AN ASSESSMENT OF THE SUITABILITY OF THE GALJOEN, *Coracinus capensis* CUVIER, FOR MARICULTURE IN SOUTH AFRICA

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CONTENTS

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Published papers arising from this thesis are listed below:

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Abstract

The suitability of the galjoen, *Coracinus capensis*, for mariculture in South Africa has been assessed from both an economic and a biological viewpoint. In terms of economic criteria galjoen shows a high potential for culture, being widely known and highly regarded as a table fish. The restaurant trade in the southwestern Cape has been identified as a possible market showing a high demand for this species.

Adult galjoen are readily available, hardy, and are easily maintained in captivity. A biochemical technique to determine the sex of live fish has been elucidated and allows sexual identification to be conducted for several months prior to and during the spawning season. Natural serial spawning with high fertilization occurs readily in captivity, with galjoen producing large numbers of good quality eggs during a single season. However induced spawning using two mammalian gonadotropins resulted in the production of poor quality eggs. The relationship between temperature and development time for fertilized galjoen eggs has been determined, and the lower lethal temperature for developing eggs was found to lie between 14° and 16°C. Newly-hatched larvae exhaust their endogenous food reserves rapidly and show a short time to irreversible starvation, with first-feeding beginning from 110 hours after hatching at 18°C.

Galjoen larvae proved to be extremely difficult to rear. Although successful first-feeding on a few live food organisms was observed, mass mortality at 4-8 days and again at 12-15 days after first-feeding resulted in exceptionally poor survival. It is surmised that galjoen larvae have very stringent nutritional requirements which cannot be met by employing commonly-used live food organisms. Growth rate estimates for galjoen that survived past metamorphosis indicate that growth under culture conditions is similiar to that in the natural environment.

Certain biological attributes of the galjoen such as simple broodstock maintenance, ease of natural spawning in captivity and high fecundity select this species for culture. However the inability to mass rear larvae through to metamorphosis drastically reduces the suitability of this species, especially since larval rearing is the key to successful mariculture. In addition, the slow growth rate under culture conditions observed also selects against this species. Because of these last two factors, it is concluded that the galjoen is not a suitable species for mariculture in South Africa.

CHAPTER 1: INTRODUCTION

1.1: Definitions of aquaculture

The term aquaculture has become recognized as meaning the husbandary of aquatic organisms (Bardach *et. al.*, 1972), and involves the culturing of aquatic organisms from an early to a later life-history stage under controlled or semi-controlled conditions (Safriel and Bruton, 1984). Products derived from aquaculture are generally utilized directly for human gain, be it nutritional (Hecht, 1988) or economic (Shang, 1981). However, aquaculture may also be used to fulfill other objectives such as the replenishment of natural stocks, the production of sport fish, bait or ornamental fish, as a means of recycling organic wastes, or for the production or commodities such as pearls or oils.

Within the term aquaculture, two further terms need to be clarified; viz. intensive and extensive aquaculture (Rabanal and Shang, 1976 in Retief, 1978). Intensive aquaculture refers to a system where the entire life-cycle of the selected organism is maintained in the culture environment. Sophisticated techniques including the use of artificial diets, induced spawning, larval rearing and genetic manipulation are utilized to ensure stringent control and result in high levels of production. Extensive aquaculture refers to a system where certain stages of the life-cycle are not maintained, such as when wild-caught juveniles are reared to production size. Traditional techniques, for example using the natural productivity of the water to supply food, are often utilized. This practice minimizes costs, but generally results in low production.

Mariculture is a sub-category of aquaculture, and distinguishes marine from fresh-water culture. This classification can be confusing where the organism (eg. salmon) being cultured migrates between fresh- and seawater during its life-cycle.

1.2: Principles of aquaculture

Several biological principles are fundamental to the practice and development of aquaculture, and include the following (Bardach *et. al.*, 1972):

1) the density of most fish and swimming crustaceans is approximately equal to the density of the water they inhabit. Energy costs of supporting their weight are thus much less than in terrestrial animals of comparable size,

2) poikilothermic animals such as fish do not expend energy on thermoregulation (apart from a few exceptions such as tuna),

3) since aquatic poikilotherms spend little energy on support and none on thermoregulation, they exhibit high food conversion efficiencies, and

4) a body of water is a three-dimensional growing space; biomass per unit volume can therefore be very high.

The major disadvantages of water as a medium for biological production are due to the general properties of liquids as a group, and to the specific property of water as a universal solvent. Contamination of water by either biological or chemical means is therefore difficult to prevent or control, and renders aquaculture extremely vulnerable with respect to pollution and organic decay in any form.

1.3: Selection of candidate species for culture

According to Bruton and Safriel (1985), a major retarding factor to the development of aquaculture worldwide has been the incorrect choice of candidate species. They make the point that in countries currently developing aquaculture, time and effort should be spent in identifying suitable candidate species so as to avoid costly mistakes. Webber and Riordan (1976) divided criteria used to select species for culture into three major categories. First-category criteria were economic, relating to consumer acceptance and processing variables in conjunction with marketing and pricing factors. Second-category criteria were biological, being factors promoting management of high density culture systems. The third group of criteria were also biological, being considered a function of the adaptability of the species examined.

Of the three categories identified by Webber and Riordan (1976), economic criteria are considered the most critical (Bruton and Safriel, 1985; Shepherd, 1988; Cook and Walmsley, 1990). This is because success of a modern intensive aquacultural enterprise is determined by its profitability. Profitability in turn is dependent on a high marketability of the product combined with a low capital expenditure. In addition, demand for the product must be sufficient to be able to absorb increases in production without excessive price erosion. The importance of market factors has been emphasized by Hecht and Britz (1990), who stated that when assessing candidate species for culture, market demand is of greater importance than any other consideration.

Biological criteria, or ease of culture of a particular species, include the following (Webber and Riordan, 1976; Bruton and Safriel, 1985; Shepherd, 1988; Cook and Walmsley, 1990):

1) seed availability,
2) simple larval development,
3) tolerance of young to high densities, disease and suboptimal conditions,
4) high growth rate and survival,
5) readiness to take artificial feed and high feed conversion efficiency,
6) high fecundity,
7) ease of inducement and control of spawning, and
8) ease of genetic selection.

Part of the development of aquaculture worldwide has been a trend towards semi-intensive or intensive culture systems. These simplified natural ecosystems are inherently unstable and often fluctuate widely (Bruton and Safriel, 1985). For a species to be suitable for intensive culture it must be tolerant of a symplified ecosystem yet able to tolerate environmental fluctuations. Thus the characteristic of adaptability, whether to increased densities or changing water quality, is perhaps the most important biological criteria determining the suitability of a particular species for culture (Shepherd, 1988).

1.4: History and current status of aquaculture

Aquaculture is an ancient practice that can be traced to the dawn of recorded history and probably began in Asia about 4000 years ago (Ling, 1977 in Liao, 1988). Reported instances of early aquaculture include carp culture in 5th century BC China and oyster culture in Ancient Gaul (Bardach *et. al.*, 1972). Until recently, aquaculture was practiced mainly on a subsistence basis, with food being produced predominantly for personal and familial consumption. With the increase in size and complexity of societies and incipient industrialization changing the socio-economic framework, aquaculture moved into a second level, the domestic production phase (Aiken, 1988). Food produced by culture systems in this stage was distributed and sold locally through established marketing channels, with the aquaculturist receiving some return (either goods or money) for the food produced. The third and most recent level of aquaculture is described as the export production phase (Aiken, 1988) and refers to a culture system where food produced is destined for non-local sale and consumption, often large distances from its origin.

Although the export production phase is a recent development, the impact of this type of culture is enormous. Because of the economic structure of this phase, some nations have hastily converted their production of high protein, low domestic value fin-fish into production of high value shrimp for the export market, generating foreign exchange at the cost of domestic protein supplies. Aiken (1988) reports that in Ecuador alone, approximately 100 000 people (out of a population of approximately 12 million) are employed in shrimp farming, whilst production of shrimp has spread around the globe along a wide tropical-subtropical band.

Export phase aquaculture has resulted in enormous market expansion and broadened species distribution. Select culture species such as the American lobster (*Homarus* spp.), Australian marron (*Cherax* spp.), Indonesian freshwater prawn (*Machrobranchium* spp.), the African bream (*Tilapia* spp.) and various species of abalone (*Haliotis* spp.) are currently being cultured far away from their native environment.

Whilst much of the East has had a long tradition of fish-farming, aquaculture in the West is still in its infancy. Few western culture systems have a history of more than 100 years, with the majority of commercial food production via aquaculture in the west occurring during the last 30 years (Sandifer, 1988). This disparity between Eastern and Western aquaculture is reflected in the proportions of the 1985 total global aquaculture production; the East (Asia and Oceania) contributing 85% of reported world harvests whilst the West (North and South America and Europe) makes up only 13% (Rhodes, 1988 in Sandifer, 1988). In recent decades however, there have been a number of success stories in western aquaculture. Examples of these include

Atlantic salmon (*Salmo salar*) culture where production has doubled every two years since 1980, and channel catfish (*Ictalurus* spp) culture where production figures of 10 000 metric tonnes (MT) in 1977 rocketed to 97 000 MT in 1986 (Sandifer, 1988).

A probable reason for the late development of aquaculture in the west has been its reliance upon traditional intensive fishing methods as the source of both marine and freshwater fish. Coupled with this is the fact that, in the west, fish has never been viewed as a major protein source; in 1985 per capita consumption of fish in the U.S. was 7.5 kg/person/year (Sandifer, 1988). Consumption of fish in the east is an order of magnitude higher, at 83 kg/person/year (Liao, 1988). The realization that traditional fishing was not harvesting an infinite resource provided the impetus required to expand and develop intensive aquaculture in the west. Estimates of maximum sustainable yield (MSY) from the world oceans range from 90×10^6 to 150×10^6 MT annually for traditional catch-species, and up to $300\text{-}500 \times 10^6$ MT if unconventional species are included (Lyubimova *et. al.*, 1973; Suda, 1973; Wheaton, 1977). Whilst this quantity remains static, worldwide demand continues to grow at an increasingly rapid rate (Figure 1.1).

In the past, most notably the 60's, aquaculture was seen as the answer to the world's food problems, being able to supply unlimited amounts of protein. More recent perspectives realize that this is impossible, but accept that the shortfall between fishery supply and market demand could partially be supplied by aquaculture (Sandifer, 1988).

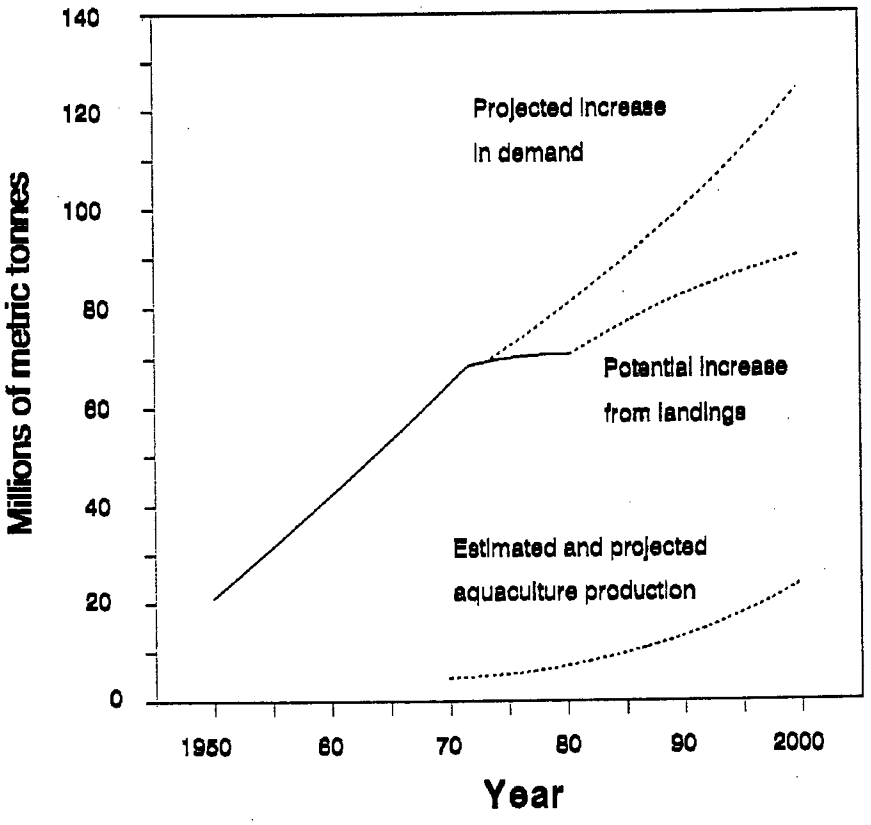


Figure 1.1: Current and future trends in global aquatic production. Redrawn from Sandifer (1988).

Factors which have been either directly or indirectly responsible for the rapid development of aquaculture worldwide include the following:

1) the dramatic increase in world population,

2) a scarcity of cheap, high quality protein which has been experienced on an expanding scale in many parts of the world,

3) traditional fisheries production is fast approaching the maximum sustainable yield,

4) agricultural production has not kept pace with population growth in several countries, and
5) the revolution of raised expectations, which has resulted in increased demands for items that create or are a part of a higher standard of living.

Current (1985) total world aquaculture production (including finfish, molluscs, crustaceans and seaweeds) is more than $10x10^6$ MT (Nash, 1988). Of this, the $4.4x10^6$ MT of finfish farmed annually accounts for 10% of world fish harvest (Shepherd, 1988).

In Japan, one of the most developed nations in terms of mariculture, production of yellowtail (*Seriola quinqueradiata*) and red seabream (*Pagrus major*) exceeds that obtained from conventional fishing (Watanabe, 1988). Yellowtail production increased from 92 352 tons in 1975 to 150 961 tons in 1985, whilst red seabream production increased from 4 303 to 28 430 tons over the same period (Foscarini, 1988). The figures for red seabream indicate an increase in production of just over 20% per year. Estimates of world aquaculture production for the year 2000 range from $22.2x10^6$ MT (Nash, 1987 <u>in</u> Sandifer, 1988) to $50x10^6$ MT (Safriel and Bruton, 1984; see Table 1.1), with Barnabe (1990) suggesting that the output from aquaculture could equal that from fishing at the turn of the century.

Chua (1986 <u>in</u> Shepherd, 1988) calculated that world aquaculture production increased at a rate of 10.5% per year from 1972 to 1983, a total increase in production of more than twofold over this period. Hecht and Britz (1990) reported that percentage increase between 1983 and 1985 was 5% and 115% for world finfish and crustacean production respectively.

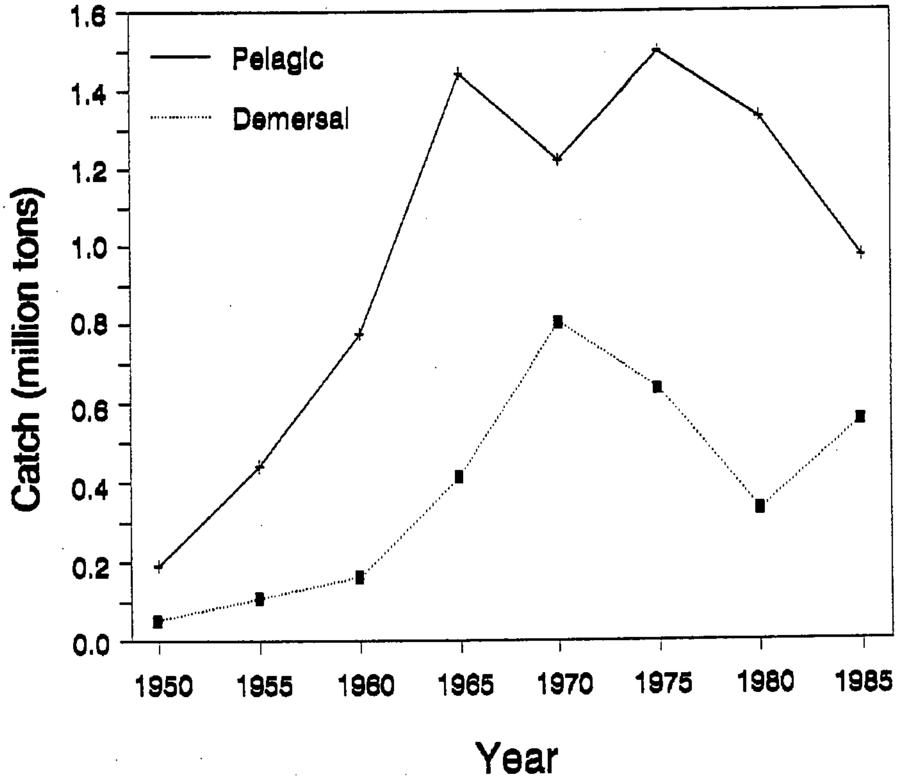
Table 1.1: Worldwide aquaculture production from 1972 onwards.

Year	**Yield** ($x10^6$ MT)	**Source**
1972	4	Bardach *et. al.*, 1972
1975	6.1	Safriel and Bruton, 1984
1980	9.4	Safriel and Bruton, 1984
1986	10.4	Shepherd, 1988
2000	22.2 (est)	Nash, 1987 in Sandifer, 1988
2000	50 (est)	Safriel and Bruton, 1984

1.5: Aquaculture in South Africa

In South Africa, fishing has only been an important industry since the beginning of the present century. The demersal trawling industry was established in the early 1900s and a pelagic fishery for pilchards, anchovy and other shoaling fish was developed in the 1940s (Payne and Crawford, 1989). The fishing industry expanded rapidly during the first six decades of its existence and by 1962 was catching over $1x10^6$ MT of fish a year (Grindley and Rabie, 1983). This meant that South Africa was the eighth largest fishing country in the world at that time. In recent years however, a marked decline in the pelagic catch has resulted in a levelling off of the catch curve (Figure 1.2) and it is clear that the MSY for the major stocks has been reached. Similarly, the most important constituent species of South

Africa's demersal fishing industry, the hakes *Merluccius capensis* and *M. paradoxus*, are suffering from overfishing. This is indicated by a dramatic reduction in size of trawled fish and by the fact that trawlers have to go ever-increasing distances to sea in order to get the best catches (Grindley and Rabie, 1983).



<u>Figure 1.2</u>: Demersal (hakes) and pelagic (pilchard, anchovy and mackerels) catches taken off southern Africa 1950-1985. Redrawn after Payne and Crawford (1989).

Fish culture in South Africa was first begun around the turn of the century, with trout hatcheries being set up at Jonkershoek, Umgeni and Potchestroom to satisfy the demands of local sport fishermen (Hecht and Britz, 1990). The first trout farm was established in the Transvaal in 1945; since then trout farming has become the primary fish culture industry in South Africa, producing 739 tons in 1988 and having a retail value of over 11 million rand (Hecht and Britz, 1990). The culture of ornamental fish for the aquarium trade is also well established in South Africa, with over 80 varieties being produced and having a retail value of 6.5 million rand (Andrews, 1989).

A relative newcomer to the aquaculture industry in South Africa is the sharptooth catfish, *Clarias gariepinus*. Although culture of this species only started in the early 1980's, the rapid growth of the catfish industry has led to the prediction that by 1994 catfish production will surpass that of trout (Hecht and Britz, 1990).

Whilst fish culture in South Africa was initiated only in this century, attempts to cultivate oysters were started more than 300 years ago. Most of these attempts were unsuccessful however, and it was not until 1948 that the first commercial oyster farm was established. Success in farming the alien Pacific oyster, *Crassostrea gigas*, has resulted in rapid development of commercial oyster farming (Genade and Hirst, 1985). Almost 300 tons of oysters were produced in 1988, giving a retail value of over 6 million rand (Hecht and Britz, 1990). Currently, the most important aquaculture organisms cultivated in South Africa are mussels, including *Choromytilis meridionalis*, *Mytilis galloprovincialis* and *Perna perna*. The first commercial mussel farm was established as recently as 1984, and mussels now contribute over 50% of South Africa's total aquaculture production. In 1988, 1575 tons with a retail value of 17,3 million rand was produced (Hecht and Britz, 1990). The spectacular growth of the mussel industry has been attributed to the successful adaptation to local conditions of raft and rope culture technology imported from Spain (Genade and Hirst, 1985). Total South African aquaculture (including mariculture) production for 1988 was approximately 3100 tons, with a retail value of over 45 million rand (Hecht and Britz, 1990).

Mariculture in South Africa is almost entirely devoted to shellfish production. The only marine finfish cultivation is that of single operations of "sea trout" (*Oncorhynchus mykiss*) and flathead mullet (*Mugil cephalus*) culture. In the "sea trout" operation, fingerling trout reared in fresh water are transferred to seawater containers for ongrowing to market size. Approximately 150 tons of sea trout (also referred to as "salmon" trout) were produced in 1986 (Genade, 1986), whilst 20 tons of mullet were produced in 1988 (Hecht and Britz, 1990).

Several other marine finfish have been identified as potential candidate species for culture in South Africa (Safriel and Bruton, 1985; Cook and Walmsley, 1990). The top ranked species include Atlantic salmon (*Salmo salar*), galjoen (*Coracinus capensis*), Dover sole (*Solea solea*), turbot (*Scophthalamus maximus*), rabbit fish (*Siganus* spp.) and rockcod (*Epinephalus* spp.). Of these species, only the galjoen and rockcod are endemic to South Africa. The other species are aliens which have been highly ranked as candidate species because they are successfully cultured in other parts of the world. It is reasoned that South

African culture of these alien species would simply require an adjustment of already developed technology to suit local conditions. In practice however, this may not be as simple as implied. In addition, the importation of alien species for culture is fraught with potential dangers to endemic ecosystems. It seems obvious that endemic species showing potential for culture should be comprehensively assessed before alien species are considered. One such species is the white steenbras (*Lithognathus lithognathus*) whose suitability for mariculture has been assessed by examination of its bioenergetics and growth under culture conditions (Harris, 1991).

This thesis is an assessment of the suitability of the galjoen for mariculture in South Africa. The galjoen is South Africa's national fish, and is regarded as one of the premier shore-angling fish of the Cape region. In addition to its recreational importance, galjoen is a much sought after table fish and is highly esteemed for its excellent flesh. Galjoen are endemic to southern Africa, extending from northern Namibia to southern Natal (van der Elst, 1981). A comprehensive study on the biology of the galjoen was reported by Bennett and Griffiths (1986) and strategies for the management of this species provided by Bennett (1988). Galjoen tend to occur close inshore and live in shallow, turbulent water where they feed on black mussels, barnacles and algae. Spawning occurs from October to March, with December being the month of peak activity.

Galjoen was initially identified as a candidate species for culture primarily on economic grounds, due to the high price commanded by this species. As well as being commercially attractive, production of galjoen could possibly be used to replenish dwindling natural stocks (van der Elst, 1981).

This thesis is divided into several chapters which examine and assess various characteristics of the galjoen in terms of mariculture. Chapter 2 of this thesis describes the economic characteristics of the galjoen and makes an estimate of the market size and demand for this species in the southwestern Cape. Chapter 3 describes broodstock maintenance and details a technique for determining the sex of live adult galjoen, whilst chapter 4 discusses both natural and induced spawning in captivity. Aspects of the early life history of the galjoen are described in chapter 5, and various attempts to mass rear larvae are listed in chapter 6. Conclusions from this study are given in chapter 7 and the possible development of mariculture in South Africa is discussed in chapter 8.

CHAPTER 2: AN EVALUATION OF THE MARKET POTENTIAL OF GALJOEN IN THE SOUTHWESTERN CAPE

2.1: Introduction

The decision to culture a particular species in a commercial aquacultural system requires a detailed evaluation of several disparate considerations. These include biological and socio-economic factors and technical and financial considerations (Avault, 1983; 1986). As discussed earlier, it is generally accepted that market and economic considerations almost always override biological and technical ones when determining the suitability of a species for aquaculture. Chacteristics affecting the marketability of an aquacultural product include taste, appearance, colour, texture, packaging, ease of processing and preparation, availability and product price in relation to perceived quality (Shang, 1981; Ferreira, 1989). However, consumer acceptance of a new product may often be influenced by traditional food habits and preferences. Market opportunity in terms of an unfulfilled demand is also important when assessing a new species for culture (Shang, 1981; Shepherd and Bromage, 1988); demand for a product being affected by its price, the price of potential substitutes, and by habits and income levels of potential consumers (Smith, 1989).

To effectively assess the suitability of the Cape galjoen for mariculture in southern Africa, a determination of the market potential and existing demand for this species has been conducted. Galjoen has long been recognised as a prime table fish and is highly esteemed for its excellent flesh. Due to exploitation pressure on dwindling natural stocks, galjoen are protected by several control measures including a closed season, minimum size limit, bag limit and a complete prohibition on the selling of this species (Bennett and Griffiths, 1986; Bennett, 1988). In spite of these restrictions, a large demand for galjoen exists, as evidenced by high black-market prices ranging from R12/kg in the southern Cape to R20/kg in the Transvaal.

Since galjoen can be considered a luxury fish (in view of prices paid on the black-market) it was felt that future production, if realized, would cater to the restaurant trade before catering to the general public as a whole. For this reason, market potential and demand for galjoen in the southwestern Cape was evaluated through the use of a consumer survey directed at the restaurant trade.

