swimbladder) grew abnormally with their spinal column bending in the region where the swimbladder is usually located. This is between vertebrae 4 and 13.

In normal fish it was noticed that there was close similarity between the shape of the lateral line and the shape of the spinal column. In those fish examined which lacked a functional swimbladder there were, broadly, four types of spinal deformity and these deformities are thought to develop as the fish grows.

The principal type, and the first to appear, is the "straight" spinal column which lacks the small curvature in the anterior portion where the swimbladder is normally positioned (see top fish in Plate 7.2). This is confirmed in Table 7.3 where the percentage with a "straight" vertebral column

Table (7.2) Growth, survival and food conversion ratio of *A. cuvieri* fingerling and juvenile with and without a functional swimbladder, experiment 2.

Treatment	Replicate	Body wt. (g) initial	Body wt. (g) final *	Daily growth rate g/fish/day **	Food conversion ratio ***	Survival (%) ****
A						
Fish with	1	5.40	41.5	0.38	1.37	84.0
swimbladder	2	5.13	40.2	0.37	1.35	85.3
	3	5.62	42.5	0.39	1.25	96.7
mean ± SD		5.38±0.24	41.4^{a}±1.2	0.38^{a}±0.01	1.32^{a}±0.06	88.7±7.0
B						
Fish without	1	4.51	35.7	0.33	1.54	83.3
swimbladder	2	5.00	36.4	0.33	1.63	73.3
	3	4.78	34.2	0.31	1.44	92.7
mean ± SD		4.76±0.24	35.4^{b}±1.1	0.32^{b}±0.01	1.54^{b}±0.1	83.1±9.7
C_1						
Fish with	1	5.51	43.0	0.37	1.38	85.5
swimbladder	2	5.15	41.3	0.39	1.27	96.3
(mixed)	3	5.43	41.2	0.39	1.36	100.0
mean ± SD		5.36±0.19	41.8^{a}±1.0	0.38^{a}±0.01	1.33^{a}±0.06	93.9±7.5
C_2						
Fish without	1	4.88	33.8	0.31		80.0
swimbladder	2	4.91	32.7	0.30	same as	98.7
(mixed)	3	4.71	35.6	0.33	C_1	98.7
mean ± SD		4.83±0.11	34.0^{b}±1.46	0.31^{b}±0.01		92.5±8.8

* Superscript with different letters are significantly different (P<0.01)
** Superscript with different letters are significantly different (P<0.05)
*** Superscript with different letters are significantly different (P<0.05)
**** Not significant (P>0.05)