2.2: Materials and methods

2.2.1: Questionnaire structure

Questionnaires were sent to 120 restaurants in Cape Town and surrounding areas which advertised themselves either as seafood specialists or offered seafood dishes on their menu. The questionnaire was divided into three sections, with the first section asking restaurateurs whether they had eaten galjoen before and how they rated it as an eating fish. The second section required restaurateurs to rank galjoen against six other commonly served fish in terms of taste, appearance, ease of preparation and versatility (how many ways it could be prepared/served). The third section questioned restaurateurs on aspects of demand, price, preferred serving method, and preferred form and size. Each questionnaire was accompanied by a pre-addressed envelope in order to facilitate prompt returns. A copy of the questionnaire is given in Table 2.1.

2.2.2: Questionnaire analysis

Section A was analysed by expressing the number of responses to each category within a question as a percentage of the total number of responses to that question. Analysis of section B was conducted using a weighted ranking method; the most preferred fish in a specific category (taste etc.) scoring 7 points whilst the least preferred fish scored 1 point. The scores for each fish in each category were summed and then divided by the number of responses received for that category. The resultant mean value was then used to rank the fish in order of perceived preference, the most preferred fish having the highest mean value for each category.

Questions 1-5 and 9 in section C were analysed using the same method used for section A.

Table 2.1: Structure of the questionnaire used in the restaurant survey.

Section A: Rating

Please tick where appropriate.

1. Have you ever eaten galjoen before ?

Yes No

2. How do you rate galjoen as an eating fish ?

Excellent Good Average Poor Inedible

Section B: Ranking

Please rank the following fish in your particular order of preference. Each fish must have a different number ranging from 1 (most preferred) to 7 (least preferred).

Y/tail Sole K/klip Kob Galjoen S/bras Snoek

Taste ....
Appearance ....
Prep. ease ....
Versatility ....

Section C:

Please tick where appropriate.

1. Assuming galjoen was available, would you offer it on your menu ?

Yes No

2. Do customers ever request galjoen in your restaurant ?

Yes No

3. Do you feel that there is a demand for fresh galjoen in your restaurant ?

Yes No

4. If so, what size demand ?

Large Medium Small

Table 2.1: Continued.

5. What would be your preferential way of serving galjoen in your restaurant ?

Whole fish   Fillet   Other - specify ....

6. What price would you be prepared to pay for fresh galjoen delivered to your restaurant ?

Under R5/kg   R5-10/kg   R10-15/kg   Above R15/kg

7. What prices do you pay for fresh fish delivered to your restaurant ?

Yellowtail	....
Sole	....
Kingklip	....
Kob	....
Steenbras	....
Snoek	....
Other (specify)	....

8. Assuming galjoen were available, what size (whole fish) would you prefer to receive ?

<20 cm   20-30 cm   30-40 cm   40-50 cm   >50 cm

9. Assuming galjoen were available, in what form would you prefer to receive them ?

Whole fish   Fillets

10. Assuming a demand for galjoen in your restaurant equal to your most frequently ordered fish, approximately what quantities of fresh galjoen would you require per week ?

<20 kg   20-50 kg   50-100 kg   >100 kg

11. Assuming a demand for galjoen in your restaurant equal to your least frequently ordered fish, approximately what quantities of fresh galjoen would you require per week ?

<20 kg   20-50 kg   50-100 kg   >100 kg

12. Are there any comments you wish to make ?

....
....
....
....

Mean values for questions 6, 8, 10 and 11 were calculated using the following formula:

$$\text{Mean} = \frac{\Sigma\ (\#\ \text{responses in cat}_i \times \text{mid-value cat}_i)}{\text{total}\ \#\ \text{responses in category}_i}$$

The >100 kg/wk category of questions 10 and 11 was assigned a mid-value of 150 kg/wk. Values obtained for question 7 were averaged for each species to determine the mean price paid by restaurateurs per species.

2.3: Results

Fifty questionnaires were received from restaurateurs, giving a return of 42%. Results from section A showed that 86.0% of respondents had eaten galjoen before, 51.1% of whom rated it an excellent eating fish and 37.8% rating it a good eating fish. The results obtained from section B are given in Table 2.2.

Results obtained from section C showed that 84.0% of restaurateurs would offer galjoen on their menu if it became available; 62.0% of them reporting that galjoen was requested by customers in their restaurants. 72.0% of respondents felt that there was a demand for fresh galjoen in their restaurants; 34.2% perceiving the demand as large, 43.9% as medium and 21.9% as small. Fillets would be the preferential method of serving galjoen for 73.3% of restaurateurs, 40.4% of whom would prefer to receive fillets whilst 59.6 would prefer to receive whole fish.

Table 2.2: Mean preference value (x) and ranking position (r.p.) for given species in various categories.

Species	Taste		Appear.		Prep. ease		Vers.	
	x	r.p.	x	r.p.	x	r.p.	x	r.p.
Galjoen	4.93	1	4.17	3	3.24	6	3.11	6
Y/tail	2.83	6	3.00	6	3.76	5	3.21	5
Sole	4.48	2	5.54	1	5.19	2	4.74	3
K/klip	4.45	3	4.96	2	5.33	1	5.58	1
Kob	4.38	5	4.17	3	4.38	3	5.16	2
S/bras	4.41	4	3.92	5	4.10	4	4.26	4
Snoek	2.72	7	2.29	7	2.05	7	2.32	7

Table 2.3: Average price (x, in rands), standard deviation (s) and sample size (n) for various fish species purchased by the restaurant trade.

Species	x	s	n
Yellowtail	6.62	1.40	40
Sole	13.14	2.45	36
Kingklip	9.44	2.11	40
Kob	6.98	1.49	40
Steenbras	7.15	1.56	37
Snoek	4.97	2.09	17

The average whole fish size that restauratuers would prefer to receive was 40cm; 20.5% of respondents preferring to receive fish of 20-30cm, 31.8% of 30-40cm, 13.6% of 40-50cm and 29.5% of >50cm. The average price restaurateurs would be prepared to pay for fresh galjoen was R7.61/kg; average prices paid for other species being shown in Table 2.3.

The average demand for the most frequently ordered fish in restaurants from which replies were received was 58.1 kg/restaurant/wk; average demand for the least frequently ordered fish being 24.4 kg/restaurant/wk.

2.4: Discussion

Surveys aimed at determining the market characteristics of a product can be divided into two categories (Shang, 1981); consumer-consumption and consumer-reaction surveys. The former is used when questioning respondents on aspects of a product with which they are familiar, whilst the latter deals with a hypothetical situation where respondents give their opinions on aspects of a product not yet available. The survey used here combines both categories; sections A and B being consumer-consumptive and section C being consumer-reaction. However, as Shang (1981) points out, information collected through the use of a consumer-reaction survey is generally not very reliable because consumers tend to reply to hypothetical situations in accord with their unconsidered judgements. In a situation where a consumer-consumption survey cannot be conducted, the consumer-reaction survey can provide a useful tool for estimating market demand and preference for a new product. It must be emphasized that a survey of this nature cannot accurately quantify market demand or preference but can at best provide ball-park estimates of these variables.

The results obtained from this survey show that galjoen is a widely-known table fish regarded as the best eating of the species listed in the questionnaire. In terms of appearance, ease of preparation and versatility however, galjoen is ranked lower than most of the other species. Reasons given by restaurateurs for the low preference for galjoen in these categories include the fact that galjoen flesh is blue in colour (due to high vascularisation) which detracts from its appearance, and is very fatty, resulting in limited ways of preparing it. In addition, difficulties in filleting galjoen mean that preparation is time-consuming and wasteful, with approximately 30% (by weight) wastage after filleting.

In spite of the low ranking of galjoen in the ease of preparation and versatility categories, almost all restaurateurs would offer this fish on their menus if it became available. Coupled with this, the fact that more than half of respondents reported that their customers requested galjoen indicates that the potential demand for this species in the southwestern Cape is large.

Assuming that the demand for galjoen lies somewhere between the demand values for the least (minimum) and most (maximum) frequently ordered fish, an estimate of the demand for varying numbers of restaurants can be obtained (Table 2.4). The monetary value of this potential demand over a range of possible prices can then be calculated (Table 2.5).

In a feasibility assessment of sole farming in South Africa, Retief (1978) concluded that a sole farm producing 125 tonnes/year would be a profitable proposition, with the breakeven point being 50 tonnes/year (ruling wholesale price in 1978 being R3.50/kg). Although it is beyond the scope of the author to conduct a comprehensive financial analysis, a galjoen farm producing 50 tonnes/year and selling fish at R7.50/kg would be able to supply approximately 25 restaurants for a return of ± R375 000. Production of 125 tonnes/year at the same price would result in a return of ± R830 000.

Table 2.4: Estimated weekly and yearly demand parameters for galjoen for varying numbers of galjoen.

# Restaurants	Weekly Demand (kg)		Yearly Demand (t)	
	Min	Max	Min	Max
10	244	581	12.7	30.2
20	488	1162	25.4	60.4
30	732	1743	38.1	90.6
50	1220	2905	63.5	151.1
100	2440	5810	127.0	302.2

Table 2.5: Estimated value (000's R/anum) of potential galjoen demand over a range of prices for varying numbers of restaurants.

# Rests.	R5.00/kg		R7.50/kg		R10.00/kg	
	Min	Max	Min	Max	Min	Max
10	63.5	151.1	95.2	226.6	126.9	302.1
20	126.9	302.1	190.4	453.2	253.8	604.2
30	190.4	453.2	285.5	679.7	380.7	906.3
50	317.3	755.3	475.9	1132.9	634.5	1510.5
100	634.5	1510.5	951.8	2265.8	1269.0	3021.0

It would appear from the results obtained in this survey that the demand for galjoen in the southwestern Cape is of sufficient size to ensure financial viability if commercial culture at reasonable production costs was achieved.

CHAPTER 3: BROODSTOCK COLLECTION, MAINTENANCE AND DETERMINATION OF THE SEX OF LIVE GALJOEN

3.1: Introduction

The first step in setting up an aquaculture enterprise is clearly the collection of broodstock of the selected species. Initially adult fish must be obtained from feral populations. However, once production of the cultured species through to sexual maturity has been achieved, broodstock can be obtained from captively-reared fish. This method of broodstock aquisition is preferable to collection from the wild since it enables selection for desirable traits such as rapid growth rate and high feed conversion efficiency. In addition, as the aquaculture enterprise becomes more operationally sophisticated, genetic selection and manipulation of broodstock can be conducted. A successful example of trait selection is shown by the Norwegian salmon industry, where the maintenance of individual pedigrees has allowed selection resulting in a 12% increase in growth rate per generation from 1975 (MacKenzie, 1989).

This chapter deals with the collection techniques used to aquire adult galjoen for broodstock, and maintenance (including disease control) of adult galjoen in captivity. In addition a biochemical technique used to determine the sex of live galjoen is described, since sexual differentiation on the basis of external morphology is unreliable.

3.2: Collection techniques

Adult galjoen, to be used as broodstock in this study, were collected by angling and by gill-netting using a 170mm stretched mesh monofilament net. Fish collected by angling were captured mainly at de Hoop Nature Reserve (34°30'S 20°30'E) and Cape Point Nature Reserve (34°20'S 18°25'E) and occasionally at other points along the Cape Peninsula. Those captured by gill netting were taken from Masbaai (34°23'S 18°52'E), a small bay east of Cape Hangklip.

Once captured, galjoen were transported to the Sea Fisheries Research Institute (SFRI) laboratories in Sea Point, Cape Town in 2001 black plastic drums. The drums were filled with sand-filtered seawater obtained from the laboratory and taken to the point of collection. Water in the drums was aerated during transportation. The number of fish per drum never exceeded 3 and in most cases was less. During long transportation periods, ice-blocks sealed in plastic bags were placed in the transportation drums in order to retard increases in water temperature.

Upon arrival at the SFRI laboratories the fish were transferred to either 4000l glass-fronted aquaria or a 20 000l portapool, supplied with a constant inflow of fresh sea-water and continuously aerated. During the week following capture, tanks containing newly-arrived fish were dosed with the antibiotic tetracycline (1.6 mg/l/day) in an attempt to combat bacterial infection (James *et. al.*, 1988). Food consisting of either shelled black (*Choromytilis* spp.) or white (*Donax serra*) mussels was provided on the fifth day after capture. If the mussels were not consumed within one day after introduction, they were removed and fresh mussels were provided the following day.

Adult galjoen proved to be extremely hardy fish, well able to withstand the rigours of capture and transportation. Survival of fish captured both by angling and gill-netting was 100% on all occasions. This fact is exceptional, especially in the case of fish captured at de Hoop Nature Reserve, since after undergoing the stress of being caught they were held in the transportation drums for a minimum of 5 hours before being transferred to laboratory tanks. No infection of recently captured fish was observed, and all fish had started to feed between 1 to 2 weeks after capture.

3.3: Broodstock maintenance

Adult galjoen used as broodstock were maintained in a series of 4000l glass-fronted spawning tanks supplied with a constant input of sea-water at a rate of 2l/min. Continuous aeration was provided through airstones placed at the bottom of each tank and illumination was regulated through the use of 4 200W light bulbs suspended above each tank and connected to a self-timer (14:10 L:D). Fish were fed daily on either shelled mussels or on a quasi-artificial diet composed of homogenised mussels and pilchards (*Sardinops ocellata*) mixed with trout pellets in the ratio 1:1:1 and stabilized with gelatine powder added at 4% of the total feed weight. Heater-thermostats were placed in each tank in an attempt to maintain constant water temperatures; temperatures were found to fluctuate between 16.5° and 20.5°C.

Initially, 2 to 3 galjoen were placed in each spawning tank. However, aggressive interactions between adults within a single tank necessitated the separation of fish using an anchovy-net partition which divided the tanks into two sections. Aggression appeared to be both sex- and size-linked, with large females commonly directing aggression towards smaller males and, on occasion, smaller females. Smaller females would also direct aggression toward males of approximately the same size. No male on male or male on female aggression was observed. Attacks by one fish on another mainly consisted of the agressor "ramming" the victim with the front of the head, generally in the region just beneath the dorsal fin in the anterior portion of the victim's body. Occasional tail- and fin-nipping by the aggressor was also observed. The victim's response to attack was to tilt the body until the raised dorsal spines were directed toward the attacking fish. Prolonged periods of aggression resulted in scale loss, surface hemmorhaging and general loss of condition of the victim, followed by death.

On one occasion an outbreak of disease occurred in captive galjoen held in the spawning tanks. Four out of the 8 fish that contracted the disease died. The symptoms of the infected fish were as follows:

1) reduced feeding,

2) changes in body colour from a dark silver-brown to a pale silver (fish stressed by handling or from being attacked turned darker brown than normal),

3) "frosting-over" of the corneas,

4) subsurface haemorrhaging, particularly in the fins,
5) increased mucus production over the surface of the body, and
6) loss of coordination.

Identification of the causative agent of the disease was carried out by microscopic examination of skin smears taken from infected fish. These examinations revealed the presence of a large ciliate, later identified as *Cryptocaryon irritans* Brown. This ciliate is regarded as the marine equivalent of the freshwater ciliate causing white spot, *Ichthyophthirius multifillis* (Needham and Wootten, 1978) and has in recent years become an increasingly frequent pest in commercial mariculture operations (Paperna, 1983; Colorni, 1985). The parasitic feeding stage of *C. irritans* (known as the trophont) ranges in size from 50 to 450 µm along the major axis, and lives primarily in and under the epithelial tissues of the skin, gills, eye and buccal cavity (Wilkie and Gordin, 1969). Infestation results in the formation of opaque papules on the surface of the host's skin which later burst to release the trophont. The trophont then sinks to the substrate where it encysts to form a tomont. Within the cyst, the tomont multiplies and produces several thousand free-swimming tomites which invade new hosts to form trophonts (Willie and Gordin, 1969).

The following treatments were used in an attempt to halt the disease:

1) a single *in situ* addition of the antibiotic tetracycline to give a concentration of 1.6 mg/l (James, pers. comm.),

2) an overnight bath in a 0.0001% solution of phenoxotol, a preservative frequently used to combat disease in marine aquaria (Tugwell, pers. comm.),

3) a 1 hour bath in a 2 mg/l solution of malachite green (Poupard, 1978),

4) direct application of Sterazin (a commercial product effective against ectoparasites) onto the skin of an infected fish (van Zyl, pers. comm.), and

5) a daily *in situ* addition of copper sulphate to maintain a concentration of 1.5 mg/l within the tank (Wilkie and Gordin, 1969).

The only treatment found to give positive results was that of daily addition of copper sulphate; no fish showing early symptoms of the disease died once this treatment had been initiated. This treatment was effective due to the fact that copper is lethal to the free-swimming stages of *C. irritans* (Wilkie and Gordin, 1969).

The high mucus production by infected fish is a normal defence against protozoan parasites (Lom, 1970 in Huff and Burns, 1981) and results in a protective covering being provided for the parasite. The efficiency of drugs used to control the infection is therefore much reduced; it is due to this fact that the parasite is so difficult to eradicate from infected fish. The copper sulphate treatment can therefore be used to prevent infection or to treat fish at an early stage of the disease, but has little effect on fish showing more advanced infections.

Techniques which have been successfully used to combat advanced infections include a formalin-copper sulphate shock treatment (Wilkie and Gordin, 1969) and exposure to either hyper- or hyposaline water (Cheung *et. al.*, 1979; Huff and Burns, 1981; Colorni, 1985). However, these treatments were not tested on infected galjoen since the need for them did not arise after the first infestation.

3.4: Sex determination of live galjoen using a biochemical technique

3.4.1: Introduction

An essential aspect of fish culture is the ability to determine the sex of adult fish required for broodstock. In many instances, species selected for culture show little or no external sexual dimorphism. When sexual dimorphism does occur, it is often limited to a short period corresponding to the pre-spawning stage of the reproductive cycle. To overcome this problem, several techniques have been developed to determine the sex of sexually monomorphic fish.

A number of invasive techniques such as catheterization (Shehadeh *et. al.*, 1973a; Ross, 1984), immunoelectrophoresis (Goedmakers and Verboom, 1974), radioimmunoassay of sex hormones (Sangalang *et. al.*, 1978) or vitellogenin (Idler *et. al.*, 1979) and immunoagglutination (le Bail and Breton, 1981) have been developed. A biochemical assay of blood plasma (Craik and Harvey,

1984) as well as a non-invasive technique using ultra-sound imaging (Martin *et. al.*, 1983) have also been described. Although invasive techniques require excessive handling of the fish which may lead to stress and high risk of injury or infection, the non-invasive method of Martin *et. al.*, (1983) requires sophisticated equipment and highly developed technological expertise. In view of the fact that such equipment is rarely available in a fish farm situation, it was felt that one of the invasive techniques listed above would be more practical for use in an aquaculture enterprise. Ideally, the technique should stress the fish as little as possible, be relatively easy to conduct, require as little sophisticated equipment as possible and be inexpensive. After examination of the various invasive techniques described, it was felt that the biochemical assay used by Craik and Harvey (1984) would best fit the required conditions.

This technique relies on the identification and determination of vitellogenin in the blood plasma. According to Wallace (1978), vitellogenin, a calcium-binding lipophosoprotein, is the precursor of much of the dry matter of the egg yolk. Vitellogenic females can therefore be positively identified by high levels of protein-linked phosphate in the plasma. The presence and quantitative estimation of alkali-labile protein phosphorous in the blood plasma forms the basis of this method.

In this section the use of the vitellogenic technique for the determination of the sex of galjoen, a sexually monomorphic fish, is described. Being a perciform (Family Coracinidae) it is related to a number of other species which are cultured commercially in other parts of the world (e.g. sparids, carangids and lutjanids) and thus the results may be applicable to other species in this order.

3.4.2: Materials and methods

Galjoen used for blood analysis were caught by angling at de Hoop Nature Reserve (34° 30'S 20° 30'E) during 1986 and 1987. On the day of capture, usually within 4 hours, blood samples of 3-5 ml were collected by cutting the branchial artery and suspending the fish in a head-down position over a funnel leading to a collection vial. Aliqouts of 0.02 ml heparin solution (10 mg/ml in 0.2 M NaCl) were mixed with the blood to prevent coagulation. Samples were then centrifuged for 5 minutes at 20000xg to separate the plasma, which was then frozen until required for analysis. The fish were then dissected to determine sex and gonad stage. Classification of the gonads into different stages of maturity was according to the method described by Bennett and Griffiths (1986).

To test the technique under laboratory conditions blood plasma was collected from captive galjoen held in seawater aquaria at the SFRI laboratories. In this case, each fish was removed from the holding tank, placed on a foam rubber mat and restrained by an assistant using a damp cloth covering the anterior two-thirds of the fish. A syringe with a 1 mm external diameter needle was then inserted midway between the anal and caudal fins. The needle was inserted towards the ventral side of the spinal column and moved gently back and forth whilst maintaining gentle suction. When the vein was pierced 2 to 3 ml of blood were removed and the fish was returned to the holding tank. 1.5 mg/l of the antibiotic tetracycline was added to the tank in an attempt to reduce infection. Blood samples were then treated as described above.

The method of plasma phosphoprotein (PPP) determination was based on that of Martin and Doty (1949) as modified by Wallace and Jared (1968 <u>in</u> Craik and Harvey, 1984). 0.5 ml of plasma was mixed with 5 ml 20% aqueous trichloroacetic acid (TCA) and allowed to stand at room temperature for 10 minutes. The tubes were then centrifuged for 10 minutes at 3000xg to precipitate the lipophosphoprotein and the supernatant was discarded. This process was repeated using a further 5 ml TCA to remove traces of acid-soluble inorganic phosphorous. The lipophosphoprotein precipitate was then extracted using the following sequence of organic solvents: absolute ethanol at 60°C, ethanol-diethyl ether-chloroform (2:2:1), acetone, and diethyl ether. In each case 3 ml of organic solvent was added to the precipitate and left to stand for 10 minutes after breaking up the solids with a glass rod to ensure penetration of the solvent. The tubes were centrifuged for 10 minutes at 3000xg between each stage of solvent extraction and the supernatant was discarded.

After the extraction process, the precipitate was dried by standing the tubes at room temperature for 2-3 hours. Blank tubes (containing nothing) and triplicate standards containing 0.5 ml standard phosphate (219.5 mg dry KH_2PO_4/l = 25 µg P), 0.25 ml standard phosphate (= 12.5 µg P) and 0.125 ml standard phosphate (= 6.25 µg P) were prepared and treated identically to the sample tubes. 1.0 ml 2N sodium hydroxide was added to each tube which was then heated in a boiling water bath for 20 minutes. The tubes were then cooled and 1.0 ml 2N hydrochloric acid, 5.0 ml isobutanol-toluene (1:1), 1.0 ml 0.2M tungstosilicic acid and 1.0 ml 40mM ammonium molybdate in 1.25M sulphuric acid were added.

After shaking, the tubes were centrifuged for 10 minutes at 3000xg to separate the phases, and 1.0 ml of the upper (organic) phase was removed. This was then treated with 0.2 ml dilute stannous chloride (1.0 ml of a solution of 1.0 g $SnCl_2.2H_2O$ in 2.5 ml conc. HCl diluted to 200 ml with N H_2SO_4) and 3.8 ml 2% ethanolic sulphuric acid. Both the concentrated and dilute stannous chloride solutions were prepared on the day of use. The absorbance of the resulting solutions at 650 nm was determined immediately after mixing, and the results were expressed as µg protein phosphorous per ml plasma.

3.4.3: Results

PPP levels for female and male galjoen at each stage of gonad development are shown in Figure 3.1. Female galjoen ranged in size from 354 to 504 mm total length (TL) whilst males were 329 to 425 mm TL. As length at 50% maturity is 340 mm TL and 310 mm TL for females and males respectively (Bennett and Griffiths, 1986), it is reasonable to assume that the majority of fish sampled were sexually mature.

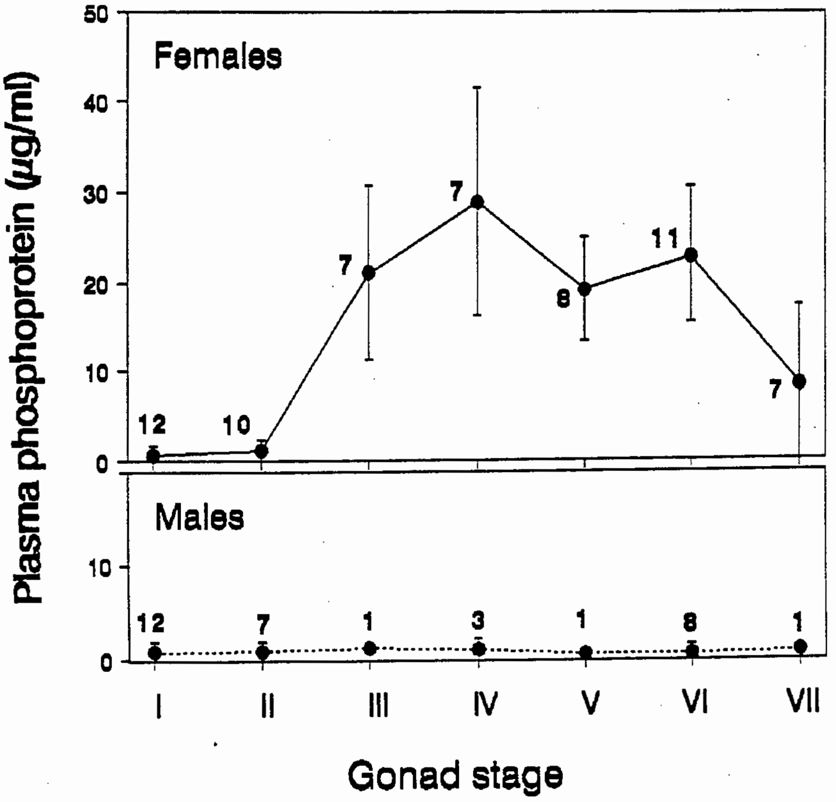


Figure 3.1: Plasma phosphoprotein levels for female and male *C. capensis* at all stages of gonadal development. Means and standard deviations are shown, with sample size given in brackets.

PPP levels in females were low for stages I and II (0.68 ± 0.83 and 1.09 ± 1.17 µg/ml respectively) but had increased substantially by stage III (21.13 ± 9.67 µg/ml). Thereafter levels remained high (28.93 ± 12.71, 19.24 ± 5.90 and 22.90 ± 7.65 µg/ml for stages IV, V and VI respectively) until stage VII where levels were reduced to 8.41 ± 9.02 µg/ml. This decrease in PPP level at stage VII corresponds to the post-vitellogenic stage of the female. The highest PPP value occurred at stage IV (max. value 43.84 µg/ml) suggesting that yolk synthesis and deposition in maximal at this stage. PPP levels in male galjoen remained low at all stages, seldom exceeding 1 µg/ml and never exceeding 2 µg/ml.

The PPP values of captive galjoen are given in Table 3.1. Two of the eleven fish tested had elevated PPP levels and were therefore identified as post stage II females. These two fish subsequently spawned thereby confirming their sex.

Table 3.1: Plasmaphosphoprotein levels in captive galjoen; (a) sex confirmed by milt production, (b) sex undetermined, (c) sex confirmed by spawning.

Fish #	PPP (g/ml)	Inferred sex	Determined sex
1	3.46	Imm F or M	Male (a)
2	3.67	Imm F or M	? (b)
3	2.92	Imm F or M	? (b)
4	26.95	F (> II)	Female (c)
5	2.58	Imm F or M	? (b)
6	0.87	Imm F or M	Male (a)
7	0.33	Imm F or M	Male (a)
8	6.84	Imm F	? (b)
9	0.64	Imm F or M	Male (a)
10	0.48	Imm F or M	Male (a)
11	21.94	F (> II)	Female (c)

The remainder of fish tested gave PPP values ranging from 0.33 to 6.84 µg/ml, suggesting that they were either males or immature females. Five of these fish produced milt when gentle pressure was applied to their abdomen, positively identifying them as males. The PPP values for four of the males were very low whilst the fifth male showed an unusually high level of 3.46 µg/ml.

All fish from which blood samples had been taken exhibited signs of stress, becoming dark in colouration and refusing to feed for two to five days following the operation. However, in no case did this stress lead to mortality.

3.4.4: Discussion

Several studies have been published which demonstrate that females and males of sexually monomorphic species may be identified by the presence or absence of high vitellogenin levels in the blood plasma. Emmerson and Petersen (1976) gave values of 4.37 ± 0.09 µg protein phosphorous per ml plasma for male and 47.9 ± 5.6 µg/ml for vitellogenic female flounder (*Platichthys flesus*). Craik and Harvey (1984) reported that vitellogenic females of a range of species had values of 20-100 µg/ml whilst males, non-vitellogenic females and immature fish of both sexes gave values of less than 7.5 µg/ml.

Whitehead *et. al.* (1978) observed an increase in plasma phosphoprotein phosphorous of rainbow trout (*Oncorhynchus mykiss*) from an initial basal level of 25 to 400 µg/ml just prior to spawning. However, the method used by these authors determined the plasma protein phosphorous by measuring the total phosphorous of the lipid-free TCA precipitate. The values obtained, therefore, include all the nucleic acid phosphorous present in the plasma and are therefore higher than would be expected if only the alkali-labile phosphorous was measured.

The results presented here suggest that the PPP technique is useful in determining the sex of live galjoen. There are however, a number of advantages and disadvantages of this method when compared to other techniques. The major advantages are that the assay is inexpensive, requires only basic biochemical materials and equipment and is relatively simple to perform. Immunochemical techniques are much more complicated in that they require antisera to be produced before the test can be performed whilst radioimmunoassay techniques require isotopically labelled antigens and counting equipment. The major disadvantages of the biochemical assay are that it requires a larger volume of plasma and is less sensitive than the other methods. Identification of the sexes becomes unreliable when the PPP difference is less than 3 µg/ml (Craik and Harvey, 1984). These disadvantages are however, unimportant when the assay is applied to galjoen since large volumes of blood can be removed from the fish without excessive stress. Vitellogenin is also present at high enough levels during vitellogenesis to offset the lack of sensitivity of this method. This would presumably also apply to other species of Perciformes.

It should be noted that it is not possible to express PPP concentration in terms of vitellogenin concentration as the protein phosphorous content of galjoen vitellogenin is unknown. Whitehead *et. al.* (1978) assumed a phosphoprotein content in vitellogenin of 1.4%, whilst Craik and Harvey (1984) assumed a value of approximately 1%. De Vlamming *et. al.* (1980) found the vitellogenin of goldfish (*Carassius auratus*) to contain 0.79% protein phosphorous. It would not seem unreasonable therefore, to assume the phosphoprotein content of galjoen vitellogenin to be about 1%; in this case the plasma vitellogenin concentration in vitellogenic females would be approximately 2.0 mg/ml.

This study has demonstrated that the sex of mature galjoen can be determined on the basis of elevated PPP levels. Because the test specifically measures yolk protein in the plasma it is unlikely to misidentify a male as a female. The test may, however, fail to distinguish pre- or post-vitellogenic females from males or immature fish of either sex. In spite of this, the test can be used to identify female fish several months prior to spawning.

CHAPTER 4: NATURAL AND INDUCED SPAWNING OF GALJOEN IN CAPTIVITY

4.1: Introduction

One of the major requirements for aquaculture programmes is that the entire life cycle of the culture species should be completed in captivity. For cultured organisms, the life cycle can be divided into four sections: breeding, larval rearing, grow-out and broodstock development. Many of the marine fish species selected for culture do not breed spontaneously in captivity, this generally being due to the female not becomming reproductively active. Whilst spermiogenesis in captive males is often complete, the oocytes of females may develop to final maturation but then undergo rapid atresia (Zohar *et. al.*, 1989) and spawning will not occur. Conditioning or induced spawning techniques must thus be utilized to stimulate reproduction in captivity.

Under natural conditions, reproductive cycles in fish are regulated by environmental influences. Of these factors, temperature and photoperiod are the two most important affecting gonadal maturation and the onset of spawning (Kuo *et. al.*, 1974; Shepherd, 1988). Captive fish can be conditioned to an advanced stage of gonadal maturity through manipulation of temperature and photoperiod. This conditioning stimulates gametogenesis and in some species is sufficient to cause spawning (eg Roberts *et. al.*, 1978; Devauchelle *et. al.*, 1987). In some culture species however, gonadal maturation and spawning are induced through the use of a variety of hormones. These hormones are introduced into the fish by injection or concealed in food, and act upon various sites along the hypothalamus-pituitary-ovary axis.

Although the primary use of hormone-induced spawning techniques is directed at species that do not reproduce in captivity, these techniques can also be used to synchronize or alter the time of spawning of species that are able to reproduce naturally in captivity. In this way, gametes may be obtained outside the normal spawning season and possibly throughout the entire year (Bryan *et. al.*, 1975; Hara *et. al.*, 1986).

Until two decades ago, techniques to induce ovulation and spawning in fish were limited to what can be referred to as first generation tehniques (Donaldson and Hunter, 1983). These induced spawning techniques initially consisted of the injection of certain gonadotropin-containing compounds. These compounds included pituitary extracts from another fish of the same or a different species (eg Kuo *et. al.*, 1974; Rothbard, 1981) or various gonadotropins of either piscine or mammalian origin, including salmon gonadotropin (SG-100; eg Shehadeh *et. al.*, 1973b; Juario *et. al.*, 1984), human chorionic gonadotropin (HCG; eg Gordin and Zohar, 1978; Rowland, 1984) and pregnant mare serum gonadotropin (PMSG; eg Hoff *et. al.*, 1972). Gonadotropins act by causing corticosteroid or progestogen secretion, which in turn stimulate ovigenesis and ultimately result in spawning (Lam, 1982). However, the use of first generation techniques is not without problems. Fish gonadotropins tend to be species specific (Bye *et. al.*, 1980), whilst other gonadotropins such as HCG are not effective in every species tested (Lam, 1982). In addition, the use of heterologous gonadotropins may also lead to an immune response in the treated fish, resulting in refractoriness to the hormone (Zohar *et. al.*, 1989). To overcome these deficiencies, a search for alternatives to gonadotropins has led to the development of second generation induced spawning techniques.

Second generation techniques employ hormones which either stimulate the production and release of gonadotropins from the pituitary, or act directly within the ovary. Those resulting in gonadotropin release include antiestrogens (eg Pandey and Hoar, 1972), lutenizing hormone releasing hormone (LHRH; eg Lam *et. al.*, 1975) or its analogue (LHRH-a; eg Lee *et. al.*, 1986) and pituitary luteinizing hormone (PLH; eg Cardeilhac, 1976). Hormones acting within the ovary include steroids such as progesterone, which induces germinal vesicle migration, breakdown and hence final maturation in teleost eggs (eg Jalabert *et. al.*, 1977) and corticosteroids, which induce ovulation (eg Hogendorn, 1979). Prostoglandins such as $PGF_{2\alpha}$ (eg Stacey and Goetz, 1982; Liley and Tan, 1985) are also included in this category, and are implicated in the control of spawning behaviour (Donaldson and Hunter, 1983). Second generation spawning inducers are small molecules relative to gonadotropins, and can be produced synthetically. In addition they are generally free of species specificity, and hence extremely applicable (Donaldson and Hunter, 1983).

Although the discovery of second generation hormones has led to greater specificity and increased control over induced spawning, a survey of the literature reveals that mammalian gonadotropins such as HCG are still widely used to stimulate spawning in captivity (Minton *et. al.*, 1983; Faranda *et. al.*, 1985; Mok, 1985; Szedlmayer, 1987). Factors responsible for the widespread use of mammalian hormones include their availability, storage properties, standardization, and cost competitiveness (Donaldson and Hunter, 1983).

This chapter discusses natural spawning in captivity, and an investigation of the use of two mammalian gonadotropins, HCG and PMSG, to induce spawning in captive galjoen.

4.2: Natural spawning in captivity

Galjoen spawned in captivity on numerous occasions when held under conditions of temperature and photoperiod approximating natural, spawning season conditions. Temperature in the 4000l spawning tanks was maintained through the use of submerged heater-thermostat units whilst a photoperiod of 15:9 (L:D) was set using 4 100-W bulbs suspended above each spawning tank and linked to a timer unit. All natural spawns occurred at night, and egg production for several of the spawnings was estimated by subsample (Table 4.1).

Spawning occurred over a range of temperatures from 16.2 to 21.0°C, with average spawning temperature being 18.5 ± 1.2°C (n = 27). Percentage fertilization for newly-spawned eggs was also determined; viable, fertilized eggs were clear and floated in the water column whilst dead, unfertilized eggs were opaque and negatively buoyant.

Table 4.1: Spawning date, water temperature, estimated egg production and fertilization of eggs from natural captive spawns.

Date	Temp (°C)	Egg prod. in 000's (± std.dev.)	Egg Prod (#/g BW)	% Fert.
22/12/85	18.0	-----	-----	-----
5/1/86	17.5	-----	-----	-----
12/1/86	18.3	-----	-----	-----
17/1/86	19.2	-----	-----	-----
28/1/86	19.0	361.6 ± 29.4	172	71
8/2/86	20.0	-----	-----	-----
17/2/86	19.5	160.9 ± 53.1	124	-----
18/2/86	20.0	190.3 ± 49.0	91	-----
27/2/86	20.0	257.5 ± 20.0	198	-----
9/3/86	19.0	276.8 ± 11.1	132	-----
13/3/86	20.1	283.5 ± 34.3	218	-----
24/3/86	21.0	260.4 ± 6.3	124	97
28/11/86	17.4	-----	-----	-----
11/12/86	16.8	373.2 ± 111.4	182	-----
25/12/86	16.2	361.2 ± 65.9	216	51
10/2/87	18.0	321.2 ± 33.8	155	98
9/1/89	19.5	412.4 ± 48.8	375	98
24/1/89	18.5	415.6 ± 75.2	378	30
7/2/89	19.0	-----	-----	-----
21/2/89	18.5	339.2 ± 10.4	308	89
23/9/89	17.0	-----	-----	-----
5/10/89	16.7	-----	-----	-----
10/10/89	17.2	63.2 ± 22.8	58	0
12/11/89	18.0	168.0 ± 35.6	30	-----
15/11/89	17.4	-----	-----	-----
1/12/89	18.1	250.8 ± 21.6	228	96
11/12/89	19.7	336.4 ± 21.2	306	98

Sex ratios in the spawning tanks ranged from 1 male to 2 females through to 1 female to 2 males. In the latter case, only one male was observed to interact with the spawning female, the second male keeping away from the spawning pair. On two occasions natural spawning occurred between two fish separated by the netting partition described in Chapter 3.

On three occasions, courtship and spawning behaviour was observed. Prior to spawning, both the male and the gravid female became darker in colouration from silver to brown. The male was frequently observed to nose against the swollen underbelly of the female. Lateral displays between the male and female were seen with both fish circling each other with spread fins, shaking their entire bodies.

Courtship coincided with hydration of the female and usually began late in the afternoon. Displays were continued intermittently until spawning occurred, which was generally between 10 pm and 2 am the following morning. Actual spawning behaviour consisted of the breeding pair positioning themselves alongside each other, with the ventral surfaces of both fish in close proximity. Release of the eggs by the female was followed by immediate milt release by the male.

Bennett and Griffiths (1986) suggested that galjoen were serial spawners, since the more advanced ovarian stages (stages 4-6) showed a bimodal distribution of oocyte size. Observations on captive natural spawning confirmed this; individual galjoen were found to spawn several times during the spawning season. Two females each spawned six times from December '85 to March '86. An estimate of spawning periodicity was determined from six females which spawned serially in captivity (Table 4.2), and was found to be 13.4 ± 2.5 days (n = 14). It is possible that in the natural environment this 2 week spawning cycle is linked to phases of the moon.

Table 4.2: Natural spawning periodicity of several captive galjoen.

Spawn date	Previous spawn date	Interval (days)
5/1/86	22/12/85	14
17/1/86	5/1/86	12
28/1/86	21/1/86	16
8/2/86	28/1/86	11
18/2/86	8/2/86	10
27/2/86	17/2/86	10
9/3/86	18/2/86	19
13/3/86	27/2/86	14
24/3/86	9/3/86	15
11/12/86	28/11/86	13
24/1/89	9/1/89	15
7/2/89	24/1/89	14
21/2/89	7/2/89	14
11/12/89	1/12/89	11

Released eggs were positively buoyant and transparent with a single prominent oil globule (Plate 4.1). The diameter of unfixed eggs ranged from 944 to 992 µm, with the oil globule diameter ranging from 240 to 250 µm.

On some occasions a few eggs with more than one oil globule were observed; abnormal eggs were never more than 10% of samples examined (average abnormality = 5.2 ± 3.6%; n = 15). Mean weight-standardised egg production was 194.8 ± 101.8 eggs/g/body weight (n = 17); the maximum values of 374 and 377 being very close to the maximum possible output calculated by Bennett and Griffiths (1986). Fertilization rate (as measured by egg viability) was high, generally over 90% (Table 4.1).

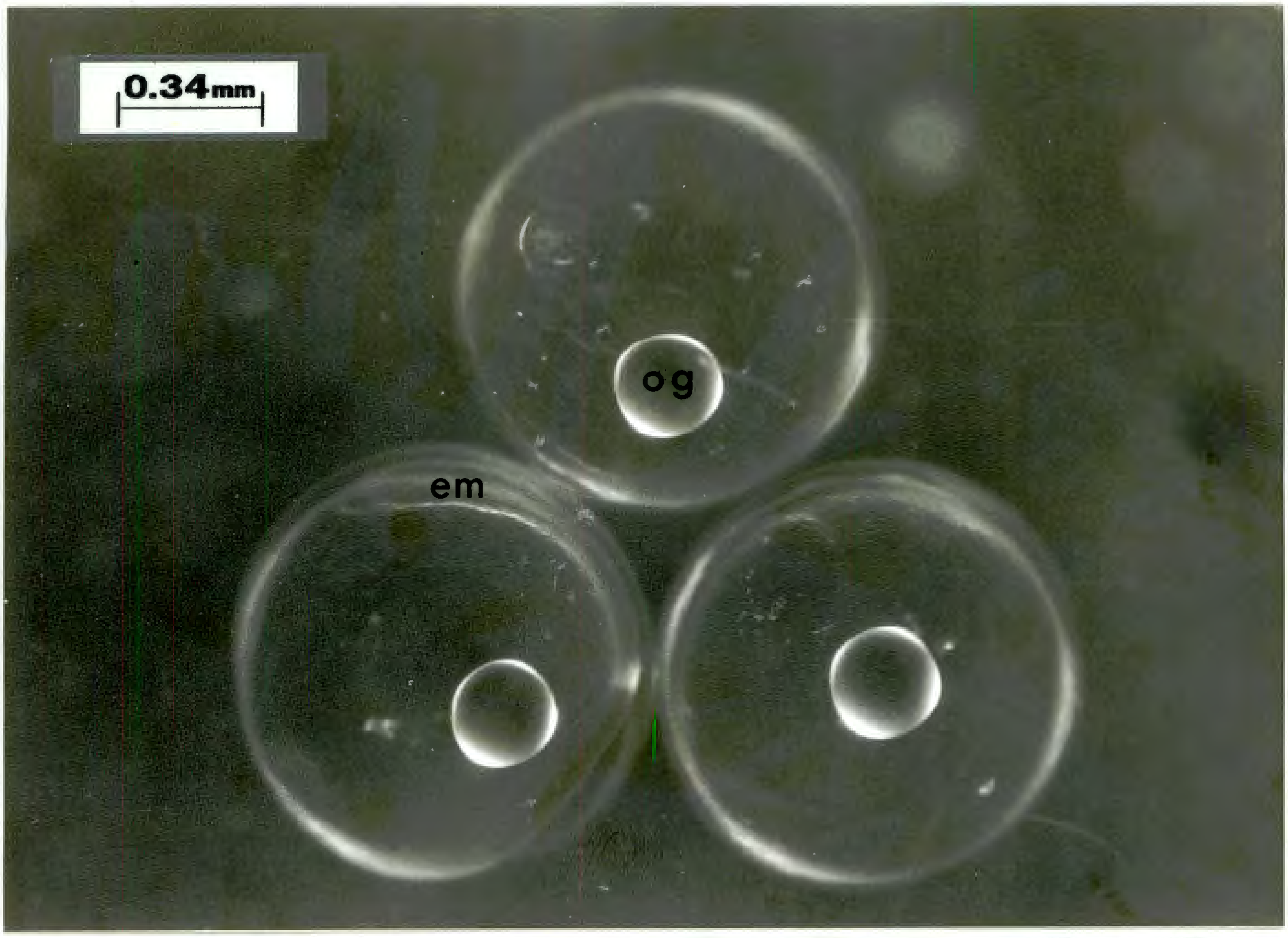


Plate 4.1: Eggs of *C. capensis* 12 hours after fertilization. Note the prominent oil globule (og) and developing embryo (em).

On two occasions attempts were made to artificially spawn naturally gravid females and ripe males using the wet method of artificial spawning (Hoff *et. al.*, 1978; Minton *et. al.* 1983). Eggs were stripped into a plastic basin containing 1l of 0.2 µm filtered seawater by applying gentle pressure to the abdomen of a female restrained by an assistant. Milt was then added by stripping the male and the contents of the bowl were swirled to mix the gametes.

Males were found to be easy to strip, producing milt after minimal pressure. Females were markedly more difficult to strip. After 3 minutes of mixing the bowl was flushed with fresh seawater to remove excess sperm, the eggs being retained by a collar of Nytex mesh. Egs were then divided into batches of approximately 200 amongst several 5l glass hatching jars filled with filtered seawater at 20°C and supplied with gentle aeration. On one occasion artificial spawning was successful, with 37% fertilization being attained. However, no eggs from this spawn survived to hatch.

4.3: Induced spawning in captivity

4.3.1: Introduction

The effect of two mammalian gonadotropins as inducers of spawning in galjoen was examined. The gonadotropins used were human chorionic gonadotropin (tradename Pregnyl) and pregnant mare serum gonadotropin (tradename Fostim). These hormones were selected because they were readily available, relatively cheap and widely used (Lam, 1982). In addition, the large volume of work reported by other authors using these hormones would allow comparisons with the results obtained on galjoen. Hormones utilized were tested on females only, as male galjoen undergo spermiogenesis and are able to spawn in captivity without requiring further stimulation.

4.3.2: Materials and Methods

Female galjoen used in these experiments were kept in isolation in several 4000l spawning tanks. Two trials using HCG (August and December 1988) and one trial using PMSG (November 1989) were conducted. Before the experiments were initiated the weight of each experimental female was determined to the nearest 25g using a spring balance. Fish were then injected intramuscularly with varying doses of the hormone tested in a constant volume of physiological saline (0.9%) just underneath the first dorsal fin. Prior to and at regular intervals after injection ovarian biopsy samples were taken using the method described by Shehadeh *et. al.* (1973a). A flame-blunted 1mm internal diameter glass catheter attached to a syringe was inserted to a depth of approximately 5cm into the genital pore of the fish and a small amount of ovarian material was aspirated out. This was immediately examined under a microscope and the diameter of 50 oocytes was measured to determine average size. The results were expressed graphically by plotting average oocyte diameter against time after injection for each trial.

Any females which became gravid and appeared to have hydrated their oocytes as a result of hormone induction during the second HCG trial were supplied with a sexually ripe and running male (one that produced milt in response to gentle pressure on the abdomen) to facilitate fertilization. Males were not placed with gravid females induced to spawn through the use of PMSG.

HCG treatment #1

This treatment examined the effect of a single dose of HCG on ovarian maturation outside the natural spawning season, and was carried out from 24 August to 21 September 1988, two months before the onset of the natural spawning season. Four adult female galjoen were used, 3 of them being injected with 1 I.U./g body weight HCG made up in 1.5ml saline whilst the control fish was injected with 1.5ml 0.9% saline. Ovarian biopsies were performed on each fish immediately before injection and after 1, 2, 3 and 4 weeks.

HCG treatment #2

This treatment examined the effect of varied strength single dose injections of HCG on ovarian maturation during the natural spawning season, and was carried out from 16 November to 8 December 1988. Ten adult female galjoen were used, two at each dose level of 0 I.U./g (control), 0.25 I.U./g, 0.5 I.U./g, 0.75 I.U./g and 1.0 I.U./g body weight HCG. All doses were injected in 1.5ml saline. Ovarian biopsies were performed on each fish immediately before injection and either 18, 30 and 42 hours or 12, 24, 36 and 42 hours after injection. The diameter of spawned eggs was measured as soon as posible after spawning.

PMSG treatment #1

This treatment examined the effect of two dose strengths of PMSG on ovarian development during the natural spawning season and was carried out from 16 to 20 November 1989. Six adult female galjoen were used, two at each dose level of 0 I.U./g (control), 0.5

I.U./g and 1.0 I.U./g body weight PMSG. All doses were injected in 1.5ml saline. Ovarian biopsies were performed on each fish immediately before injection and 24, 48 and 72 hours after injection. The diameter of spawned eggs was measured as soon as possible after spawning.

4.3.3: Results

Ovarian samples collected by catheterization were found to consist of discernible oocytes combined with amorphous ovarian material (Plate 4.2). Oocytes showed a large size range within each sample taken.

HCG Treatment #1

HCG was observed to be ineffective in stimulating ovarian development when injected into galjoen with small initial oocyte diameters of approximately 0.2mm prior to the natural spawning season (Figure 4.1). Oocyte growth in injected fish was minimal over the month-long experimental period, with diameters increasing by only 0.1mm. Oocytes from the control fish also showed minimal growth.

HCG Treatment #2

Doses of 0.5 I.U./g body weight HCG and higher were effective in stimulating ovarian development leading to spawning when injected into galjoen with initial oocyte diameters of approximately 0.5mm (Figure 4.2).