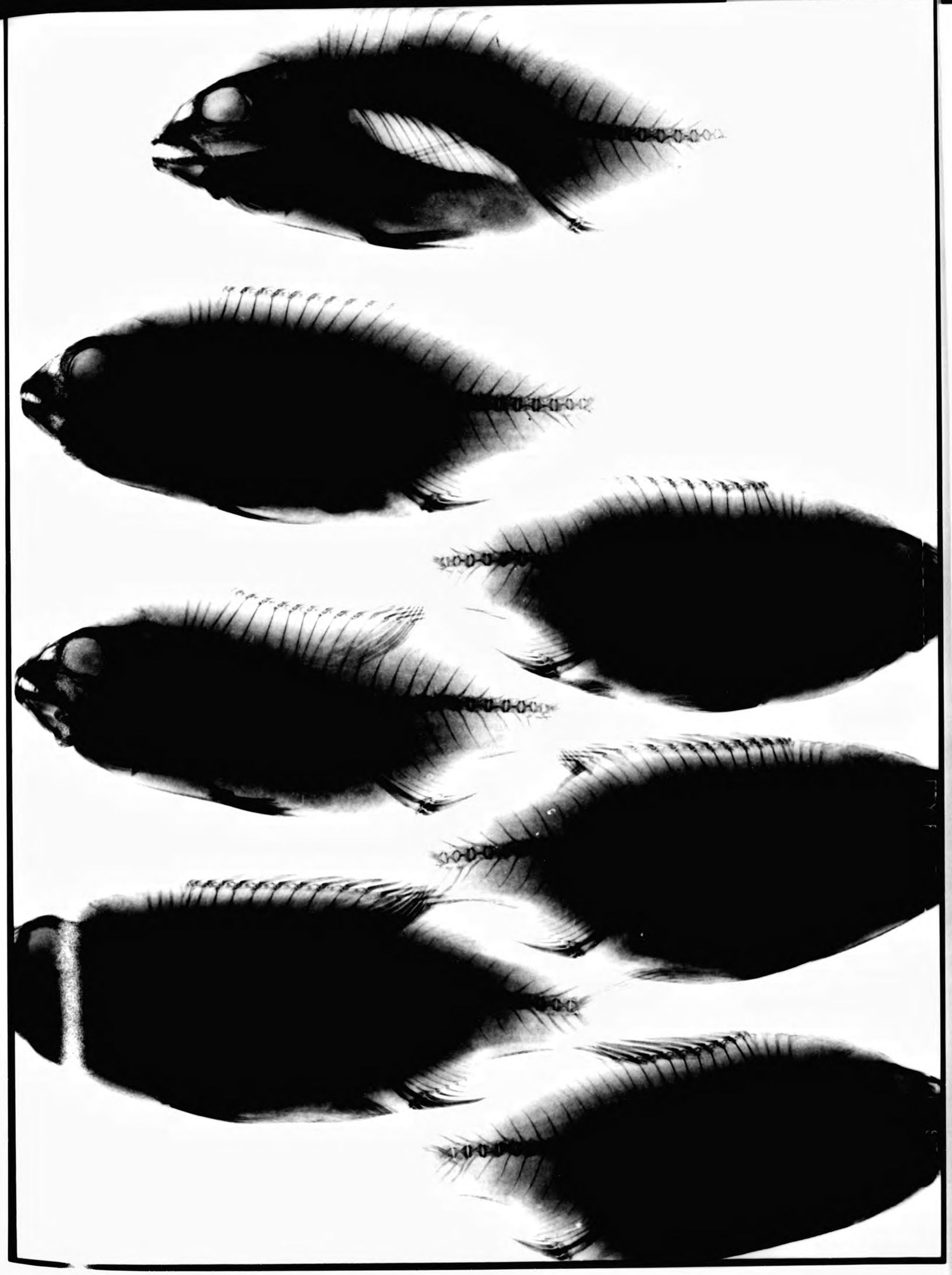


Plate (7.2). Radiography of normal (top) and deformed *A. cuvieri* juveniles.

Table (7.3) The types of spinal deformity observed among *A. cuvieri* fingerling and juvenile of different sizes which lack a functional swimbladder.

Size Total length(mm) (Body weight,g)	Types of spinal deformity	No of deformed fish	Percentage	Approximate bending angle of column	Fish general appearance
4-8 (1-8)	Straight	14	58	-	Normal and slim
	Single front bend*	10	42	150-170°	Head-up
	Single middle bend**	None	-	-	-
	Double bends (Front and back)	None	-	-	-
9-12 (10-20)	Straight	2	4.6	-	Normal and slim
	Single front bend	28	65.1	140-160°	Head-up
	Single middle bend	5	11.6	130-140°	curved upward
	Double bends (Front and back)	4	9.3	140-160°	curved upward
15-21 (70-170)	Straight	3	3	-	Normal and slim
	Single front bend	64	60	130-150°	Head-up
	Single middle bend	27	25	110-130°	curved upward
	Double bends (Front and back)	13	12	130-150°	curved upward

* The bending occurs at vertabrae 5 to 9.
** The bending occurs at vertebrae 10 to 13.

is high in small fingerlings, decreasing drastically as the fish grow. The second and third types of spinal deformity develop from the "straight" vertebral column as the fish increases in weight and as the muscular system develops and becomes more used. The continuous muscular activities by the sinking fish to stay in the water column probably puts a lot of strain on the front and middle portions of the spinal column. Generally, the vertebral column bending worsens with growth as the angle of bending decreases (Table 7.3). The single front-bending type occurs more frequently and appears earlier than the single middle-bending type in the larger fish.