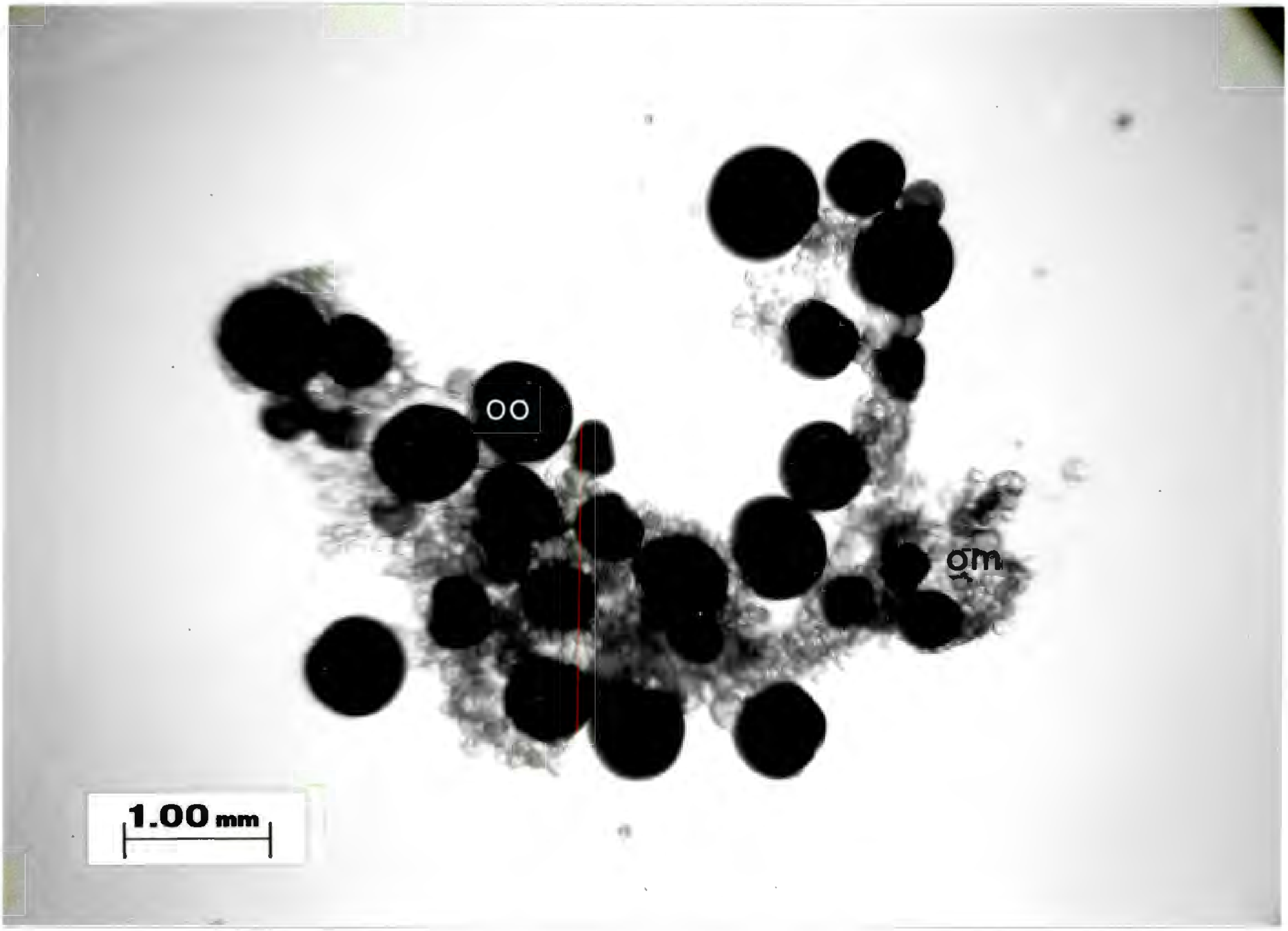
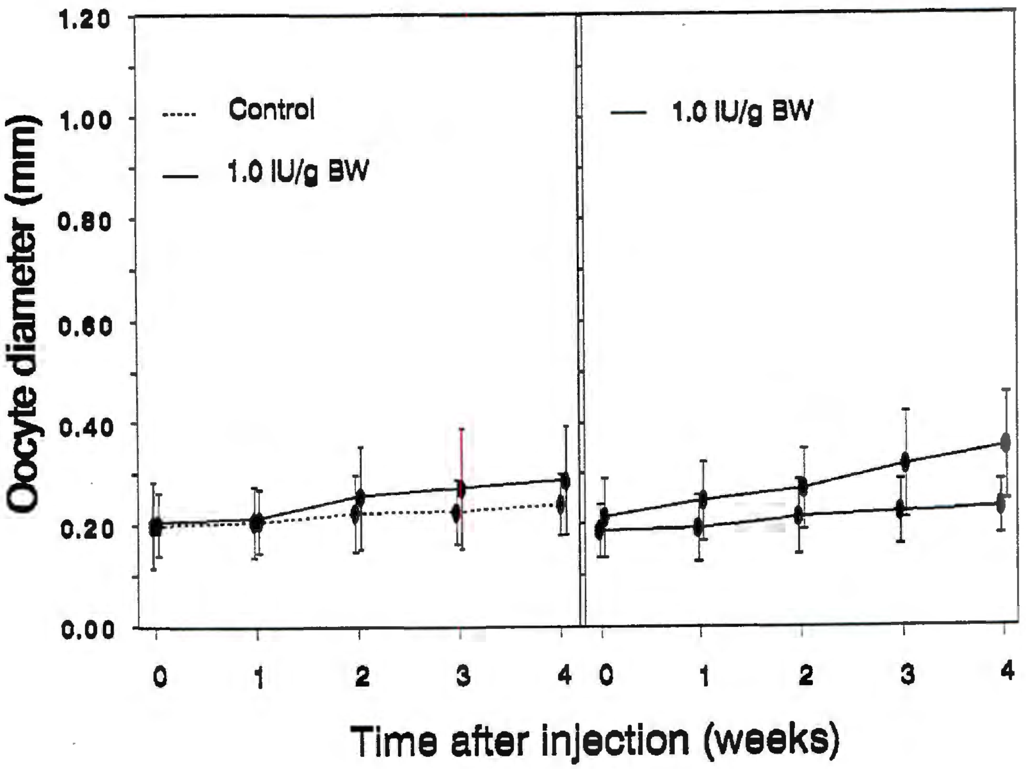


Plate 4.2: Ovarian biopsy sample from a female *C. capensis* taken 12 hours after injection with 1.0 IU/g body weight HCG. Note oocytes (oo) and amorphous ovarian material (om).

At doses of 0.50 and 0.75 I.U./g body weight, oocyte diameters increased steadily from 0-24h after injection and showed maximal increases from 24-36h after injection, by which time they were approaching full size. The rate of oocyte increase was then much reduced until spawning. On a few occasions, spawned eggs had a smaller average diameter than oocytes from the last sample before spawning. At a dose of 1.0 I.U./g body weight oocyte diameter increased at an almost steady rate from injection to spawning.



<u>Figure 4.1</u>: Oocyte diameter as a function of time after injection for female *C. capensis* injected with 1.0 IU/g body weight HCG. Mean values and standard deviations are shown.

Fish injected with 0.25 I.U./g body weight showed no increase in oocyte diameter until 24h after injection. From 24h onwards, oocytes increased in diameter, reaching approximately 0.75mm by 40h. After 40h however, oocyte diameter decreased. No growth in oocytes of control fish was observed.

The time from injection to spawning for galjoen injected with doses of 0.50 I.U./g body weight and higher ranged from 52-59h (55.2 ± 3.0 hours; Table 4.3). This period was not markedly different for different doses. Whilst all fish injected with 0.5 I.U./g HCG or more spawned, not all spawnings produced fertile eggs (Table 4.3). Whether this was due to defficiencies in either the eggs or sperm or to the stress experienced by the spawning fish is uncertain.

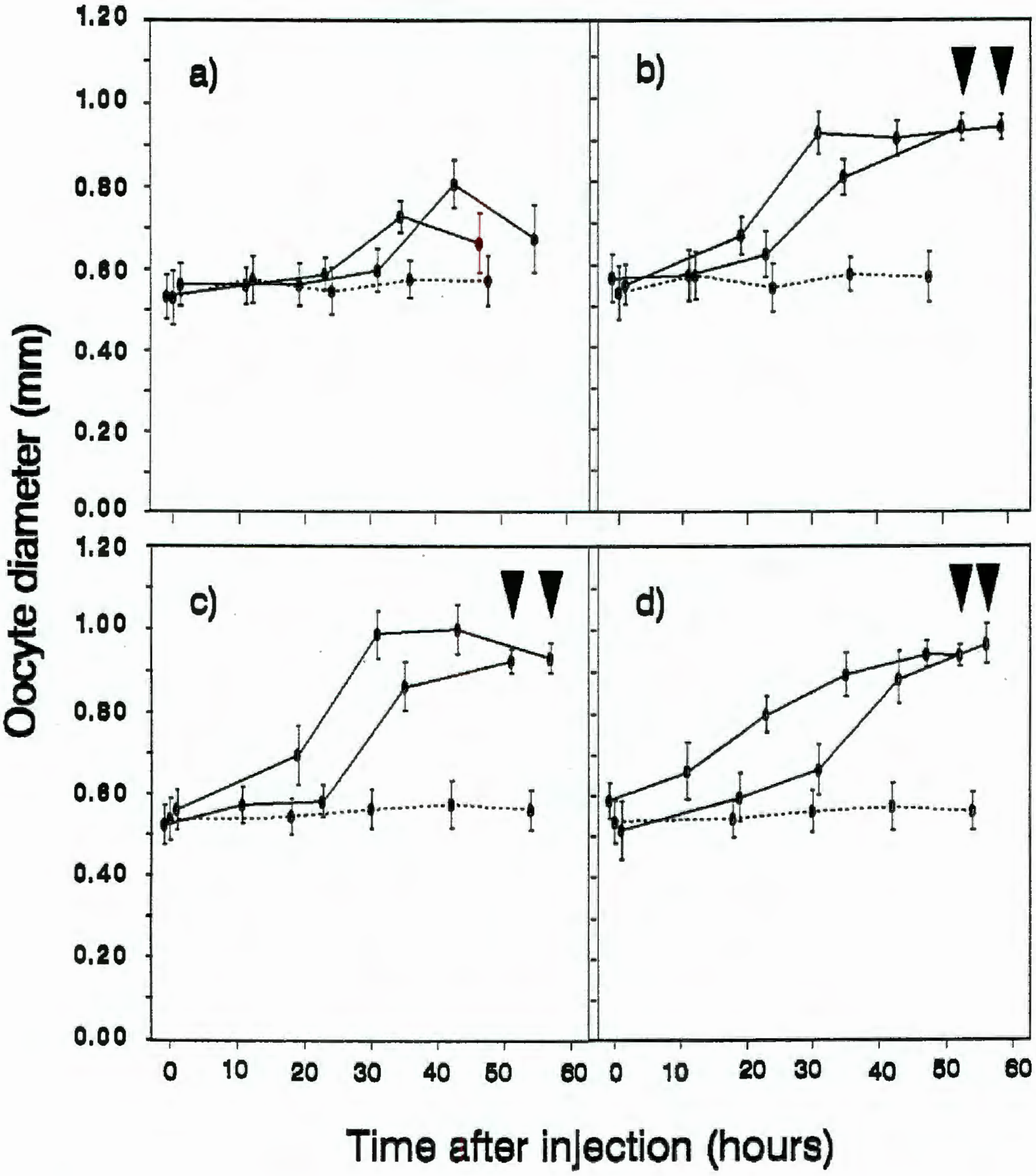


Figure 4.2: Oocyte diameter as a function of time after injection for female *C. capensis* injected with varying doses of HCG. (a) 0.25 IU/g, (b) 0.50 IU/g, (c) 0.75 IU/g, (d) 1.0 IU/g. Mean values and standard deviations are shown. Control values are shown connected by a dotted line. Arrows indicate spawning.

Furthermore, only in one treatment (0.75 I.U./g HCG) did fertilized eggs survive to hatch. In all of the treatments some released eggs showed more than one small oil globule (Plate 4.2), with higher incidences of abnormality at increased dose levels. Egg production also appeared to be reduced at higher dose levels.

Table 4.3: Egg production, percentage fertilization, egg hormality (single oil globule), survival to hatch and time to spawn for galjoen injected with various doses of HCG.

HCG dose (I.U./g)	Egg prod. (#/g BW)	% fert.	% normality (±std.dev.)	Survival to hatch	Time to spawn(h)
0.50	301.2	0	42.4 ± 9.2	No	54
0.50	271.3	52	91.2 ± 6.7	No	59
0.75	292.1	37	59.1 ± 8.8	No	58
0.75	121.6	98	72.4 ± 3.9	Yes	52
1.00	124.9	0	37.8 ± 7.1	No	56
1.00	130.9	52	54.6 ± 6.6	No	52

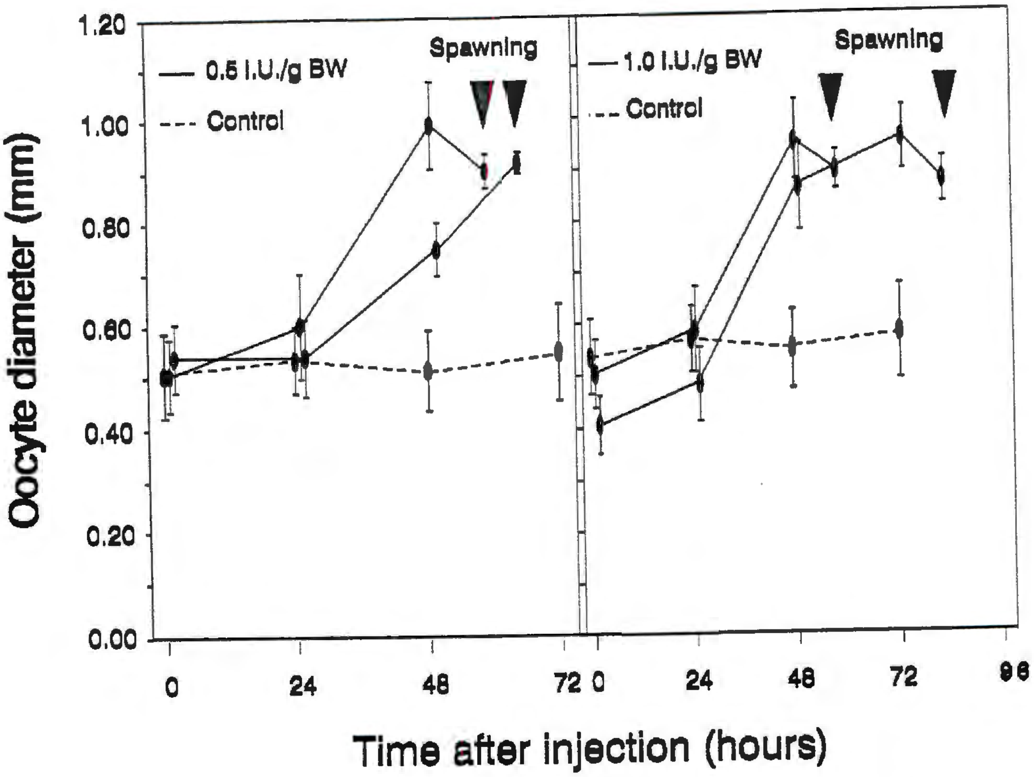
PMSG Treatment #1

PMSG stimulated ovarian maturation leading to egg release at both tested doses, whilst control oocytes showed no increase in growth (Figure 4.4). The rate of oocyte diameter increase was maximal from 24-48h after injection. Egg production was much reduced at both hormone levels when compared to values for normal and low dose HCG-induced spawns. The percentage of normal eggs was reduced at the higher hormone dose (Table 4.4). The time from injection to spawning ranged from 56-80h (65.0 ± 10.7 hours; Table 4.4).

<u>Plate 4.2</u>: Spawned eggs from an HCG-induced female *C. capensis*. Note the presence of several oil globules (og) in each egg.

Table 4.4: Egg production, egg normality and time to spawn for galjoen injected with varying doses of PMSG.

PMSG dose (I.U./g)	Egg production (#/g BW)	% normality (± std.dev.)	Time to spawn (h)
0.50	33.3	75.2 ± 3.4	59
0.50	106.8	84.8 ± 5.4	65
1.00	16.3	58.4 ± 6.8	80
1.00	33.8	51.6 ± 5.2	56



<u>Figure 4.4</u>: Oocyte diameter as a function of time after injection for female *C. capensis* injected with varying doses of PMSG. (a) 0.50 IU/g, (b) 1.0 IU/g. Mean values and standard deviations are shown. Control values are shown connected with a dotted line. Arrows indicate spawning.

4.3.4: Discussion

Human chorionic gonadotropin has been successfully used to induce spawning in a variety of fish species (see Lam, 1982 and Donaldson and Hunter, 1983 for reviews). Whilst dosages used range from as low as 0.1 IU/g body weight (Gordin and Zohar, 1978) to as high as 40 or even 60 IU/g body weight (Kuo *et. al.*, 1973; Alvarez-Lajonchere *et. al.*, 1988), a general rule of thumb is to use 1 IU HCG/g body weight (Lam, 1982; Cavaliere *et. al.*, 1985; Faranda *et. al.*, 1985; Tucker and Barbera, 1987). In several instances a single injection of HCG suffices to induce spawning (eg Hirose *et. al.*, 1979). Often however, the hormone is applied in several doses; either a second, smaller dose is given some time after the first (eg Minton *et. al.*, 1983), or similiar sized doses are given at constant intervals until ovulation and spawning occur (eg Mok, 1985).

In contrast to HCG, pregnant mare serum gonadotropin has not been extensively used to induce spawning. Successful induced spawning using PMSG has only been reported for a few species, including gulf croaker, *Bairdiella icistia* (Haydock, 1971) and winter flounder, *Pseudopleuronectes americanus* (Smigielski, 1975). Regular injections of PMSG at 1 IU/g body weight were found to be effective in initiating vitellogenesis in mullet, *Mugil cephalus* (Kuo *et. al.*, 1974), although this did not lead to spawning. Oocyte diameter of Florida pompano, *Trachinotus carolinus*, did not appear to be affected by PMSG at a dose of 0.055 IU/g body weight (Hoff *et. al.*, 1972).

The presence of multiple oil globules in eggs which normally possess only one has been reported for several species induced to spawn using HCG (Kuo *et. al.*, 1973; Cardeheilac, 1976; Hoff *et. al.*, 1978; Roberts and Schlieder, 1983). These authors have suggested that premature spawning resulting from a hormone overdose is characterized by multiple oil globules in each oocyte. Under natural conditions of development, the oocytes of several species of fish possess multiple oil globules which coalesce before ovulation (eg *Lutjanus campechanus*; Minton *et. al.*, 1983), coalescence occurring at approximately stage IV of the gonadal maturation cycle in mullet (*Mugil cephalus*; Kuo *et.*

al., 1974). In galjoen, multiple oil globules were found to start coalescing from 30hrs after injection in fish dosed with 1.0 IU/g body weight HCG. Eggs containing two or more oil globules are occasionally found from naturally spawning galjoen (see above), but at a much reduced incidence of approximately 5%. Thus the presence of multiple oil globules in several eggs from hormone-induced spawnings implies that accelerated development due to hormone overdose occurred at all doses resulting in egg release.

It has been suggested that hormone treatments have a negative influence on the quality of spawned eggs (Garcia, 1989). In this study, eggs from only one HCG-induced spawn survived to hatch. Poor survival and fertilization of eggs from hormone-induced spawns has been reported by other authors. Cardeheilac (1976) found that pituitary lutenizing hormone-treated pinfish (*Lagodon rhomboides*) produced eggs containing more than the usual single oil globule which did not survive to hatch. Similiarly, Atlantic salmon (*Salmo salar*) eggs from spawners treated with a lutenizing hormone releasing hormone analogue showed low survival (Crim and Glebe, 1984). The fertilization rate of eggs of sole (*Solea solea*) induced to spawn with HCG was reduced at higher doses (Ramos, 1986). Whilst the viability of PMSG-induced eggs was not assessed, the relatively high proportions of abnormal eggs encountered would suggest that they too suffered decreased viability.

The size of the dose required to induce spawning depends on how closely the hormone used mimics the endogenous gonadotropin, and also upon the stage of gonadal maturation prior to injection (Lam, 1982). In the sea bream, *Sparus aurata*, the amount of HCG required to induce spawning was found to vary inversely with the diameter of the vitellogenic oocytes (Gordin and Zohar, 1978). A similiar inversely proportional relationship was reported for mullet, *Mugil cephalus*, induced to spawn using fractionated salmon pituitary extract (Shehadeh *et. al.*, 1973b). Whilst fish with smaller sized oocytes require larger amounts of gonadotropin to induce spawning, a cutoff point where the oocytes are too small and immature to respond to hormonal manipulation exists, and has been termed the critical or minimum oocyte diameter. In addition to critical diameters, optimum oocyte diameters, at which hormone-induced spawning is optimized, have been determined for several species, and are given in Table 4.5. These values range from 32-82% of the spawned egg diameter, with an average optimum diameter of 57% of the spawned egg size.

The failure of galjoen oocytes of 0.2mm diameter to respond to HCG in the first trial combined with the fact that oocytes of 0.5mm diameter responded to all doses of both hormones, indicates that the minimum oocyte diameter for galjoen lies between 0.2 and 0.5mm. However, the number of fish subjected to hormone treatment was not sufficient to enable accurate determinations of either minimum oocyte diameter or the relationship between oocyte maturity and required dose. An estimation of optimum oocyte diameter for galjoen can be made using the percentage value averaged from the literature (57%) multiplied by the spawned egg size. This gives a value of 0.55mm for the optimum diameter, approximately the size of oocytes in fish used in the second two trials and corresponding to stages IV-VI of gonadal maturity (Bennett and Griffiths, 1986).

Table 4.5: Optimum oocyte and spawned egg diameter for several marine fish induced to spawn using various hormones. Numbers in brackets indicate sources.

Species	**Optimal oocyte diameter (mm)**	**Spawned egg diameter (mm)**
Archosargus probatocephalus	0.40 (1)	0.80 (2)
Centropristis striata	0.40 (3)	0.80-1.00 (2)
Chanos chanos	0.66 (4)	1.20 (5)
Cynoscion nebulosus	0.46 (6)	0.75-0.87 (2)
Dicentrarchus labrax	0.70 (7)	1.20 (8)
Lagodon rhomboides	0.50 (9)	0.99-1.05 (9)
Lutjanus campechanus	0.40 (10)	0.80 (11)
Mugil cephalus	0.65 (12)	0.94-1.03 (13)
Siganus oramin	0.41-0.46 (14)	0.57 (15)
Sparus aurata	0.30-0.63 (16)	0.93 (8)
Trachinotus carolinus	0.58-0.72 (17)	0.87-1.00 (17)

(1) Tucker and Barbera, 1987; (2) Fahay, 1983; (3) Tucker, 1984; (4) Juario *et. al.*, 1984; (5) Lee *et. al.*, 1986; (6) Colura, 1974; (7) Barnabe, 1975 in Lam, 1982; (8) Faranda *et. al.*, 1985; (9) Cardeilhac, 1976; (10) Minton *et. al.*, 1983; (11) Arnold *et. al.*, 1978; (12) Kuo *et. al.*, 1974; (13) Brownell, 1979; (14) Soh and Lam, 1973 in Lam, 1982; (15) Hara *et. al.*, 1986; (16) Gordin and Zohar, 1978; (17) Hoff *et. al.*, 1978.

Whilst diameter is frequently used as the sole criteria for determining oocyte development (eg Shehadeh *et. al.*, 1973a), other characteristics including membrane width, the number, size and distribution of oil droplets, yolk appearance, and position of the nucleus have been suggested as criteria necessary to determine maturity (Alvarez-Lajonchere *et. al.*, 1988). These criteria were not used in this study, since they require detailed microscopic examination of the oocytes which was felt to be unnecessary in view of the rapid changes in oocyte diameter observed after injection.

The surprising observation that spawned eggs were often smaller than oocytes measured immediately prior to spawning has been noted previously by Hoff *et. al.* (1978), who found that fertilized eggs of Florida pompano (*Trachinotus carolinus*) shrunk by as much as 16% within 1 hour of fertilization. Spawned galjoen eggs were observed to show shrinkage from 6 to 10%. The mechanism causing shrinkage is unclear.

Latency periods from injection to spawning did not differ between doses within hormones or between hormones used. Other species induced to spawn with HCG applied in a single injection at doses of approximately 1 IU/g body weight showed similiar latency periods; 42-56h for red snapper (*Lutjanus campechanus*) at 1.1 IU/g (Minton *et. al.*, 1983), 47-48h for sheepshead bream (*Puntazzo puntazzo*) at 1-1.2 IU/g (Faranda *et. al.*, 1985) and 70-75h for sea bass (*Dicentrarchus labrax*) at 0.8 IU/g (Barnabe, 1975 in Lam, 1982).

The results obtained from this study suggest that neither HCG nor PMSG are suitable hormones with which to induce spawning in galjoen. Doses of either hormone which led to successful ovulation and spawning resulted in decreased fertilization and increased egg abnormality compared to eggs from natural captive spawns. Egg production was also much reduced at higher hormone doses. In addition, on only one occasion did fertile HCG-induced eggs survive to hatch.