The fourth type of spinal column deformity is the double bend in which two v-shaped bends are found at the anterior and posterior ends of the vertebral column. The deformity can range from a relatively smooth curve to an abrupt angle.

Within all types of deformity a wide variety of different shapes occur. A single v-shaped bend at the posterior and of the spinal column was found in some fingerlings having a functional swimbladder suggesting that this posterior bend is not related to its absence. The appearance of the double bend deformity was observed at a body weight of about 10g. This double v-shaped bend deformity is a complex one and may be due to more than one cause. Other deformities such as vertebral fusion, shortened operculum, pughead, and deformed jaws were seen to occur in combination with vertebral deformity caused by the absence of a functional swimbladder.

IV. DISCUSSION

The lower growth rate of the fish lacking a functional swimbladder in both trials A and B is most probably due to the high rate of energy expenditure as these fish swim actively to feed and maintain a position in the

water column. This was reflected in the lower specific growth rate and higher food conversion ratios obtained. Chaptain (1987) also found that growth rate was lowered in European Sea Bass and Gilthead Sea Bream lacking a functional swimbladder. Paperna (1978) working on Gilthead Sea Bream noted marked growth retardation in those fish with abnormal swimbladders and having skeletal deformations.

It was noted that the abnormal fish in the mixed treatment occupied the lower portion of the water column and the tank bottom, while the normal ones were generally in the upper portion of the tanks. A further consequence of this is that swimbladder-deficient fish frequently rest on the tank bottom and develop skin damage and hemorrhage at the base of the pelvic and anal fins. This has also been reported by Paperna (1984) in European Sea Bass where a secondary myxobacterial infection later occurred.

The survival results were not as initially expected where abnormal fish may be expected to have a lower survival. Chatain and Dewavrin (1989) worked on European Sea Bass and found that most of the mortalities (86-100%) were from fish having no functional swimbladder. They studied the mortality during the weaning period where small, pre-metamorphosed, fish constitute the majority. Pre-metamorphosed larvae without a swimbladder swim in the upper portion of the water column, and it was noted that those were prone to being bitten by the normal fry already weaned and established in the lower column. The fish used in the study described here were much larger and more well established. The better survival of the abnormal fish may be explained by the fact that normal fish are very mobil and are more easily excited to jump than the abnormal ones. They thus, become caught in the tank covering net or are injured by striking the tank wall or the water values. A further reason could be that the overall rearing conditions were very favourable and thus masked

any differential mortality which may otherwise occur. A cage-net culture operation during winter in the Kuwaiti environment would be a good way to show whether such a difference in survival exists.

The fact that there was no secondary inflation due to gas gland secretion in those fish with abnormally deflated swimbladders after reaching a total length of 5 cm in both trials in this study shows that *A. cuvieri* is quite different to *P. major*. In *Pagrus*, a secondary inflation of fish with an abnormal swimbladder will occur by about 44 days or 2.7 cm in total body length by internal secretion of the gas gland (Kitajima *et al.*, 1981). Both *A. cuvieri* and *P.major* will lose the pneumatic duct very early in their larval development. Kitajima *et al.* (1981) also reported that lordosis linked to the absence of a functional swimbladder will be noted by day 44 after hatching in *P.major*.

Overall, the data obtained in this Chapter strongly confirm that in the absence of a functional swimbladder a deformed vertebral column will develop within the area above the location of the swimbladder. It also confirms that in *A. cuvieri* the abnormally deflated swimbladder is permanent and can never inflate as fish grow bigger. These data further suggest that it may be best to avoid stocking fingerlings without a functional swimbladder into an on-growing system as their presence will be reflected by slower growth rates and higher food conversion ratios. In addition a proportion of the harvested product will be of low quality in term of appearance and hence marketability.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

This thesis is essentially concerned with a specific abnormality, the lack of a functional swimbladder, which is only one of a number of problems encountered from time to time in the mass production of Sparids and other marine fish species world wide. Such difficulties have been reported in the Red Sea Bream, *S.major*, in Japan (Kitajima *et al.*, 1981; Iseda, 1982; Fukusho, 1985), the Gilthead Sea Bream, *S.aurata*, in Europe (Paperna, 1978, 1984; Annanomous, 1987; Chatain, 1987), the European Sea Bass *D.labrax* (Paperna, 1984; Chatain, 1987), and the Striped Bass, *M.saxatilis*, in the USA (Doroshov and Cornacchia, 1979; Bulak and Heidinger, 1980). The Blue-finned Sea Bream, *A. cuvieri*, which is being developed for culture in Kuwait (Al-Abdul-Elah *et al.*, 1982) also has this abnormality which has been linked with massive mortalities during weaning, low growth and deformed spinal column (Lordosis).

The objective of this work was to try to understand and perhaps resolve, the phenomenon of absence of a functional swimbladder in artificially reared *A. cuvieri* larvae. The work has thus concentrated on two areas of study. Firstly, the mechanism of initial inflation and the development of larval swimbladder and pneumatic duct. Secondly, the overall behaviour of larvae, including vertical migration, aggregation, and response to light and darkness and the relationship between these phenomena and larval buoyancy. The notion that several abiotic and biotic factors may have an effect on initial swimbladder inflation were also tested and the working hypotheses for this were partly based on the structural and developmental studies, which helped in setting the experimental design.

This work has shown that the larval swimbladder of *A. cuvieri* originates as an outgrowth of the dorsal wall of the foregut on the second day after hatching at 25°C. It develops further, increasing in complexity, and appears to become functional when the larvae are 4 or 5 days old. At this

time, the pneumatic duct, gas gland, and rete mirabile have all developed and are probably functional. The pneumatic duct develops and appears simultaneously with the first swimbladder outgrowth, and it is probably functional from days 3 to 4 onwards. It atrophies and becomes disconnected from the oesophagus by day 10-12, thus ending any communication with the outside environment (Table 3.2). Since it was confirmed that *A. cuvieri* larvae require access to atmospheric air to initially inflate their swimbladders, this disconnection of the pneumatic duct clearly ends the possibility of normal initial swimbladder inflation.

The Blue-Finned Sea Bream larvae begin to inflate their swimbladder on the third or fourth day after hatching at 25°C. This coincides with a growth plateau at the completion of the yolk sac resorption and the initiation of external feeding. It also coincides, at this age, with the larvae forming dense aggregations just under the water surface during day-light hours. The larvae at this time have a strong phototactic behaviour and are slightly negatively buoyant (Table 4.4). This negative buoyancy was considered to provide the trigger for the larvae to swim in an upward direction during day-light.

It was expected that increasing water temperature would increase the developmental rate of the larval swimbladder, pneumatic duct, gas gland, and rete mirabile and that this may either enhance or retard the initial swimbladder inflation. It was found that, in terms of swimbladder inflation alone, the higher temperatures used (28 and 31°C) gave the worst results, while 25°C later emerged as the optimum temperature (Figure 5.1). In term of overall yield of normal larvae,25°C again, gave the best results (Table 5.2). The effect of water temperature on the initial swimbladder inflation is a complex one. It could effect the rate of yolk and oil resorption, the

development of an external feeding response and cropping rate of rotifers. The duration of potency of the pneumatic duct, development of gas gland and rete mirabile may also be effected, as well the strength of the surface tension of rearing water. These effects are discussed in chapter 5. The optimum rearing temperature for mass rearing of the *A. cuvieri* larvae seems to be 25°C.