CHAPTER 5: ASPECTS OF THE EARLY LIFE HISTORY OF GALJOEN

5.1: The effect of varying incubation temperatures on embryonic duration and survival of fertilized galjoen eggs.

5.1.1: Introduction

The determination of optimum conditions for the hatching and rearing of larvae is of paramount importance if large scale culture is to be attempted, since successful aquaculture is dependent on a reliable and sufficient supply of fry (Popper *et. al.*, 1973; May *et. al.*, 1974; Hecht, 1985; Devauchelle and Coves, 1988). Hatching success and larval viability are influenced by several factors, including environmental conditions during fertilization and development (Roubaud *et. al.*, 1984 in Wiegand *et. al.*, 1988; Van der Wal, 1985) and intrinsic quality of the spawn products (Kjorsvik and Lonning, 1983; Watanabe *et. al.*, 1985). Of the environmental factors affecting fertilized eggs of marine fish, temperature and salinity exert the most profound effects (Blaxter, 1969; Alderdice, 1972; Santerre and May, 1977; Akatsu *et. al.*, 1983; Herzig and Winkler, 1986). Since fish are poikilothermic, the effects of temperature are felt both directly (Alabaster and Lloyd, 1980) or indirectly through its influence on water quality parameters such as dissolved oxygen (Alderdice and Forrester, 1968) and un-ionized ammonia concentration (Holt and Arnold, 1983).

A knowledge of the relationship between incubation temperature and embryonic duration is of practical importance in a culture situation since it allows prediction of the time to hatch at any given incubation temperature. In addition, a higher level of control can be maintained over the period of early development from fertilization to hatching and, to a certain extent, synchronization of hatching of embryos from separate spawns can be achieved.

Of perhaps more importance is a determination of the temperature tolerance ranges and optima for all life history stages of a culture species. Knowledge of this tolerance range is fundamental to successful and efficient propagation.

This investigation of the effect of temperature on embryonic duration and survival of fertilized galjoen eggs was conducted in order to determine the optimum incubation temperature necessary for rapid normal development with concurrent high survival.

5.1.2: Materials and methods

Batches of eggs were incubated at 8 nominal experimental temperatures from 12-26°C in 2° increments. Eggs were obtained from a natural captive spawn (spawning temperature 19°C) within 1 hour of spawning and placed in a 25 litre bucket of seawater for 10 minutes to allow dead or infertile eggs to sink. Floating fertilized eggs were removed and transferred in 15 batches of 5 eggs each into test-tubes containing 50ml of 0,2µm-filtered sea water at 18° and 20°C. These were then placed in polystyrene holding trays, which were transferred consecutively in 2°C increments from 20°-26°C or from 18°-12°C. Trays were maintained at each temperature for at least 1 hour before being transferred to the next. This stepwise transfer either up or down from the spawning temperature provided an opportunity for at least partial acclimation of the eggs.

Experimental temperatures were obtained using an array of 150 litre rearing tanks (Brownell and Horstman, 1987) used as water baths and maintained at specific temperatures by the use of heater-thermostats. Constant aeration from an air stone at the bottom of each water bath ensured complete mixing and temperature homogeneity. The water baths were operated simultaneously in a constant-temperature room held at 12°C and provided with 24h fluorescent illumination.

Eggs were examined 2-hourly around the clock for a period of 80 hours to determine the time taken to hatch. Dead eggs (opaque and lying on the bottom of the test-tube as opposed to clear, floating live eggs) were counted and removed. Temperatures in all water baths were measured at each 2-hourly interval using standard mercury-in-glass thermometers read to 0,1°C. Water in the test-tubes was not changed during the experiment in view of the relatively short experimental period and low density of eggs within each test-tube.

Maximum and minimum possible development times linked to the observation intervals used were averaged to provide a mean embryonic duration for each egg. Percent survival at hatching for eggs in each test-tube was averaged within each treatment to determine mean survival at each temperature. Statistical analysis for differences in survival values at each temperature was conducted using the Kruskal-Wallis analysis for non-parametric values (Zar, 1984).

5.1.3: Results

Average actual temperatures did not differ from nominal experimental temperatures by more than 0.1°C (maximum standard deviation 0.7°C) except for the 12°C water bath, which showed an average temperature of 12.8° ± 0.3°C.

The relationship between embryonic duration and temperature for fertilized galjoen eggs is illustrated in Figure 5.1. A power equation of the form

$$D = aT^b$$

where D = embryonic duration, T = temperature and a and b = constants (Laurence and Howell, 1981; Camus and Koutsikopoulos, 1984) was used as the best regression equation describing the relationship of embryonic duration to temperature. Embryonic duration decreased with increasing temperature according to the predictive equation given in Figure 5.1.

No eggs survived to hatch at either 12° or 14°C, whilst average egg survival was above 80% at all temperatures from 16° to 26°C (Table 5.2). No statistically significant difference was observed in egg survival from 16° to 26°C (p>0.05). No abnormal larvae were observed after hatching at any of the experimental temperatures.

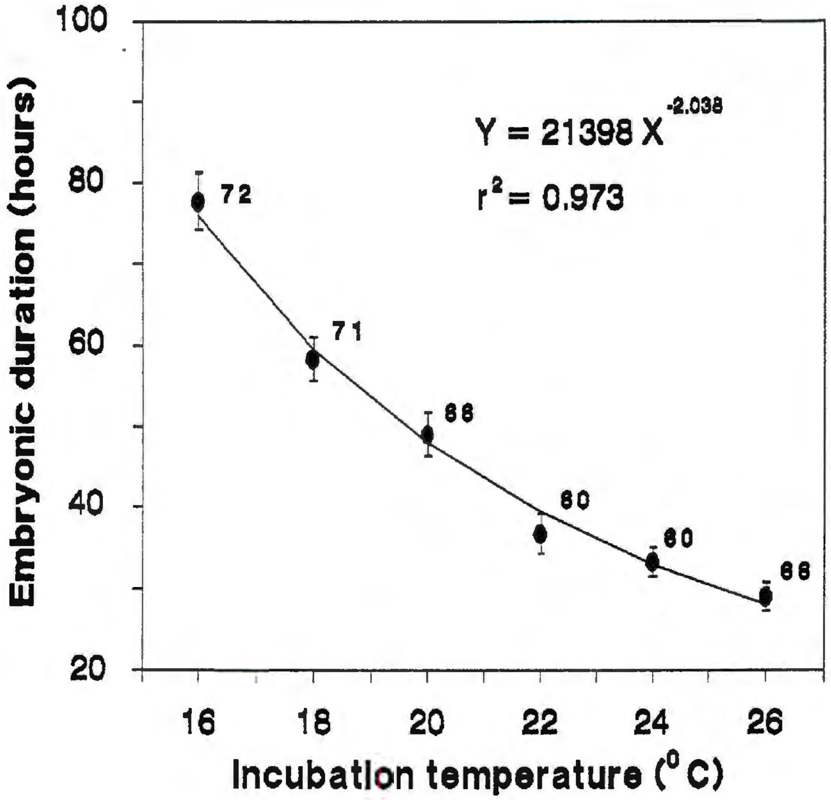


Figure 5.1: Embryonic duration as a function of temperature for fertilized *C. capensis* eggs. Standard deviations are shown above and below mean values, with the number of observations for each temperature given in brackets.

Table 5.1: Survival to hatch of fertilized *C. capensis* eggs incubated at various temperatures.

Nominal exp. temp (°C)	Mean % survival (± std.dev.)
12	0
14	0
16	94.7 ± 9.2
18	96.0 ± 15.5
20	90.7 ± 24.9
22	82.7 ± 23.4
24	81.3 ± 20.7
26	90.7 ± 12.8

5.1.4: Discussion

Several studies concerning the relationship between incubation temperature and embryonic duration for a variety of fish species have been published (see Crisp, 1981 and Elliot *et. al.*, 1987 for extensive reviews; Murray and McPhail, 1988; Wiegand *et. al.*, 1988). All have demonstrated that embryonic duration decreases with increasing temperature; i.e. that developmental rate increases with increasing temperature. This relationship is a consequence of the temperature-dependent metabolism of poikilothermic animals and is due to the fact that the rate at which biochemical reactions occur and are catalyzed is directly related to temperature.

In the studies cited above, various models have been used to describe mathematically the relationship between embryonic duration and temperature. Of these models, the most common form is that of a power relationship (eg Camus and Koutsikopoulos, 1984; Luczynski and Kirklewska, 1984). The power relationship may also include a temperature correction factor, termed the Belehradek constant (eg Jungwirth and Winkler, 1984; Herzig and Winkler, 1986). Other functions such as hyperbolic (eg Guma'a, 1978), semi-logarithmic quadratic (eg Colby and Brooke, 1973) or exponentional decay curves (Austin *et. al.*, 1975; Sylvester and Nash, 1975; Nakatani and Maeda, 1984) have also been described.

In a review assessing the applicability of various mathematical models used to describe the embryonic duration/temperature relationship, Crisp (1981) stated that a simple power-law with a temperature correction factor was the preferred model. Similiarly, of the two-parameter models available, Elliot *et. al.* (1987) found the power-law provided the best description.

The predictive power equation (without a temperature correction factor) derived from the results obtained in this study explained 97% of the variance in embryonic duration over the range of temperatures used. It was therefore felt that this equation was more than adequate to describe the embryonic duration/temperature relationship for fertilized galjoen eggs.

Any attempt to determine the optimum incubation temperature for developing embryos must aim at maximizing survival and producing robust, viable larvae. Long incubation periods are inadvisable since bacterial build-up may become a problem, especially when high density incubation is practised (Devauchelle and Coves, 1988b). The survival-to-hatch results obtained in this study suggest that galjoen embryos are eurythermal, being able to tolerate temperatures from 16°-26°C. At temperatures of 14°C and lower however, 100% mortality is encountered. The highest temperature used in this study did not significantly affect embryonic survival. This suggests that the upper limit of the temperature tolerance range for galjoen embryos exceeds 26°C. Although it would appear that the lower limit of the temperature tolerance range for early galjoen larvae lies between 14° and 16°C, this may not necessarily be the case for later embryonic stages.

A recent study by Cloud *et. al.* (1988) demonstrated that tolerance of fathead minnow (*Primephales promelas*) embryos to low temperatures changed according to developmental stage, with early developmental stages being less tolerant than later stages. Cloud *et. al.* (1988) suggested that embryos of different species of fish may have some developmental periods that are more tolerant to low temperatures than others. In effect, this means that the various developmental stages through which a fertilized egg passes have different temperature tolerance ranges. Although this suggestion may apply to galjoen embryos, the experimental design used here did not take this possibility into account. Further work is therefore required to elucidate this point.

A knowledge of the temperature tolerance range of galjoen embryos is of importance regarding spawning location of feral galjoen stocks. Results presented by Bennett and Griffiths (1986) in a study of galjoen biology off the southwestern Cape indicate that spawning occurs in the immediate vicinity of the shore from Lamberts Bay to Cape Infanta during the October to March spawning season. Typical summer sea surface temperatures for this region are provided by Shelton and Hutchings (1990). Inshore temperatures of 11° to 14°C are encountered from Cape Point to Lamberts Bay, whilst temperatures from Cape Point to Cape Infanta range from 15° to 22°C. Thus galjoen spawning along the west coast could possibly be exposing their eggs and early embryos to temperatures below the lower temperature tolerance limit determined in this study. However, strong southeasterly winds occurring in summer (Shannon, 1985) could displace warm coastal water containing eggs and embryos offshore, prior to the upwelling of cold water. In these warmer offshore regions temperatures are above the lower lethal limit (Shelton and Hutchings, 1990), and would allow normal development to occur. Galjoen eggs and embryos could also survive until a later development stage in shallow sun-warmed west coast bays such as St Helena. It is also possible that west coast galjoen may have a lower optimum temperature range than the fish used as broodstock in these experiments, which were of south coast stock.

Incubation temperature has been shown to affect the size of embryos at hatching (Beacham and Murray, 1985). Gunnes (1979) and Murray and McPhail (1988) demonstrated that high incubation temperatures reduced embryo size for several salmonid species, and suggested that development at high temperatures was too rapid, resulting in inefficient conversion of yolk to fish tissue. However, Alderdice and Velsen (1978) and Laurence and Rogers (1976) found a tendency for larger larvae of *Clupea pallasi, Gadus morhua* and *Melanogrammus aeglefinus* respectively to develop at higher temperatures, whilst Laurence and Howell (1981) concluded that yellowtail flounder (*Limanda ferruginea*) hatching length was not significantly altered by temperature.

The size of larvae at hatching may have significance in a culture situation, assuming that a correlation between hatching size and subsequent size at first feeding exists. Larger first feeding larvae may be more efficient predators than their smaller counterparts and may be able to ingest larger food organisms.

This could be of practical importance with respect to the size of food organisms offerred to first feeding larvae.

5.2: Endogenous food utilization in newly-hatched galjoen larvae

5.2.1: Introduction

Many marine fish species , including galjoen, produce large numbers of pelagic eggs at spawning. These eggs hatch into larvae that are generally more elementary in structural development than the early larvae of less fecund species (Blaxter, 1969). Development proceeds as the larvae float in the water column using nutrients obtained from their yolk to provide energy for development as well as for maintenance and growth. Larvae are yolk-dependent until such time as they make the transition from endogenous to exogenous feeding, and successfully capture prey organisms. It has long been recognized that mortality during this early life history stage is much greater than during any other stage (Beyer and Laurence, 1980). As early as 1914 Hjort (<u>in</u> McGurk, 1984) proposed the "critical period" concept, suggesting that a brief period of high mortality occurred very early in the life history of marine fish. Mortality during this critical period was due to catastrophic starvation resulting from a lack of sufficient or suitable food at the time of yolk sac exhaustion. Thus the transition period from endogenous to exogenous feeding was seen as being exceptionally important in terms of larval survival.

Most marine larvae are capable of exogenous feeding shortly before their yolk is completely absorbed (Blaxter, 1969; Theilacker and Dorsey, 1980). Once larval yolk reserves are completely utilized however, survival is dependent on the larva's ability to find and capture sufficient prey. The time after yolk absorption at which a larvae is too weak to feed and death is inevitable has been termed the "point of no return" (Blaxter and Hempel, 1963).

Since the transition phase between endogenous and exogenous feeding is critical to larval survival, a knowledge of the pattern of early development of a species selected for culture is crucial. Information concerning energy utilization at various stages of development can enable accurate determinations of the time of first feeding and the time to the point of no return to be made. This experiment was conducted to examine endogenous energy utilization in newly-hatched, unfed galjoen larvae.

5.2.2: Materials and methods

Newly-hatched larvae used in this experiment were obtained from a natural captive spawn on 1st December 1989. Water temperature at spawning was 18.1°C and hatching of fertilized eggs was completed after 60 hours. Prior to hatching, fertilized eggs were placed in 5 litre glass beakers containing 0.2µm-filtered seawater and kept in a constant temperature room maintained at 18°C. Water in the beakers was changed every second day. Approximately 200 fertilized eggs were placed in each of 5 beakers.

Immediately after hatching and at approximately 10 hour intervals thereafter, six larvae from each beaker were removed and examined under a binocular compound microscope fitted with an ocular micrometer. Measurements to the nearest 0.01 mm of total length (TL; measured from the tip of the snout to the end of the notochord), yolk sac length and height and oil globule diameter were then taken. Larvae were measured as soon after collection as possible whilst they were still in a fresh state, since autolysis leading to deformities is rapid in larval tissues (Theilacker, 1978). Photographs of the various stages were taken using a Contax 139 camera mounted on a Wild M3Z microscope.

Yolk sac and oil globule volumes were computed based on their shape as follows (Blaxter and Hempel, 1963):

$$\mathrm{Vol} = \pi/6.lh^2$$

and

$$\mathrm{Vol} = 4/3.\pi r^3$$

where l is the length and h is the height of an ellipsoid (yolk sac) and r is the radius of a sphere (oil globule).

A measure of the efficiency of development (Ed) was also determined by examining the ratio of total endogenous food resorption rate to growth rate. Growth rate (g) and endogenous food resorption rate (u) were calculated and expressed as a % of total length per hour using the following formulae (Klaoudatos *et. al.*, 1990):

$$g = \frac{(L_2-L_1)*100}{dt*((L_2+L_1)/2)}$$

and

$$u = \frac{((V_{ys1}+V_{og1})-(V_{ys2}+V_{og2}))*100}{dt*((L_2+L_1)/2)}$$

where L_2 and L_1 are total length at times 2 and 1 respectively and dt is the change in time in hours between times 1 and 2 and where V_{ys1} and V_{ys2} are yolk sac volumes and V_{og1} and V_{og2} are oil globule volumes at times 1 and 2 respectively.

A total of 450 larvae were sampled at 14 different periods after hatching. Water temperature inside the beakers was taken at each sampling time. No attempt to feed the larvae was made during the experiment. The experiment was terminated after 152 hours, by which time mortalities and larval deformation due to starvation were apparent.

5.2.3: Results

Mean water temperature during the experiment was 17.9° ± 0.4°C (n=15). The length increase of larvae from 0h time after hatching (TAH) to 152h TAH is shown in Figure 5.2. Newly-hatched larvae were 2.04mm TL and showed rapid growth after hatching up to 32h TAH, by which time they were 3.11mm TL. Thereafter the larvae showed slower growth until 110h TAH at which they measured 3.55mm TL. After 110h TAH larval size decreased. Because of the inherent variability in micromeasurements (Howell, 1980), data for each growth phase were smoothed using a semi-logarithmic plot of ln(TL) vs TAH (see insert in Figure 5.2). The regression analyses for all three growth phases are given in Table 5.2.

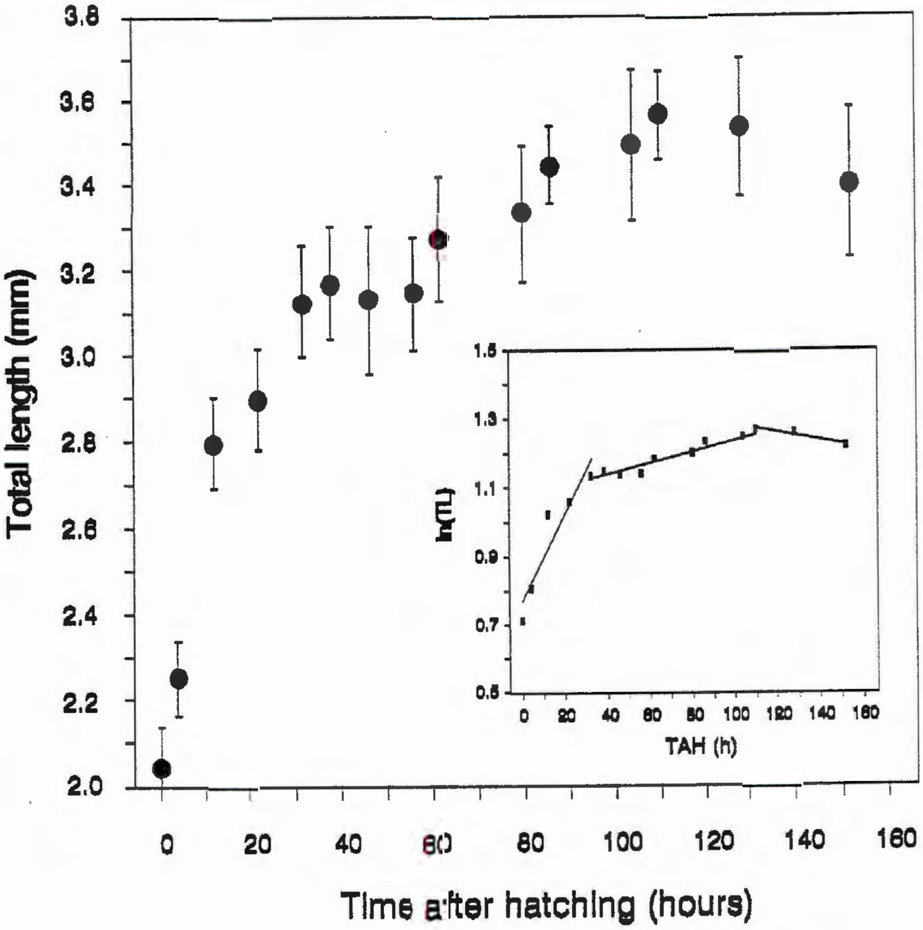


Figure 5.2: Growth of early *C. capensis* larvae from 0-152h TAH. Means and standard deviations in total length are plotted. The insert shows a semi-logarithmic plot of growth against TAH (see Table 5.2).

After hatching, yolk sac volume was found to increase initially to a maximum value of 0.23 mm^3 at 4h TAH and then decrease rapidly until 56h TAH, by which time only 8% of the yolk sac was left (Figure 5.3). Thereafter, yolk sac volume decreased minimally with time until termination of the experiment. A semi-logarithmic plot of ln(YSV+1) vs TAH was constructed to provide linear regressions of the two phases of yolk resorption (see insert in Figure 5.3); regression analyses are given in Table

5.2.

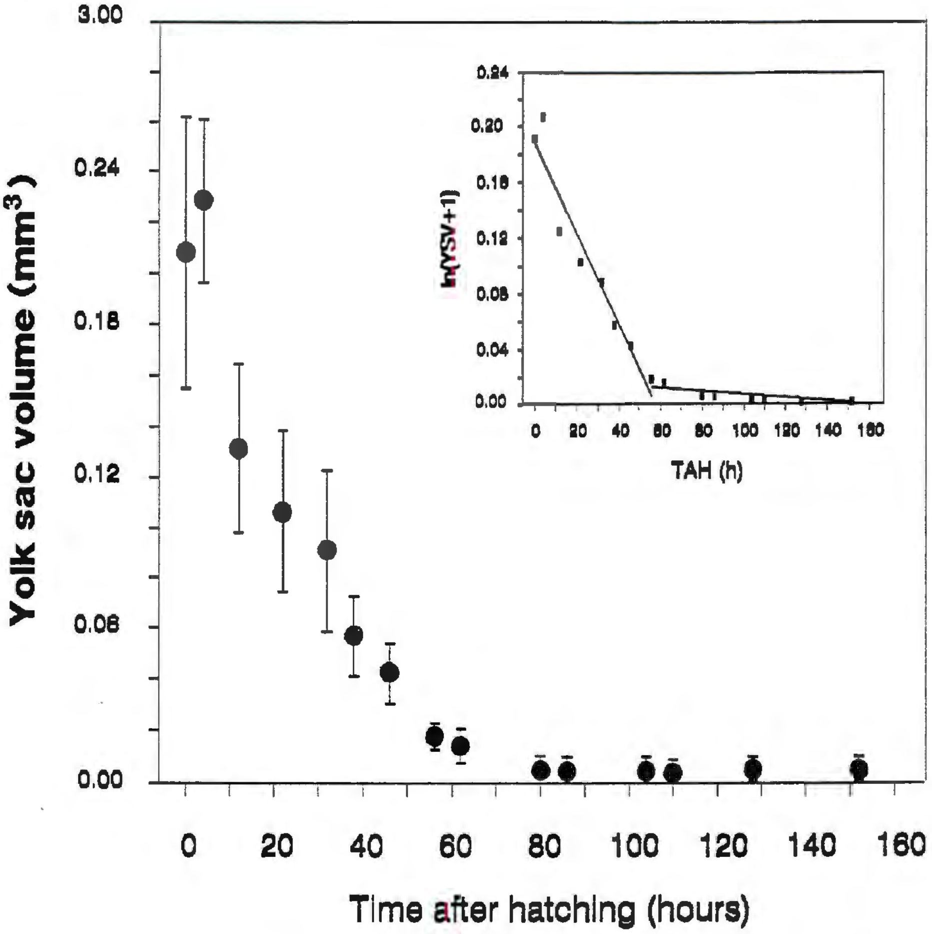


Figure 5.3: Yolk sac resorption in early *C. capensis* larvae from 0-152h TAH. Means and standard deviations of yolk volume are plotted. The insert shows a semi-logarithmic plot of resorption against TAH (see Table 5.2).

Oil globule volume was found to decrease at a slower rate than that shown by the yolk sac. 5% of the oil globule remained at 110h TAH (Figure 5.4). Thereafter, oil globule volume decreased minimally. A semi-logarithmic plot of ln(OGV+1) vs TAH was constructed and linear regressions of the two phases of oil globule resorption were determined (see insert in Figure 5.4 and Table 5.2 for regression analysis).