The use of low salinities may induce the larvae to swim upward more than in the ambient sea water due to their higher sinking rate. It is also thought that low salinity could reduce the osmotic stress and hence energy expenditure. This may channel more of the food reserves (yolk and oil globule) into tissue growth. The trial on the effect of salinity in chapter 5 showed that low salinity has no effect on initial swimbladder inflation up to day 15 of age, but by day 21 a clear difference was observed at 20 ppt (Table 5.4). In terms of overall percentage yield of normal larvae there was no difference between 20, 30 and 40 ppt (Table 5.5). *A. cuvieri* larvae are negatively buoyant in the ambient sea water of 40 ppt and it seems that the sinking rate of *A. cuvieri* larvae in 40 ppt is enough to trigger swimming and drive the larvae to the upper layer of surface water. Thus , ambient sea water of 39-40 ppt will be the best to use.

The phototactic behaviour observed in *A. cuvieri* larvae and their aggregation near the water surface during the first week promoted the idea of testing and identifying the light intensity at which they exhibit best phototactic behaviour and best survival. Best initial swimbladder inflation was attained at 250 to 1000 lux (Table 5.11), while, the percentage yield of normal larvae was the same for all tested treatments (Table 5.12). It seems that the larvae will aggregate densely near the water surface at a light intensity below 1000 lux (fluorescent day-light) and that this favours higher

initial swimbladder inflation. By contrast, higher light intensities cause the larvae to spread more evenly through the water column (Figure 5.18), which seems to promote higher survival. Therefore, on balance, a light intensity of 1000 lux was selected as optimum for mass rearing of *A. cuvieri* larvae.

It was considered that the use of continuous illumination as a photoperiod regime for rearing *A. cuvieri* larvae would help in keeping the larvae aggregated near the surface for maximum duration, thus, facilitating higher percent of initial swimbladder inflation. It was also possible that, in continuous light, the larvae will have a longer time to train to feed and so eventually a higher percentage of external feeders will become established. These feeding larvae are stronger and have a better chance to inflate their swimbladders than starved and weak ones. The photoperiod trial reported in chapter 5 seems to confirm this hypothesis both in terms of survival and initial swimbladder inflation. Thus, continuous illumination can be recommended for mass larval rearing of *A. cuvieri* for the first two weeks.

The idea behind a proper aeration rate is that, in addition to providing oxygen, it could create the right mechanical force which cause surface instability. This may result in reduction of water surface tension and could help to clean the surface water by gently pushing any scum or debris to the side of the tank. It seems probable that this would ensure a higher percentage initial swimbladder inflation. The aeration rate trial (chapter 5) showed that the mechanical effect obtained from an aeration rate of 50 to 300 ml/min was best in giving high percentage of initial swimbladder inflation and larval survival (Tables 5.17 and 5.18). The mechanical effect of aeration differs with different tank sizes, shapes and size of air bubbles, therefore it is not easy to fix a specific value for aeration rate. For the

experimental conditions used in this trial, it is only possible to say that 50 to 300 ml/min gave the best swimbladder inflation and survival.

The water-exchange trial (chapter 5) was based on the notion that the addition of new water to the water surface will disturb the surface tension and weaken it. The new, clean, water will also replace the old water and ensure a surface layer free of any debris or film that may impede free air access for the initial gulp of atmospheric air. This trial was also considered as a test trial combining the optimum results from all the biotic and abiotic factors examined. The results showed that water-exchange rate have no real effect on initial swimbladder inflation but a strong effect on larval survival (Figures 5.27 and 5.28). It is possible that use of a non-oily enrichment for rotifers gave film-free water surface thus masking any effect of the water-exchange. The high percentage swimbladder inflation in all treatments is a strong indication of success in combining these optima of the biotic and abiotic factors. The effect of water-exchange rate will differ according to size of tank used and its surface area although, overall, it is possible to say that an 8-hr flow of 250 ml/min gave best results.

Table 5.21 lists the values of parameters tested which gave the best yield of normal *A. cuvieri* larvae.

The process of initial swimbladder inflation requires a certain level of larval strength and muscular activity for swimming and breaking the water surface tension. Therefore, larval fitness and well-being is a prerequisite for successful initial swimbladder inflation and the three biotic factors examined all promote such fitness.