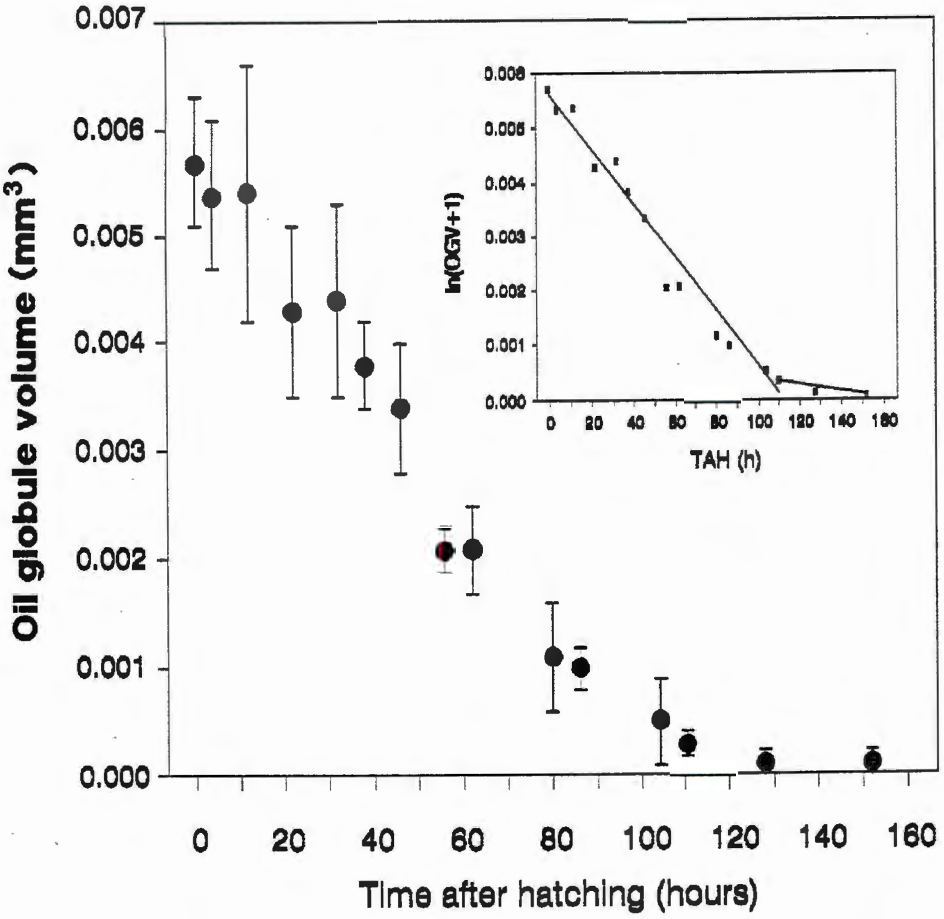


Figure 5.4: Oil globule resorption in early *C. capensis* larvae from 0-152h TAH. Means and standard deviations are plotted. The insert shows a semi-logarithmic plot of resorption against TAH 2 (see Table 5.2).

Table 5.2: Regression analyses for growth, yolk sac and oil globule resorption in larval *C. capensis*. The equations are of the form ln(x)=a+b(TAH) where a and b are constants. r^2 = regression coefficient.

	TAH	n	a	b	r^2
Length	0-32	5	0.76777	0.01283	0.88
	32-110	9	1.06953	0.00175	0.94
	110-152	3	1.40134	-0.00117	0.93
Yolk	0-56	8	0.18834	-0.00324	0.94
	56-152	8	0.02314	-0.00016	0.76
Oil globule	0-110	13	0.00564	-0.00005	0.97
	110-152	3	0.00101	-0.00001	0.85

Growth rates and endogenous food resorption rates were calculated using measured values of TL and measured values of yolk sac and oil globule volumes combined, from the beginning and end of each growth phase (i.e. 0-32, 32-110 and 110-152h TAH). Growth rate was maximal during the first phase, being 1.304 %TL/h (0.034 mm/h) whilst that for the second phase was much reduced at 0.169 %TL/h (0.006 mm/h). Growth rate during the third phase was negative due to the decrease in TL of starved larvae.

Total endogenous food resorption rate during the first phase was higher than during the second phase (0.145 and 0.036 %TL/h or 0.0037 and 0.0012 mm^3/h respectively) and was minimal during the third phase (0.001 %TL/h).

Efficiencies of development for each phase were determined using Ed = u/g (Klaoudatos *et. al.*, 1990), and are presented in Table 5.3. Development was found to be most efficient during the second phase from 32-110h TAH, being approximately twice the value for the first phase. The third phase showed a negative value due to the negative growth rate.

Table 5.3: Growth rates (g), endogenous food utilization rates (u) and efficiency of development (Ed) for the three growth phases observed in unfed *C. capensis* larvae.

TAH	g	u	Ed
0-32	1.3039	0.1451	0.1113
32-110	0.1692	0.0355	0.2098
110-152	-0.1145	0.0013	-0.0114

Larval development from just prior to hatching through to 152h TAH (at 18.0°C) is shown in Plates 5.1 to 5.11. Prior to hatching embryos possessed discernable eye lenses and diffuse pigmentation over the entire head and body was evident (Plate 5.1).

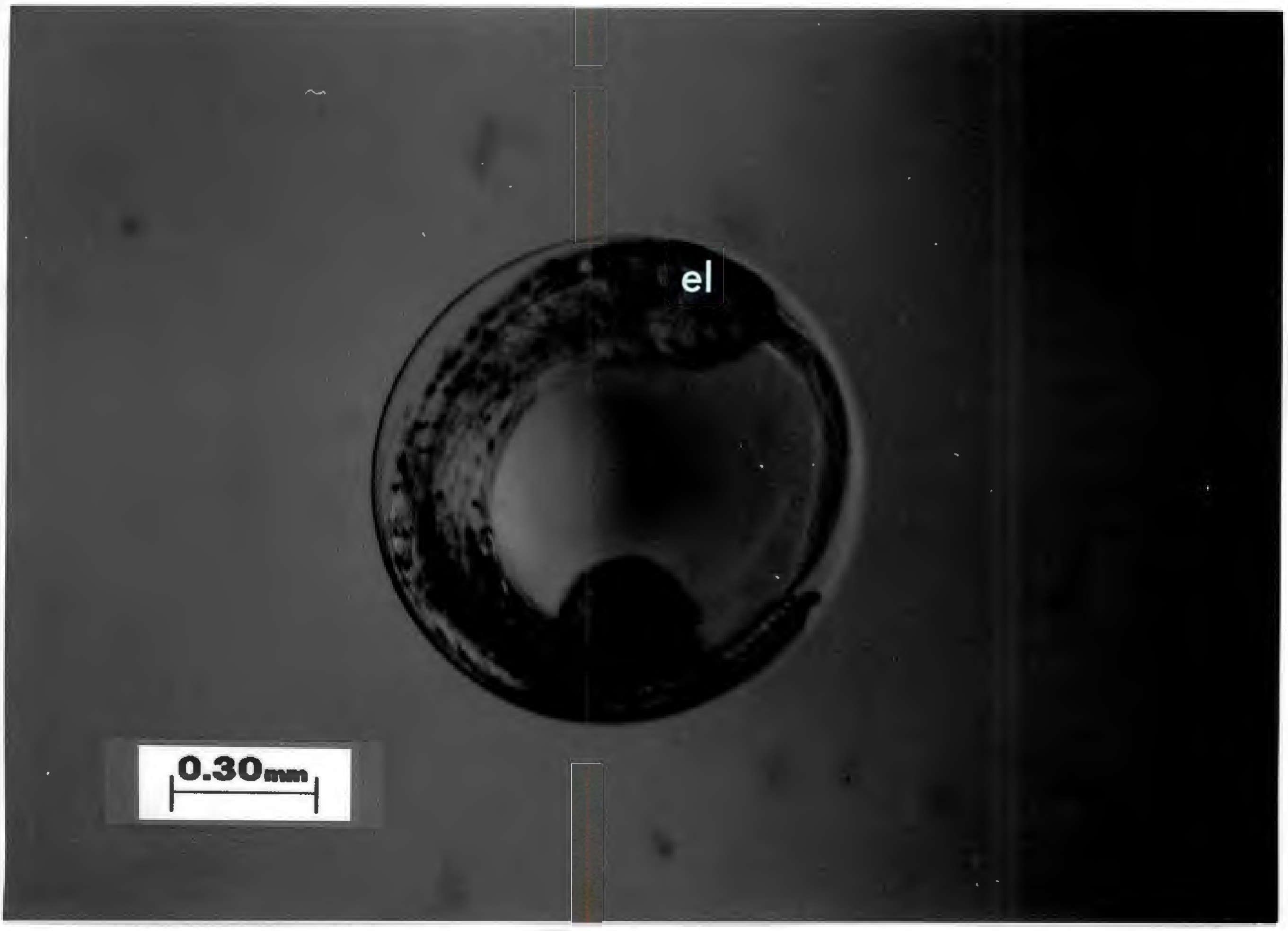


Plate 5.1: Fertilized *C. capensis* egg just prior to hatching. el = eye lens.

Newly-hatched larvae exhibited a slight curvature of the body in the region posterior to the head, and the digestive tract appeared as a straight tube with slight pigmentation at the posterior end (Plate 5.2). Myomeres were chevron shaped with the convex side pointing anteriorly. The fin-fold was present in newly-hatched larvae and remained uniform at all stages observed. The yolk sac was oval in shape with the spherical oil globule located in a ventral posterior position.

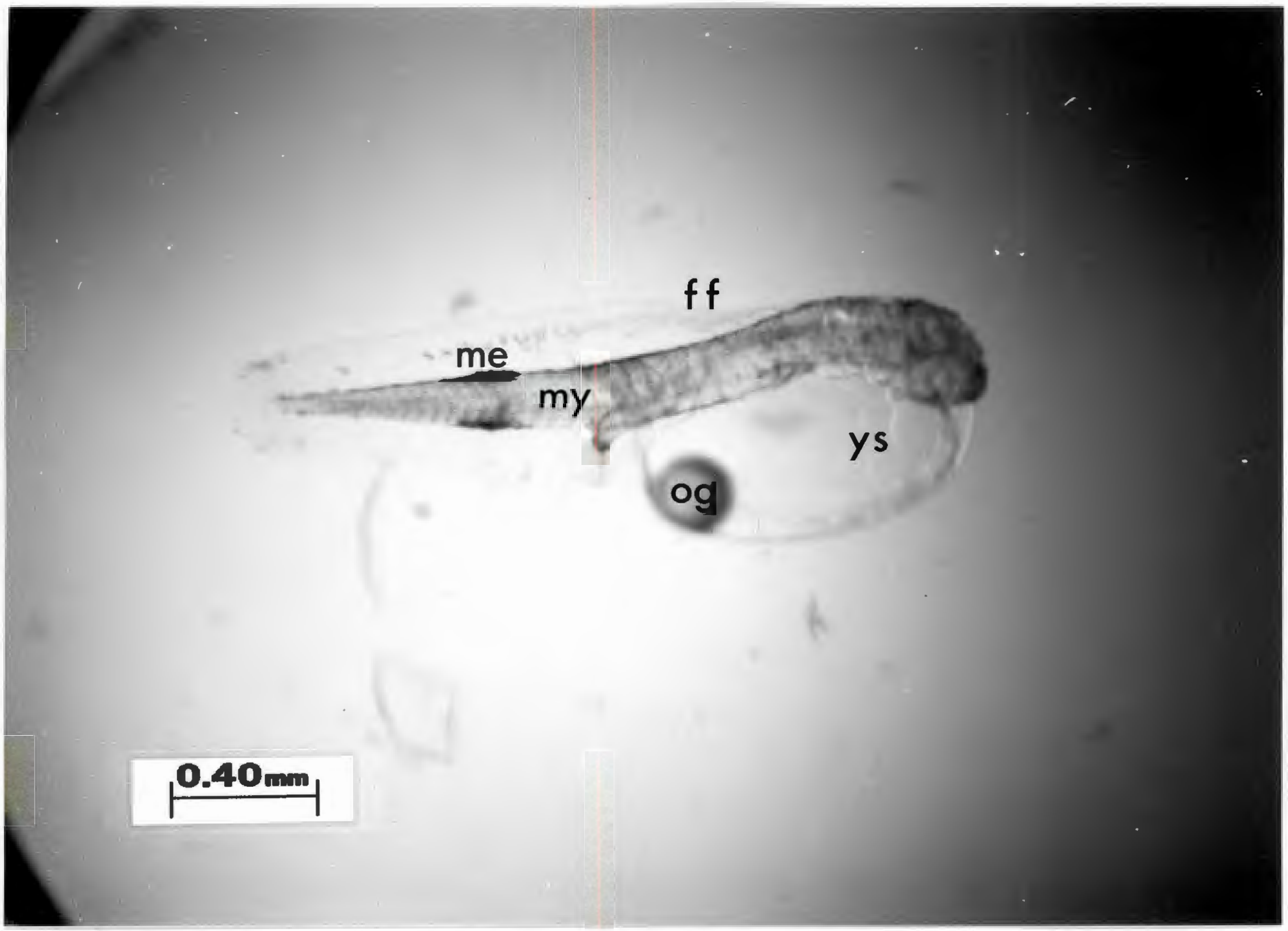


Plate 5.2: A newly-hatched *C. capensis* larva. my = myomeres, ff = fin-fold, ys = yolk sac, og = oil globule, me = melanophores.

Melanophores of newly-hatched were sparsely distributed over the head and body but were concentrated in a band approximately 2/3 of the body length away from the head. Some pigmentation of the dorsal portion of the fin-fold was also observed. Larvae floated with their head down, probably due to the posterior position of the buoyant oil globule. Although unable to swim actively, larvae exhibited sporadic wriggling motions which had the effect of moving them downwards in the water column.

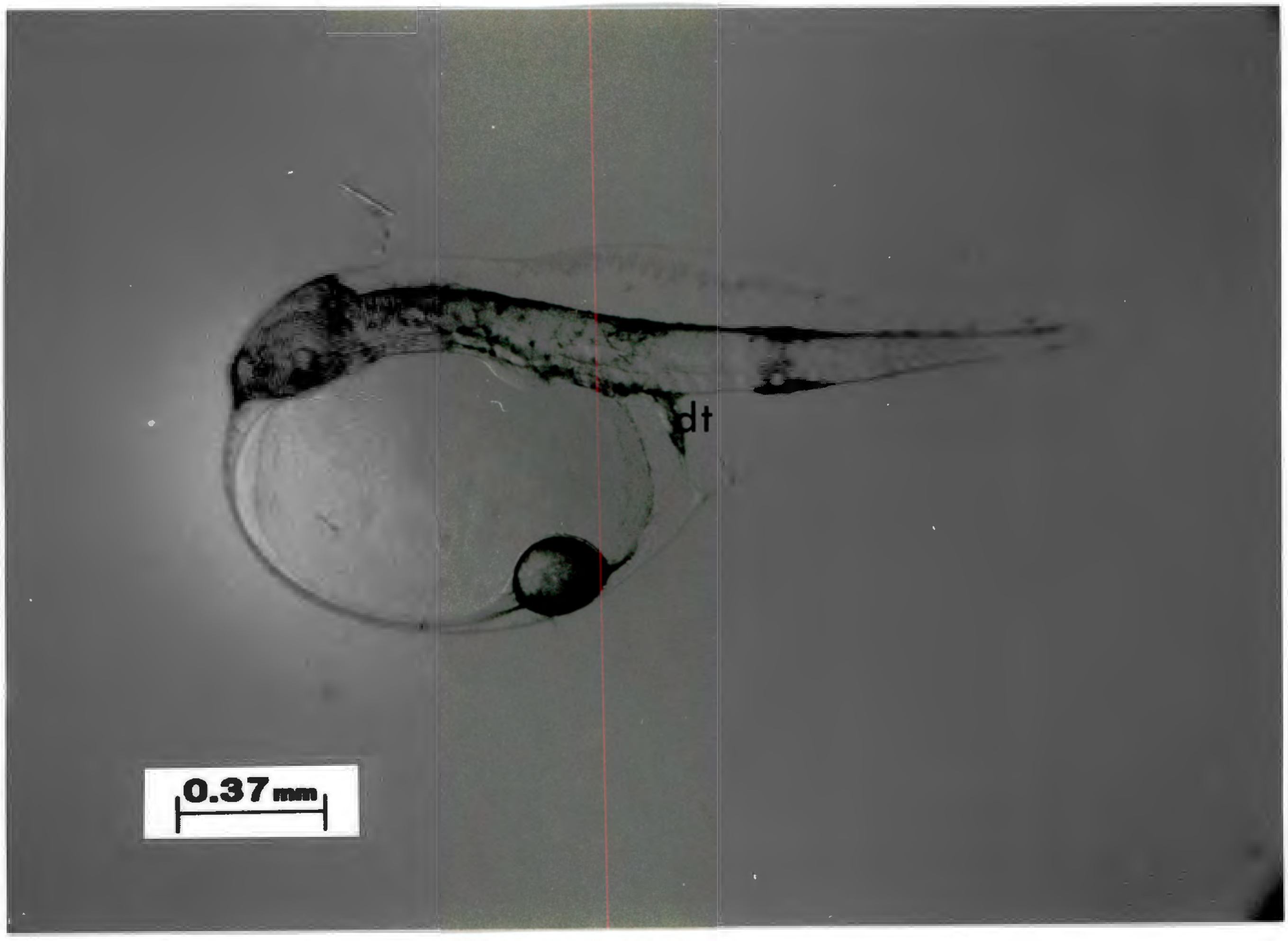
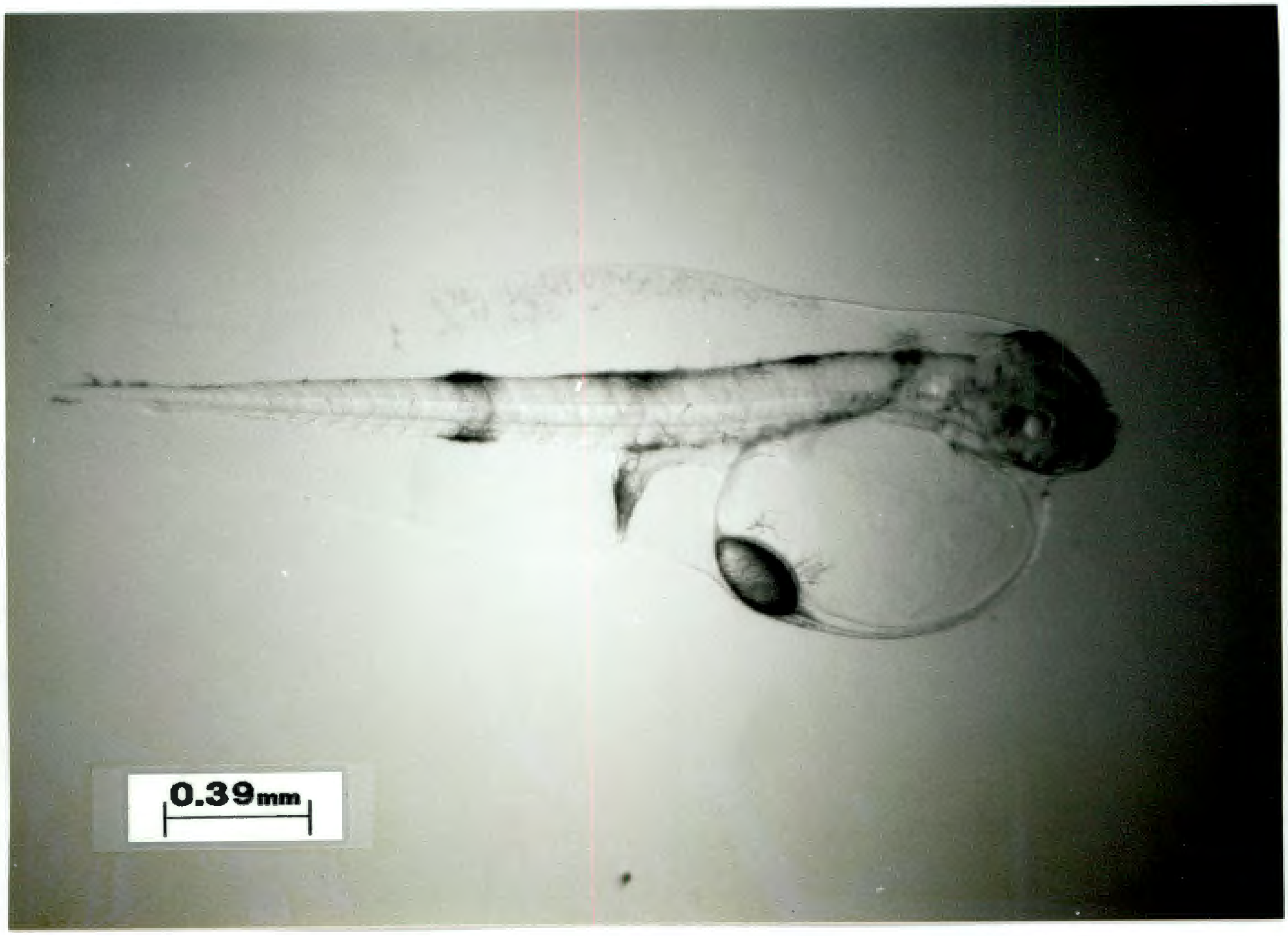


Plate 5.3: *C. capensis* larva at 4h TAH. dt = digestive tract.

At 4h TAH larvae were still slightly curved (Plate 5.3). The posterior end of the digestive tract had not yet reached the ventral edge of the fin-fold. At this stage the head appeared distinct from the body and development of the eyes had begun. A second band of melanophore concentration just anterior to the end of the digestive tract was observed and pigmentation of the dorsal portion of the fin-fold was still evident. Larvae at 22h

TAH were almost straight and the end of the digestive tract had reached the edge of the fin-fold (Plate 5.4). A third and fourth band of pigmentation above the yolk sac and behind the head became apparent. Pigmentation of the posterior end of the digestive tract was marked, whilst pigmentation of the dorsal portion of the fin-fold appeared to be lessening.



<u>Plate 5.4</u>: *C. capensis* larva at 22h TAH.

Larvae at 32h and 46h TAH appeared similiar to those at 22h TAH but showed an increase in melanophore concentration in the four pigment bands (Plates 5.5 and 5.6). Further development of the eyes was noted and by 46h TAH pigmentation on the dorsal portion of the fin-fold had almost disappeared. Larvae at this stage were able to maintain themselves horizontally in the water column.

Plate 5.5: *C. capensis* larva at 32h TAH.

By 60h TAH resorption of the yolk sac was almost complete (Plate 5.7). Development of the digestive tract had begun and light pigmentation on the anterior of the stomach and the eyes was observed.

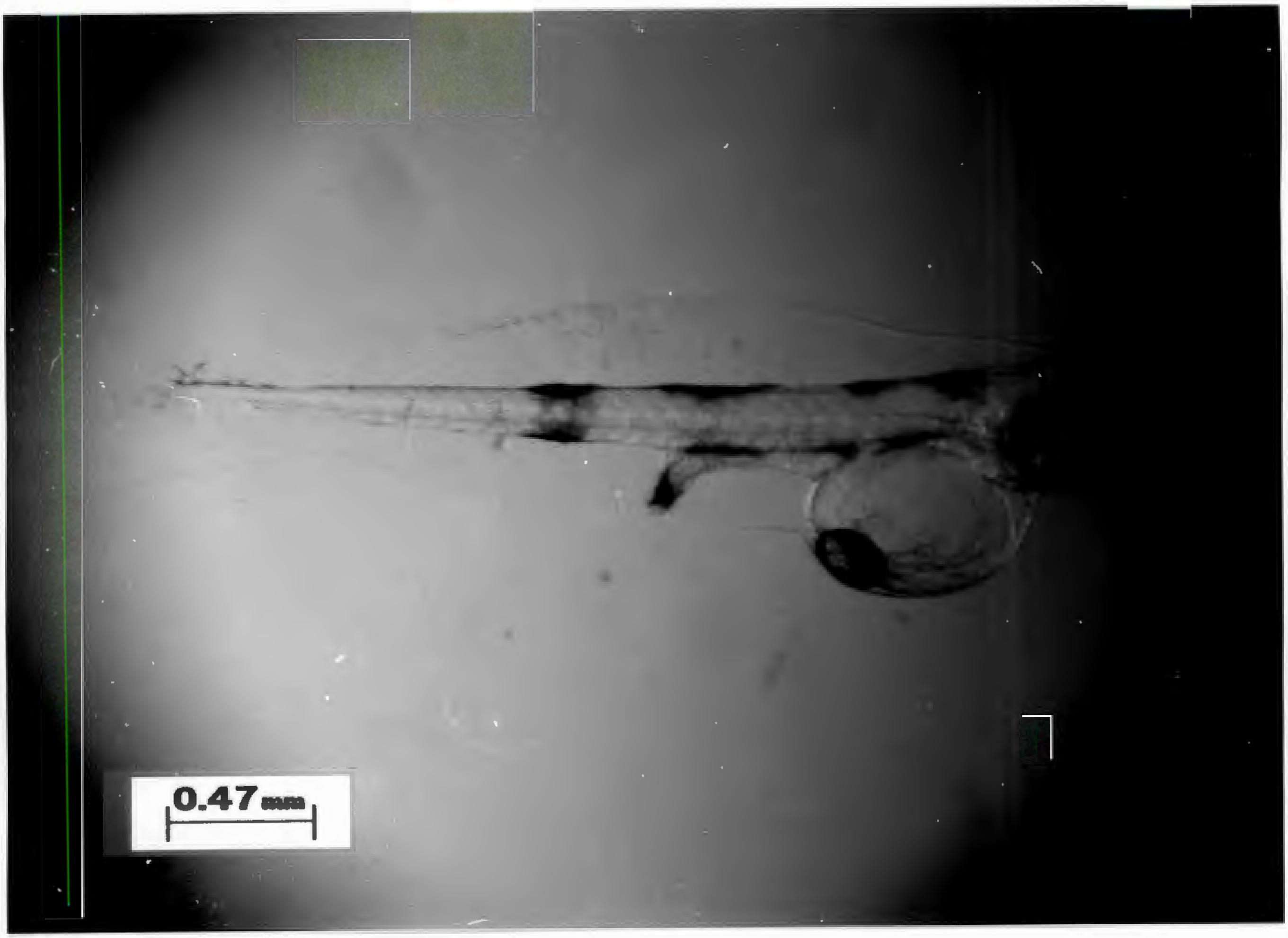


Plate 5.6: *C. capensis* larva at 46h TAH.