It is commonly known that more suitable food available to the larvae the higher the ingestion rate, resulting in a higher larval fitness and

swimming ability. The optimum rotifer: larva ratio was 100 to 1000 rotifer:larva (Table 6.2). The exact cut off point of how many rotifers should be made available initially to each larva is probably governed by other factors in addition to the ingestion rate. These may include the actual percent of normal larvae, water quality of rearing media, the number of remaining uneaten rotifers,larval survival,and general wastage. Based on the data obtained, the optimum ratio for mass rearing of *A. cuvieri* is probably 100-200 rotifer:larva.

It was thought that the use of a smaller size rotifer (S-type) may help in attaining higher success in establishing external feeding which would then be reflected in a higher percent of fit and strong larvae. The rotifer type trial in chapter 6 suggested that this hypothesis was not true although it seems probable that the larvae fed on S-type rotifers were infact underfed. While there was no difference in initial swimbladder inflation noted between the two rotifer types (Table 6.5), larval survival was strongly affected in favour of the L-type rotifer (Table 6.6). This trial needs developing further by supplying the two types of rotifer by weight and not by number per unit volume and that W3HUFA should be high in both of the rotifers.

The initial capabilities of larvae to swim and search for food and eventually establish external feeding comes from the condition of the embryo which is controlled by several factors. This condition, referred to as embryo quality or egg quality, is strongly related to the condition of the broodstock. The compiled trial data in chapter 6 shows that larvae hatched from early-spawned egg batches are far better than those hatched from later egg batches from the same broodstock. This superiority is in terms of both initial swimbladder inflation and larval survival (Table 6.7 and 6.8). *A. cuvieri* broodstock spawn continuously for 6-8 weeks with a high percentage of

floating eggs and a high hatching rate during the first two weeks. It may be possible to increase the egg quality in terms of percent swimbladder inflation and survival in the mid and late egg batches by broodstock diet manipulation, since *A. cuvieri* is an active feeder during spawning. This idea was suggested indirectly by Watanabe *et al.* (1984d, 1985b) who were working on *P.major* which also feeds actively during spawning.

Table 8.1 list the optimum biotic factors recommended for maximum production of normal *A. cuvieri* larvae.

Table 8.1 Summary of the optimum values for the biotic factors tested achieving maximum yield of normal *A. cuvieri* larvae

Factor	Recommendation
Rotifer:larva ratio	100 - 200
Rotifer type	L - type
Egg batch	early egg batch

Based upon the data generated in these trials, and the discussions from the previous chapters, the nine abiotic and biotic factors examined can be grouped into three major strategies which contribute to successful initial swimbladder inflation. These three strategies are; reduction of water surface tension, enhancement of larval fitness and the management of the vertical aggregation of larvae.

The reduction of surface tension by continuous disturbance can be achieved by weak aeration or by gentle addition of fresh sea water to the surface layer, or a combination of both. The mechanical cleaning effect of the aeration and the continuous replacement of old surface water by the

addition of fresh sea water will keep the surface tension at its minimum value as it will be free from any oily film or other debris. Another way to reduce surface tension, providing that it is basically clean, is to increase rearing water temperature to the highest possible within the range. The physical properties of water dictate that the surface tension at 24-25°C is less than at 18-19°C (Cox, 1965).

Since many hatcheries use oil-based enrichment media to increase the W3HUFA in the rotifers, it has been found necessary to clean the oily film from the water surface thereby assuring free access of the larvae for initial swimbladder inflation. There are three common methods used in hatcheries to remove this oily film or other debris floating at the water surface. These are; a surface drainage system, use of an air blower and use of a synthetic hydrophobic mat. In CENMAR, Yugoslavia, a cylindrical surface drain is used which is positioned vertically in the center of the rearing tank. An aeration ring is fixed around the surface drain just below the water surface with holes on the outer side of the aeration ring in order to drive the larvae away from the drain. An air blower can be used and arranged to collect all the surface scum into a trap. This method has been well described by Chatain and Ounais-Guschemann (1990). A synthetic hydrophobic mat with a high affinity to oily scum (3M corporation) is found in practice in the USA in the culture of Striped Bass (personal comm., Shehadeh, Z.). All of these methods can have a positive effect and it may be that some combination of these would work out best in practice.