Larvae at 80h TAH showed further development of the digestive tract with a constriction at the posterior end forming the rectum (Plate 5.8). The oil globule was located anterior to the stomach. At this stage larvae were observed to swim for short periods and moved away from a pipette brought near them.

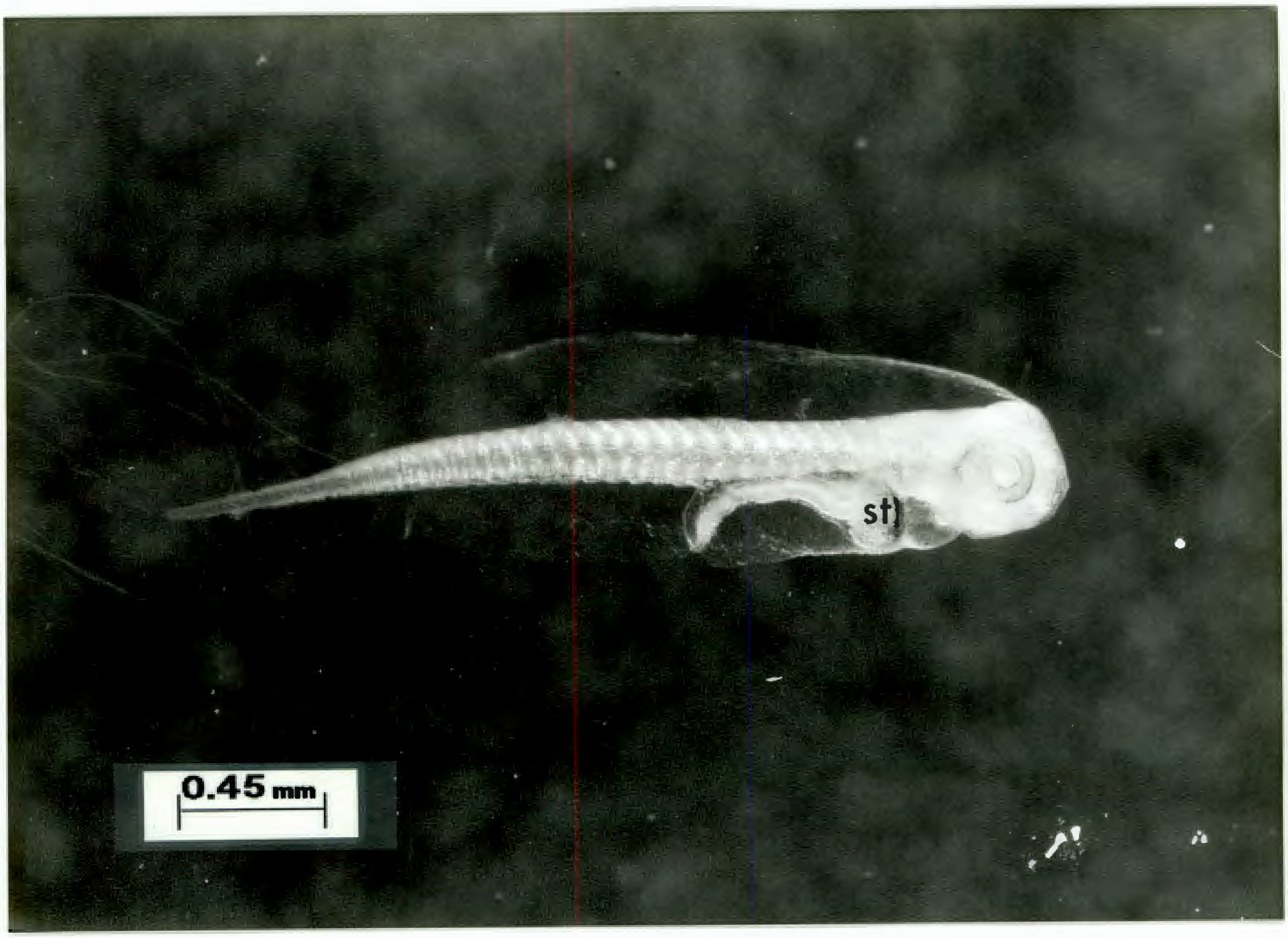


Plate 5.7: *C. capensis* larva at 60h TAH. st = stomach.

After 104h TAH pigmentation of the eyes was complete and the digestive tract appeared fully developed (Plate 5.9). The mouth had become movable and dense pigmentation along the dorsal surface of the digestive tract just below the myomeres was apparent. At this stage larvae exhibited vigorous swimming and appeared to be actively searching for prey.

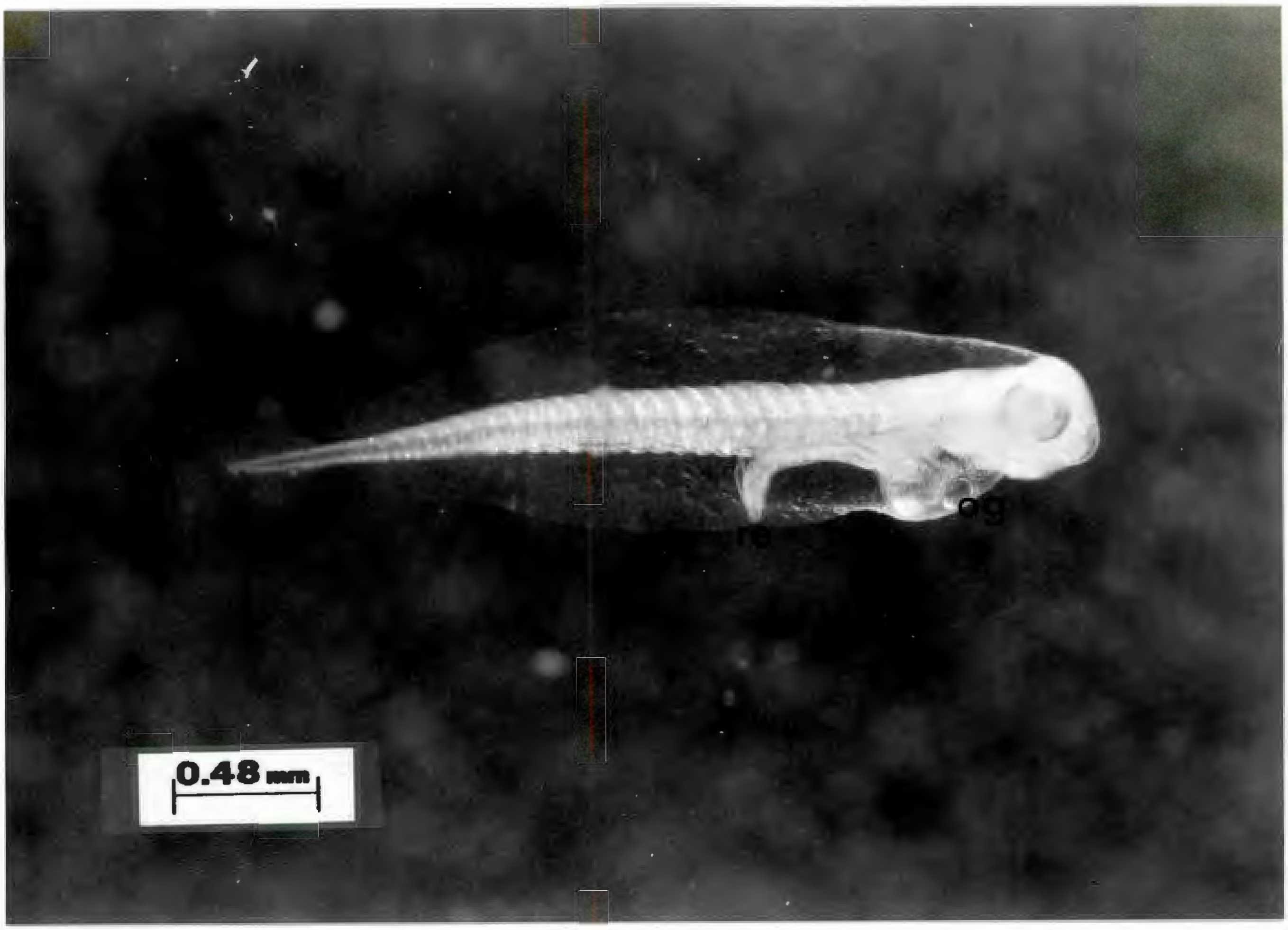


Plate 5.8: *C. capensis* larva at 80h TAH. re = rectum, og = oil globule.

By 128h TAH larvae possessed a fully developed mouth (Plate 5.10). A line of pigment extending from the digestive tract along the ventral surface of the myomeres was apparent.

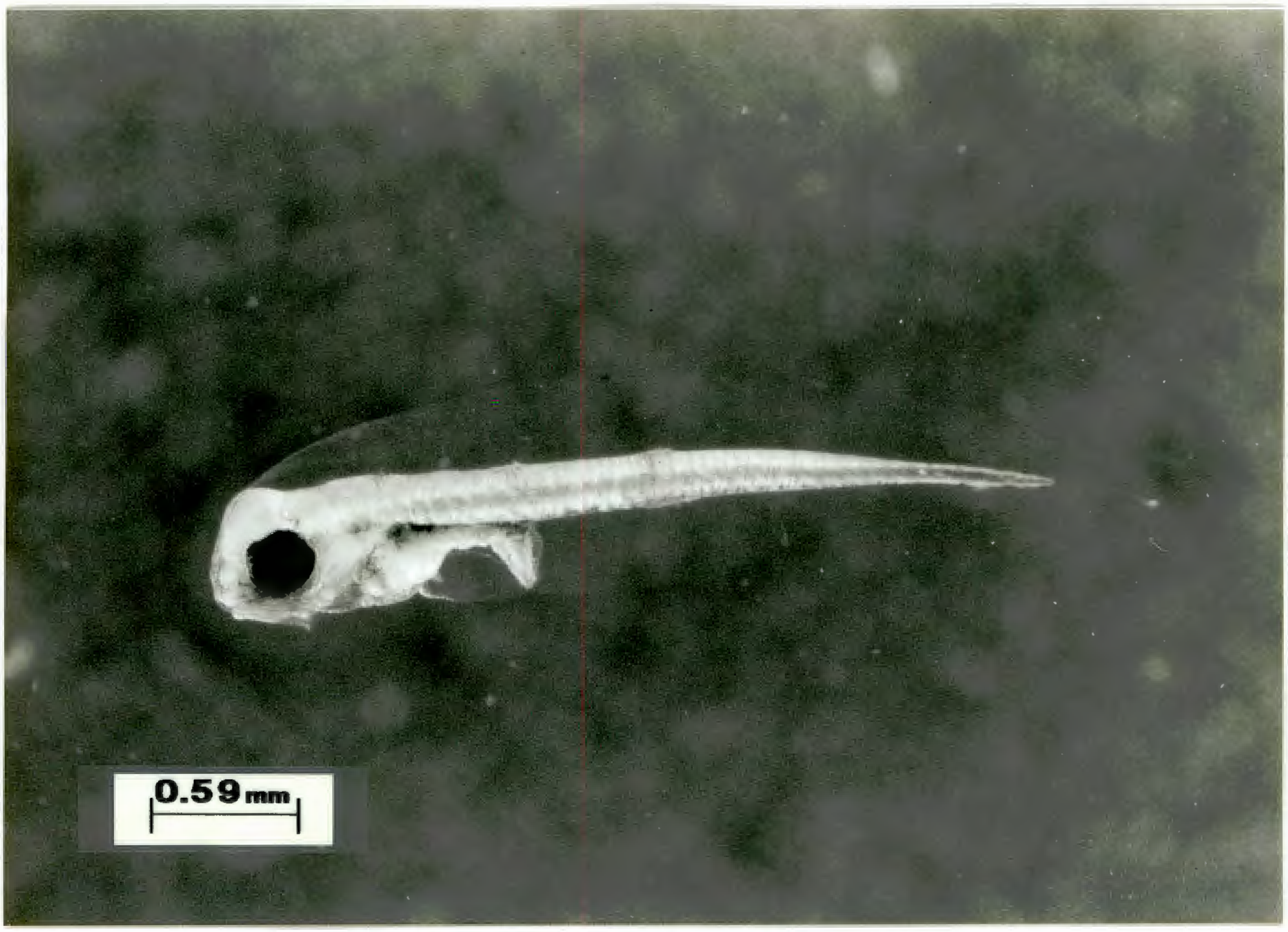


Plate 5.9: *C. capensis* larva at 104h TAH.

At 152h TAH deformities in body shape and most noticeably the fin-fold were seen, with the head appearing disproportionately large (Plate 5.11). The larvae appeared listless and did not show active swimming, with several of them being either moribund or dead.

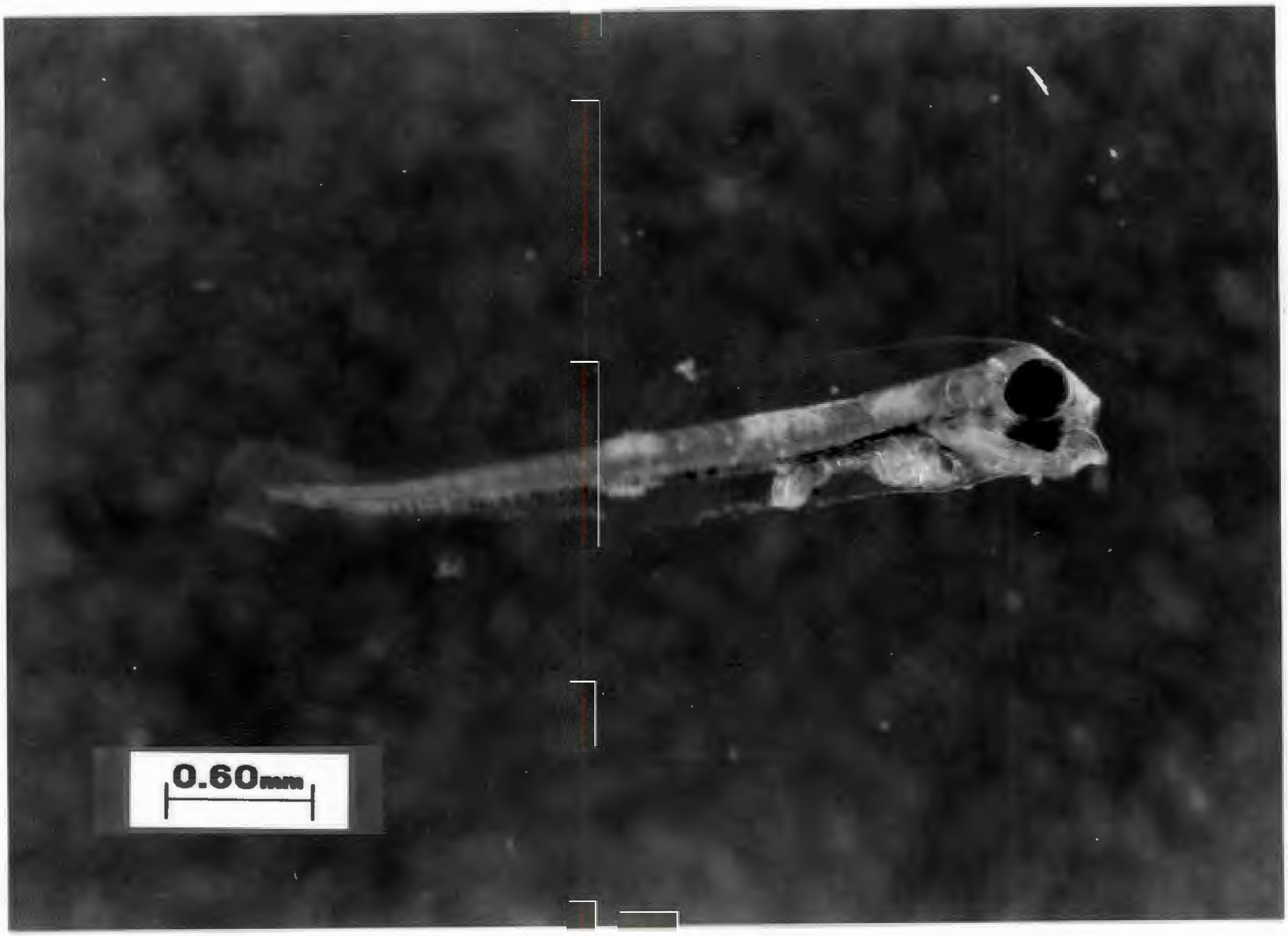


Plate 5.10: *C. capensis* larva at 128h TAH.

5.2.4: Discussion

Growth in unfed early larvae of galjoen is divided into three phases: an initial phase of rapid growth from 0-32h TAH; a second phase of slower growth from 32-110h TAH; and a third phase of negative growth from 110-152h TAH. During the initial period, organs are undeveloped and the larvae are inactive. The concomitant rapid resorption of yolk indicates that most of the yolk nutrients are channeled into growth at this time. During the second growth phase development of various organs including the eyes, digestive tract and mouth occurs. In addition, the larvae develop the musculature and structures necessary for active swimming. Yolk resorption is rapid at the beginning of this period but becomes minimal after 56h TAH, by which time most of the yolk has been utilized.

Plate 5.11: Deformed *C. capensis* larva at 152h TAH.

The rapid initial and slower second growth phases observed for newly-hatched galjoen larvae correspond well to those described for other marine larvae (Pacific mackerel, *Scomber japonicus*, Hunter and Kimbrell, 1980; seabass, *Lates calcarifer*, Kohno *et*.

al., 1986; Black sea bream, *Acanthopagrus schlegeli*, Fukuhara, 1987). Bagarinao (in press in Kohno *et. al.*, 1986) considered this initial rapid growth observed for smaller-sized larvae at hatching a compensatory mechanism, and suggested that small larvae that do not achieve such rapid growth would have reduced chances for survival.

Negative growth in the third phase is due to starvation of the larvae. Starvation in early larvae results in shrinking and the collapse of soft tissues, with the digestive tract and associated glands being the first tissues affected (Theilacker, 1978). Features exhibited by starved jack mackerel, *Trachurus symmetricus*, included a bent body and a disproportionately large head (Theilacker, 1978). These features are similiar to those shown by starving galjoen larvae.

Oil globule resorption in larval galjoen proceeds at a steady rate from 0h until 110h TAH at 18°C, by which time 95% of the globule has been exhausted. Similar steady-rate resorption has been reported for Pacific mackerel (*Scomber japonicus*, Hunter and Kimbrell, 1980) and Black Sea bream (*Acanthopagrus schlegeli*, Fukuhara, 1987). A different resorption pattern where oil globule utilization begins only when most of the yolk has been used has been reported by some authors. Kohno *et. al.* (1986) observed that the oil globule of larval seabass (*Lates calcarifer*) was not resorbed until 60h TAH, by which time the yolk was almost completely exhausted. Oil globule resorption in the European seabass (*Dicentrarchus labrax*) started when 94.6% of the yolk had been utilized (Klaoudatos *et. al.*, 1990). In both cases, the oil globule was found to persist long after the yolk was depleted. It has been suggested that oil globule persistence helps to prolong the period before irreversibile starvation, and results in robust larvae which show high survival rates at a very early life history stage (Eldridge *et. al.*, 1981; Rogers and Westin, 1981; Kohno *et. al.* 1986).

McGurk (1984) suggested that the period from exhaustion of endogenous nutrients to the point of no return was an approximate index of larval susceptibility to starvation in nature. He further proposed that this susceptibility index could be used to rank marine fish species. A plot of the time from fertilization to irreversible starvation (t_s; days) against time from fertilization to complete yolk and oil absorption (t_y; days) for 25 species of marine fish was linear, and gave the following regression equation (McGurk, 1984);

$$t_s = 0.5 + 1.3t_y \qquad (r^2=0.98,\ P<0.001)$$

McGurk (1984) also showed a high correlation between temperature (T) and t_s, the relationship being best described as a negative exponential of the form;

$$\ln\ (t_s) = 3.9643 - 0.0892T \qquad (r^2=0.91,\ P<0.001)$$

For both regressions, data fall into either a temperate or tropical group. In the temperate group, development occurs at low temperatures of 7 to 15°C and larvae show a relatively long time to irreversible starvation (eg *Clupea harengus*, *Pleuronectes platessa* and *Solea solea*). Larvae in the tropical group develop at temperatures of 15 to 29°C and exhibit short times to irreversibile starvation (eg *Mugil cephalus*, *Chanos chanos*, *Epinephalus tauvina* and *Siganus rivulatus*). Species of fish whose larvae do not fit the pattern described by McGurk (1984) include California grunion (*Leuresthes tenuis*; May, 1971), striped bass (*Morone saxatilis*; Rogers and Westin, 1981), seabass (*Lates calcarifer*, Kohno *et. al.*, 1986) and European seabass (*Dicentrarchus labrax*, Klaoudatos *et. al.*, 1990). All of these larvae show persistence of the oil globule long after the yolk sac has been totally resorbed.

McGurk (1984) proposed that marine fish larvae could be divided into two classes on the basis of endogenous food utilization: the first containing pelagic species that have a brief period after yolk exhaustion in which to learn how to feed successfully, and the second containing inshore or estuarine species that have much longer survival periods after yolk resorption due to the presence of a large, persistent oil globule. Thus the response of larval fish to a delay in the onset of feeding following yolk resorption can be considered to occur along a continuum ranging from extreme dependence on early feeding to near indifference to the timely arrival of suitable prey (Rogers and Westin, 1981).

Using the value of 170h (7.1 days) for t_y for early galjoen larvae and substituting into the predictive equation produced by

McGurk (1984) gives a t_s value of 233h (9.7 days). Time from hatching to the point of irreversible starvation is therefore 173h, a value in accordance with the results obtained in this experiment since the effects of starvation were observed by 152h TAH. Substitution into the temperature/t_s predictive equation of McGurk (1984) gives a value of 256h, or 194h TAH. Galjoen larvae thus show characteristics typical of tropical species lacking a persistant oil globule, and exhibit a short time to irreversible starvation of between 173-196h TAH. Brownell (1980a) observed similiar values of the time to irreversible starvation for the larvae of several local species, including blacktail, *Diplodus sargus* (time to irreversible starvation of 210h TAH), lace sole, *Synaptera kleini* (189h TAH) and the Cape sole, *Heteromycteris capensis* (212h TAH).

The efficiency of development of early galjoen larvae was found to be highest during the second growth period from 32h-110h TAH. This result seems somewhat surprising, given the rapid growth shown during the first phase. During the first phase however, total endogenous food utilization was approximately 4 times as fast as during the second phase. Although growth in the latter phase was reduced, endogenous food utilization was also much reduced and this may explain the higher calculated efficiency of development. Klaoudatos *et. al.* (1990) showed that larvae of European sea bass (*Dicentrarchus labrax*) exhibited a rapid initial (0-24h TAH) and a slower second (24-72h TAH) growth phase. Using their data, efficiency of development for each of these phases was calculated, and was found to be higher in the second phase (0.1131 and 0.1303 for the first and second phases respectively). The development efficiency value for the first phase was similiar to that calculated for the first phase in galjoen larvae.

Other studies of yolk utilization have shown that the efficiency of yolk utilization is temperature dependent, being more efficient at higher temperatures for larval yellowtail flounder (*Limanda ferruginea*; Howell, 1980). Efficiency was also found to decrease during the course of development for both yellowtail flounder and herring (*Clupea harengus*) larvae (Blaxter and Hempel, 1966). Blaxter and Hempel (1966) suggested that the decrease in development efficiency was due to the relatively higher metabolic demands of heavier, more developed larvae.

The method used to calculate efficiency of development of larval galjoen in this experiment did not take the weight of either the yolk or the larvae into consideration, but related yolk utilization to changes in larval length. Thus whilst growth in length was slight during the second phase, it is probable that the larvae increased more rapidly in weight, given that morphological development during this period was high. The accuracy of this method of determining efficiency of development is therefore questionable; a more rigorous analysis by weight would certainly provide more accurate estimates and could well demonstrate a different pattern of efficiency of development, possibly similiar to that reported by Blaxter and Hempel (1966) and Howell (1980).