The minimum level of larval fitness desirable is that which will enable the larvae to swim actively and to break water surface tension mechanically for their initial swimbladder initial. Larval fitness is clearly

addition of fresh sea water will keep the surface tension at its minimum value as it will be free from any oily film or other debris. Another way to reduce surface tension, providing that it is basically clean, is to increase rearing water temperature to the highest possible within the range. The physical properties of water dictate that the surface tension at 24-25°C is less than at 18-19°C (Cox, 1965).

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The minimum level of larval fitness desirable is that which will enable the larvae to swim actively and to break water surface tension mechanically for their initial swimbladder initial. Larval fitness is clearly

connected to egg quality and consequently to larval quality, the quality and quantity of first live-food, and the provision of optimum rearing parameters.

The larvae used in this work were spawned naturally without any hormonal induction and those larvae obtained from the early egg batches seemed to perform best. In order other species, where spawning is induced hormonally, the quality of eggs released is governed by many other factors related to the technique itself. In addition, the nutrition and general maintenance of the broodstock is thought to have an effect on egg quality and hence larval performance (Watanabe *et al.*, 1984a). The conditions during egg incubation and yolk resorption may also play a part in determining larval fitness, for example, the use of optimum temperature and salinity could lead to a bigger larva at the end of yolk resorption.

Optimum larval rearing parameters are also important to ensure fitness and good overall performance. A suggested combination of parameters is shown in Table 5.21 and 8.1.

The quantity and presentation of first-feeding is most critical in establishing strong, active larvae which are fit for physical activities. The quality of live-food is also important especially the level of W3HUFA (Kitajima *et al.*, 1979).

The vertical aggregation and dispersion of larvae are influenced by, and could be managed via, two behavioural reflexes. The larvae are clearly phototactic and also exhibit the tendency to swim in the opposite direction to their buoyancy. Thus, within limits, the larvae can be manipulated to be at any required position in the water column by the use of different light intensities and photoperiod regimes. The lower light intensities (250-1000 lux) gave higher swimbladder inflation and lower

survival and the larvae were aggregated near the water surface. By contrast, the high light intensities (2000-5000 lux) gave higher survival but lower swimbladder inflation and the larvae were away from the water surface and well dispersed. The advantage of the 24-hr light is that the larvae will act as they would in day-time, remaining at the surface for a longer time and increasing the opportunity for air-gulping. The use of a low aeration rate may also be important to assist free larval vertical movements and to promote aggregation near the water surface.

It is important to realise that fit larvae, aggregated near the water surface, at the right time will not necessarily inflate their swimbladder if there is no free access to atmospheric air. Thus, the overall assurance of successful initial swimbladder inflation depends on the utilization of all of the three strategies discussed, but particularly free access to the air-water interface.

A marked variability in the results of swimbladder inflation and survival trials in marine and brackish-water hatcheries from year to year and batch to batch is a common fact. This variability may occur for two main reasons; the general lack of knowledge on larval behaviour, physiology and development, and uncontrolled biotic factors such as egg quality and the unwanted microbial and macrobial populations that tend to develop in the rearing tanks. This variability in the quality and quantity of artificially produced larvae has a strong negative economic impact on this industry. Thus, if it is assumed that 50% of the fry produced have no functional swimbladder and that they will not be stocked for on-growing, this will cut the hatchery production in half and double the production cost per fry. Similarly, even if it is assumed that those fry without a functional swimbladder will be used

in on-growing, this will also lead to a lower production because of the poorer FCR's and lower product quality in terms of appearance and marketability.

The work reported here summarises the development of the swimbladder in *A. cuvieri* larvae and clearly indicates how this first inflation may be maximised through careful husbandry and an understanding of the biological processes involved. Although many questions remain unanswered, it is hoped that some of the data will be of practical value in this rapidly developing and economically important field.

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