It is generally held that the optimum time for first-feeding in marine larvae is at or slightly before the time of yolk sac resorption (Theilacker and Dorsey, 1980; Fukuhara, 1987). An estimation of the time of first-feeding for larval galjoen based on the development of morphological features such as the mouth and the digestive tract would suggest that first-feeding occurs between 104 and 128h TAH. By this time, virtually all of the endogenous nutrients have been resorbed. Galjoen larvae thus have a limited period of time of approximately 70h in which to make the transition from endogenous to exogenous feeding. Mouth size at the time of first-feeding was approximately 0.3mm, thus restricting the size of suitable live food organisms to <300µm.

Several studies have examined the effect of delayed feeding in early larvae (Roberts *et. al.*, 1978; Rogers and Westin, 1981; McGurk, 1984). Roberts *et. al.* (1978) observed that red drum (*Sciaenops ocellata*) larvae not fed within 5 days from hatching experienced 100% mortality. Survival in a small percentage of larvae offered food on day 6 was prolonged by a few days, but none of these larvae were able to recover. Rogers and Westin (1981) observed that some unfed larval striped bass (*Morone saxatilis*), although showing a decrease in length and dry weight resulting from tissue breakdown, were able to recover and continue development after food was provided. This recovery was due to the presence of a persistent oil globule which acted as an energy store during starvation. The oil globule remained mostly unused even whilst tissue breakdown occurred. The effects of feeding, delayed or otherwise were not examined in this experiment, but it is suspected that larval galjoen would show a response similiar to red drum larvae, since neither possess a persistent oil globule.

The results obtained have demonstrated that larval galjoen deplete their endogenous food resources rapidly. The transition from endogenous to exogenous feeding is thus of paramount importance, since the time from depletion to irreversible starvation is a scant 70 hours. Since most marine larvae, including galjoen, are visual predators (Stoeker and Govoni, 1984) they can only search for food during daylight hours. Thus actual time available for successful feeding is further reduced, to approximately 40 hours for galjoen. Provision of sufficient suitable food at the correct time is therefore crucial if successful propagation of galjoen is to be achieved.

CHAPTER 6: MASS REARING OF GALJOEN LARVAE

6.1: Introduction

As successful aquaculture is dependent upon a readily available supply of larvae and fry, seed production must surely rank as the most necessary biological characteristic of a candidate species. In a culture situation, larvae are obtained from natural captive spawns or from artificially induced spawns, whilst fry are sometimes collected from the wild. This latter method of seed aquisition is generally used in extensive culture only, due to the seasonality and unreliability of the supply (Ben Yami and Grofit, 1981). Once sufficient seed has been obtained however, the larvae must be reared to a size suitable for ongrowing. The early life history stages of marine fish are generally the least robust, with the highly plastic early embryo and the stage of larval transition between reliance upon endogenous and exogenous food sources being the most susceptible to environmental and pollutant stress (Rosenthal and Alderdice, 1976). Consequently, it is at this time that mortality is highest (Beyer and Laurence, 1980), and a determination of the environmental factors affecting larval survival and growth, and how these factors may be manipulated to maximise production is therefore a priority for culture.

A major constraint upon the development of mariculture worldwide has been the difficulty in ensuring reliable and sufficient supplies of post-larvae for ongrowing. Most of the problems encountered in the mass propagation of marine finfish larvae have been due to the small size and fragility of the larvae (Nash and

Kuo, 1975; Shepherd, 1988). As was discussed earlier, the majority of marine fish produce small pelagic eggs which hatch into diminutive larvae. These show a short yolk-utilization period, a brief period of time to irreversible starvation and have a requirement for very small food organisms which are nutritionally adequate.

Whilst it is generally agreed that nutrition of marine larvae is the major determinant of survival (May, 1970; Stepien, 1976; Beyer and Laurence, 1980; Tandler and Sherman, 1981; Watanabe, 1988b), numerous other factors have been shown to affect both survival and growth. These factors are reviewed in detail in the discussion section, and include several water quality parameters such as temperature, salinity, dissolved oxygen, pH and levels of ammonia, nitrite and nitrate. In addition tank size, stocking density, light intensity, photoperiod, the presence of algae in the rearing tanks and the presence of disease-causing organisms are also implicated in affecting larval survival and growth.

Several rearing trials, aimed at assessing the effects of some of these factors and attempting to mass produce galjoen larvae, were conducted. The first trial examined the effect of water sterilization by the use of ultra-violet light or antibiotics upon the survival and hatching success of fertilized galjoen eggs, and upon subsequent larval survival. The following trial attempted to identify suitable food organisms for first-feeding larval galjoen. Trials 3 and 4 were designed to examine the effects of stocking density and tank size on early larval size and growth. Several attempts to raise galjoen larvae extensively with minimal control or monitoring have been grouped together as trial 5.

6.2: Materials and Methods

6.2.1: Production of live food organisms

Live food organisms used in various feeding trials are listed in Table 6.1. Rotifer cultures were initiated in 20l plastic bags filled with a strong green algal culture of either *Tetraselmis* or *Chlorella* and held in a constant temperature and light room. When rotifer densities were sufficient, the contents of the bag were transferred to a 300l asbestos tank, supplied with gentle aeration and seawater at a flow rate of approximately 0.2l/minute and maintained outside at a mean ambient temperature of 18.5°C. 10 litres of dense *Chlorella* or *Tetraselmis* was added to the tanks every second or third day. Rotifers achieved higher production when fed *Tetraselmis*. Prior to being offered to larvae, rotifers were collected using a 37µm mesh and rinsed with 0.2µm-filtered seawater.

Artemia nauplii were obtained from dehydrated eggs suspended in strongly aerated seawater. Since nauplii hatch from eggs after 30h at 25°C, they can be produced almost on demand. Nauplii were separated from hatching debris through the use of a light-proof box which exploited their positive phototactic response and hence allowed separation. Newly-hatched nauplii were cultured in plastic bags and were fed either *Tetraselmis*, *Chlorella* or a suspension of baker's yeast for one day before being offered to larval galjoen. Nauplii were collected using a 67µm mesh and were rinsed using 0.2µm-filtered seawater before being offered as food.

Table 6.1: Type and size of live food organisms used in C. capensis larval rearing trials.

	Length	**Width**
Brachionus plicatilis typicus (1) (L-strain)	230-320µm	115-159µm
Brachionus plicatilis rotundipes (1) (S-strain)	140-220µm	55-102µm
Artemia franciscana (2) nauplii	400µm	200µm
Paracalanus scotti (3,4) nauplii	90-250µm	50-120µm

(1) Fukusho and Okauchi (1982); (2) Sorgeloos (1980); (3) Ogilvie (1953); (4) Faber (1966).

Paracalanus were cultured in 150l fibreglass tanks or 3000l portapools and were fed on diatoms (*Thallassiosira weissflogii*) or green algae. Nauplii and copepodites were collected by sieving initially through a 300µm mesh to remove later stages and adults, and then through a 37µm mesh, and were rinsed using 0.2µm-filtered seawater before being offered to larvae.

Algal cultures used to feed live food organisms were maintained in either 5, 20 or 300l plastic bags held in a constant temperature and light room and supplied with Walnes medium and sodium metasilicate as nutrients to promote log phase growth.

6.2.2: Rearing trial #1

This trial examined the effect of antibiotic and ultra-violet sterilized water on survival and hatching success of fertilized galjoen eggs. The effects of these two treatments on larval survival subsequent to hatching were also assessed. Viable, fertilized eggs were selected from the products of a natural captive spawn and were transferred in batches of 200 to six 20-litre hatching tanks within 12 hours of spawning. Two of the tanks were dosed with veterinarian tetracycline (active ingredient 5,5% oxytetracycline-hydochloride by weight, manufactured by Glaxo (Pty) Ltd) at a level of 1.6 mg/l (James *et. al.*, 1988), two received UV sterilized water and two tanks were without antibiotic or UV sterilization and acted as controls. One control and both antibiotic-treated tanks were static with no seawater input, whilst the other control and both UV-treated tanks were supplied with 0.2µm-filtered seawater at a rate of 0.1l/minute. Ultra violet sterilization was attained by using a suspended sterilizer consisting of three UV tubes hung above a shallow (1cm) reflective trough through which filtered seawater passed. All tanks received constant gentle aeration and 24h illumination from overhead fluorescent tubes. Dead eggs and larvae which had sunk to the bottom of the tanks were removed and counted daily to determine survival rates and hatching success. Food was not supplied to the larvae at the time of first-feeding, and the trial was terminated after all larvae had died.

6.2.3: Rearing trial #2

This trial examined the suitability of the various organisms listed in Table 6.1 as food for first-feeding galjoen larvae. Food organisms offered individually included L- and S-strain rotifers, *Paracalanus* nauplii and copepodites and *Artemia* nauplii. Combinations of approximately equal numbers of L-strain rotifers with copepod nauplii and copepodites, and L-strain rotifers with brine shrimp nauplii were also offered to the larvae.

Fertilized eggs were obtained from natural captive spawns and were placed in several 20l plastic bowls at densities of approximately 15 eggs/litre. The bowls were supplied with constant gentle aeration and 0.2µm-filtered seawater at a rate of 0.1l/minute. Larvae hatched two days after being transferred into the bowls.

Food organisms were introduced into two bowls per food type or combination thereof on the third day after hatching at an approximate density of 15 organisms/ml. On the day of first-feeding (4d post hatch), 5 larvae from each tank were captured using a clear plastic siphon pipe, and the total length from the tip of the snout to the end of the notochord was measured using a microscope with an ocular micrometer. The larvae were then placed together on a gauze mat and were kept at 60°C for 4 days to dry to a constant weight. The dry weight of all 20 larvae was then determined using a Cahn 26 automatic electrobalance. At successive 2d intervals from first feeding, 10 larvae from each of two tanks per food type or combination were measured, examined for the presence of food in the gut, and the dry weight determined. Quantification of survival rates was not made as it was impossible to accurately determine this without killing all larvae. The experiment was conducted for 8 days after first-feeding, by which time high mortalities were evident and the results obtained showed that the larvae were starved.

6.2.4: Rearing trial #3

This trial was conducted to examine the effect of density on survival and growth of galjoen larvae. Fertilized eggs were obtained from a natural captive spawn and were placed at densities of approximately 10, 30 and 50 eggs/litre in 800 litre asbestos tanks. The tanks were supplied with gentle aeration and 0.45µm-filtered seawater at a rate of 2l/minute. Larvae hatched on the second day after being placed in the tanks.

Larval densities were determined by using a section of PVC piping (55mm internal diameter) in conjunction with 64µm mesh cups lying on the bottom of the tank. The piping was held vertically and lowered into the tank until it mated with one of the cups, after which the combination was slowly withdrawn. During withdrawl, larvae captured inside the piping were concentrated on the mesh, and 30 from each tank were then counted, measured (TL) and dried for dry weight determinations. Because the water level in the tanks remained constant, the volume of water encased by the piping and subsequently filtered through the mesh was known, allowing an estimate of larval density to be made. Prior to sampling, the tops of the tanks were covered with black plastic sheeting for a period of 30 minutes. The reduction in light caused larvae to become disorientated and resulted in even dispersion throughout the tank. Sampling was repeated three times per tank every two days after hatching. Measurements of water quality including temperature, dissolved oxygen, pH and salinity were taken daily from each tank. The experiment was conducted for 13 days after hatching of the larvae, by which time densities had dropped to almost zero, and remaining larvae were moribund.

Larvae were offered a mixture of rotifers and copepod nauplii as food organisms at densities of 10-15/ml from 3 days after hatching. Food organism mixtures were replenished every 2 days to maintain these concentrations.

6.2.5: Rearing trial #4

This trial was conducted to examine the effect of rearing tank size and associated features on larval survival and growth. Rearing containers of several sizes were used, including 5l glass beakers, 20l rectangular glass aquaria, 300l and 800l asbestos tanks and 60l, 100l and 1000l conical-bottom fibreglass tanks. The beakers and glass aquaria were kept inside a constant temperature room maintained at 18.0°C whilst the asbestos and fibreglass tanks were kept outside and held at ambient water temperatures of 19.0-21.0°C. In general, the smaller containers experienced faster water turnover rates ranging from a 90% exchange every 0.5-3 hours whilst 90% exchange in the larger tanks required 0.5-8 hours. Fertilized eggs obtained from natural captive spawns were placed in the various rearing tanks at a density of approximately 10 eggs/litre on the morning following spawning.

Live food organisms offered to the larvae at first-feeding included *Paracalanus* nauplii and copepodites when available and both L- and S-strain rotifers. *Artemia* nauplii were added to rearing tanks where larvae were still present one week after first-feeding. Food organisms were maintained at approximately 10 organisms/ml. In some of the experiments using the larger rearing tanks, live food organisms were enriched with w3-highly unsaturated fatty acids (w3-HUFA) using the direct method of Watanabe *et. al.* (1982). L-strain rotifers, previously fed green algae, were collected by the method outlined above and were placed in 20l of 0.2µm-filtered seawater. A mixture of 1.5g cuttlefish liver oil, 0.3g raw egg yolk and 20ml sea water was homogenised in a blender for 3 minutes and added to the rotifer culture together with 0.5g baker's yeast. Rotifers were allowed to feed on this emulsion for 15-20 hours before being concentrated and rinsed with seawater prior to being offered to first-feeding larvae. Newly-hatched *Artemia* nauplii were also enriched by adding the same emulsion and allowing the nauplii to feed for 15- 20 hours before being concentrated and offered to larvae. The effect of dense algal cultures of either *Chlorella* or *Tetraselmis* in some of the rearing tanks was also examined.

6.2.6: Rearing trial #5

After most natural captive spawns, fertilized eggs which were not used in any of the trials detailed above were placed in 3000l portapools containing sand-filtered seawater. The pools were supplied with constant aeration and seawater at a rate of 2l/minute. The pools were maintained indoors, with 24h illumination being provided by fluorescent lights. Eggs were placed in the pools at an estimated density of 10/l. Food organisms not required for other rearing trials were added to the portapools when available, but food densities were never determined. The portapools were generally uncontrolled and unmonitored, and were viewed as experiments in extensive larval rearing.

6.3: Results

6.3.1: Rearing trial #1

The results obtained from this trial are presented in Figure 6.1. Survival to hatch was high in all tanks, ranging from 83-96%, with neither ultra-violet sterilization nor the addition of antibiotics being found to appreciably improve hatching success. The high survival to hatch rates observed were similiar to those reported in chapter 5 (see Table 5.1).

Larval survival subsequent to hatching was similiar in all tanks regardless of treatment. Survival curves exhibited a gradual decrease from hatching to the time of first-feeding, followed by an abrupt decrease over the 2 days following the time of first-feeding, at which stage survival was less than 25% in all tanks. No larvae survived longer than 11 days after spawning.

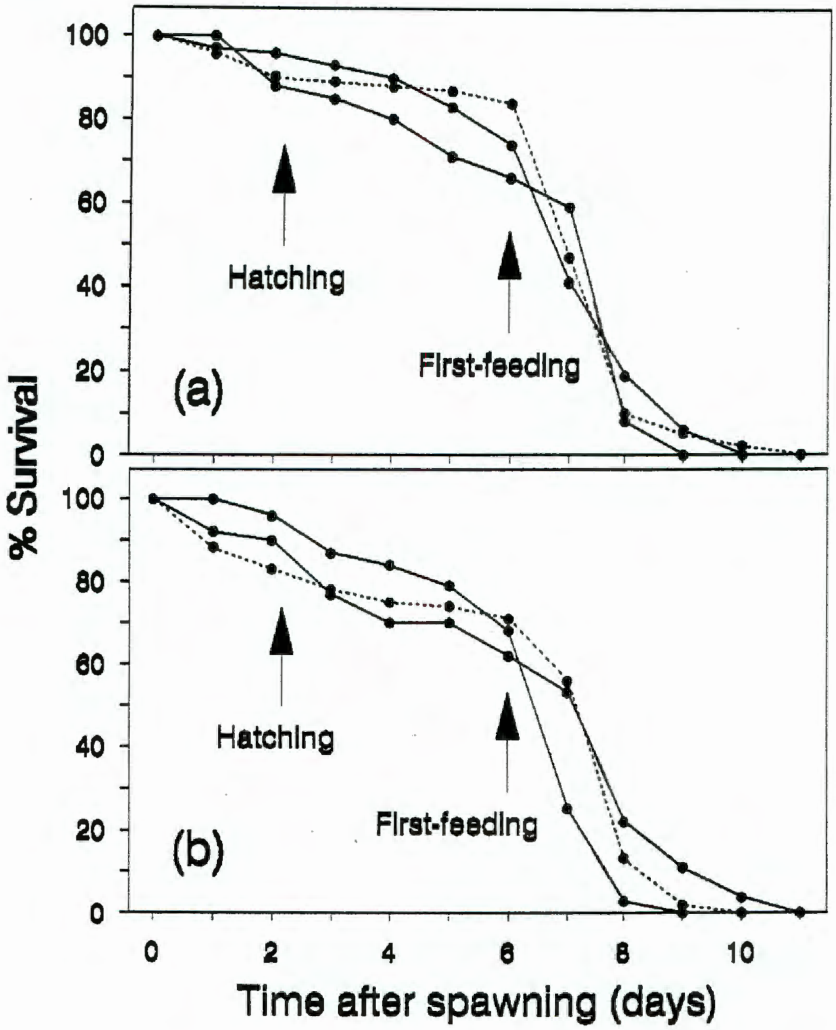


Figure 6.1: Survival curves for egg and unfed larval *C. capensis* from spawning to 5 days post first-feeding. (a) shows survival for larvae in antibiotic-treated tanks, (b) shows survival for larvae in ultra-violet treated tanks; the dotted lines in each graph indicate responses from larvae in the control tanks.

6.3.2: Rearing Trial #2

The percentages of larvae with food in the gut at various times after first-feeding for each treatment are given in Table 6.2. Feeding success was high for larvae offered all types and combinations, with the exception of those offered *Artemia* nauplii. Two days after first-feeding, only 10% of larvae from each tank supplied with *Artemia* nauplii had successfully ingested food particles. Feeding success at this time for those offered other food types ranged from 50-80%, with larvae from tanks supplied *Paracalanus* nauplii and copepodites and S-strain rotifers showing the highest values. By four days post first-feeding, none of the larvae offered *Artemia* nauplii had food in their guts.

Table 6.2: Percentages of *C. capensis* larvae with food in the gut offered different food types at various times after first-feeding. n=10 for all samples; - indicates no larvae were sampled.

Food type		Percentage of larvae with food in the gut at various times after first-feeding (days)			
		2	4	6	8
L-rotifers	a:	50	50	30	0
	b:	70	30	30	10
S-rotifers	a:	80	50	50	10
	b:	60	60	30	0
Paracalanus naup/copes	a:	70	70	40	20
	b:	70	70	50	0
Artemia nauplii	a:	10	0	0	-
	b:	10	0	0	-
Paracalanus + L-rotifers	a:	70	60	50	10
	b:	50	60	40	10
Artemia + L-rotifers	a:	50	50	20	0
	b:	60	30	10	0

The percentage of larvae with food in their guts decreased during the course of the experiment for larvae offered all other food types, with those from tanks supplied with *Paracalanus* nauplii and copepodites and S-strain rotifers decreasing the slowest.

Differences in appearance and behaviour were apparent between larvae offered different food types. By four days post first-feeding, several larvae from each tank displayed curved backs, an indication of starvation (Theilacker, 1978; see Chapter 5). However, the incidence of curved larvae was lower in the tanks where *Paracalanus* nauplii and copepodites were offered either singly or in combination with L-strain rotifers. In these latter tanks, 25-30% of larvae sampled appeared very healthy, being well formed and exhibiting an irridescent blue/green sheen when examined immediately after capture. Only 10% of those from tanks supplied with L- and S-strain rotifers displayed the blue/green sheen, whilst none of the larvae sampled from any of the other tanks showed this colouration. In addition, at four days post first-feeding, larvae from tanks supplied with *Paracalanus* nauplii and copepodites still showed a good escape response, vigorously swimming away from the siphon pipe. At this stage, larvae from all the other tanks were listless and showed little or no escape response. No larvae offered *Artemia* nauplii survived beyond six days after first-feeding, whilst those supplied with other food types did not survive for longer than 9 days.

At first-feeding, average larval total length was 3.63 ± 0.15mm. Total length measurements were pooled for larvae fed each food type, since with the exception of larvae fed a combination of L-strain rotifers with *Paracalanus* nauplii and copepodites four days after first-feeding, there was no significant difference between the variances for each of the food type replicates (p>0.05; F distribution; Zar, 1984). Average total length was unchanged 2 days after first-feeding for larvae fed each food type, but decreased thereafter despite the fact that some of the larvae had successfully ingested food particles of various types. The decrease in total length after first-feeding for larvae fed three of the food types (copepod nauplii and copepodites, a combination of L-strain rotifers with copepods or L-strain rotifers) is illustrated in Figure 6.2a.

The response of larvae fed the other food organisms was similiar, there being no significant difference between larval total lengths for any food type at each sampling time (t test; P>0.05; Zar, 1984). Although differences were not significant, larvae fed copepod nauplii and copepodites showed the slowest decrease in total length compared to those fed the other food types, whilst those fed *Artemia* nauplii only decreased in total length most rapidly and also showed the largest variation.

Average larval dry weight at first-feeding was 0.031mg. Dry weight decreased rapidly up to four days after first-feeding, beyond which the rate of decrease diminished. Because larvae were weighed together on a single gauze mat, variations in dry weight were not determined, so no statistical test could be applied. The average dry weight for larvae fed three of the food types is illustrated in Figure 6.2b. The response of larvae fed the other food organisms appeared similiar. Again, those fed *Paracalanus* nauplii and copepodites showed the slowest decline in dry weight when compared to those fed other organisms.

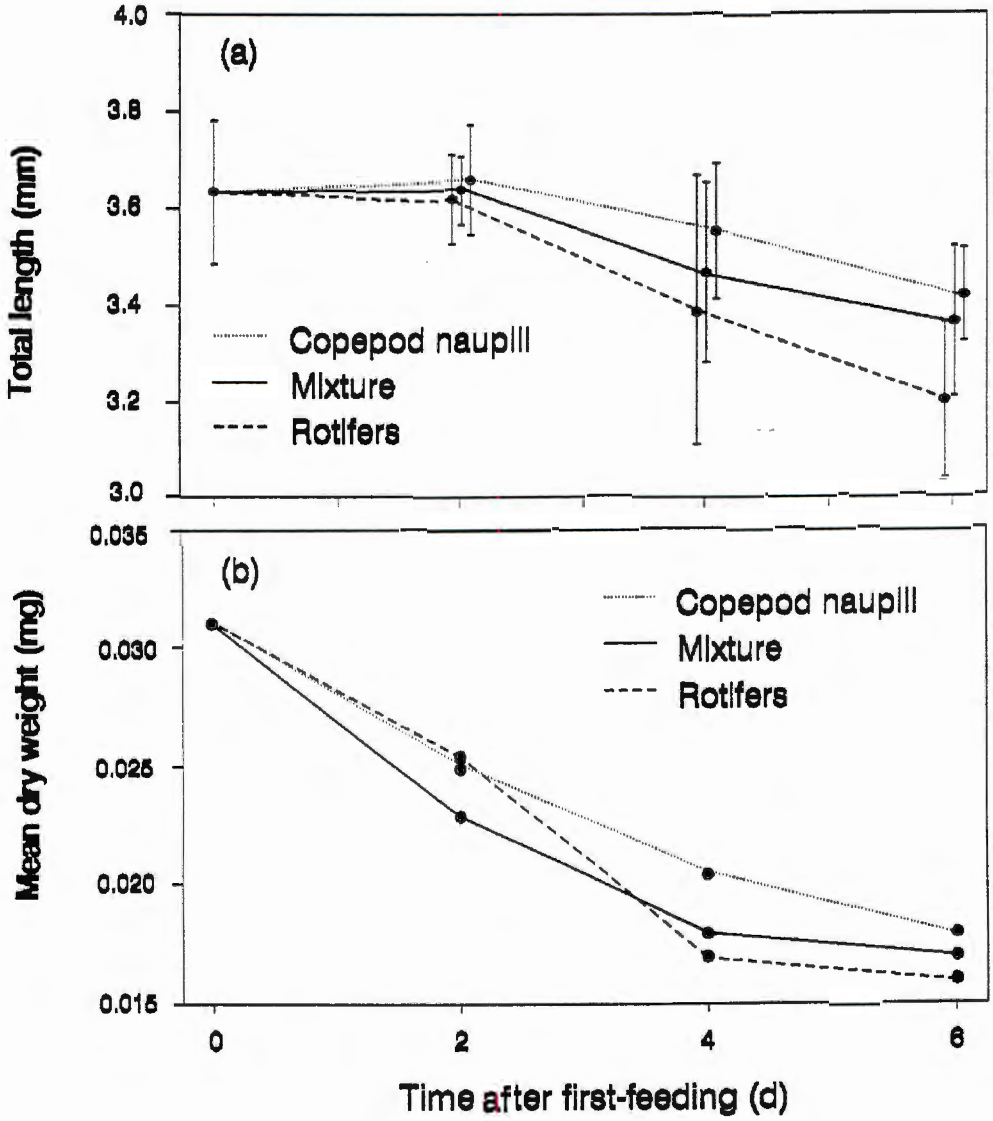


Figure 6.2: Mean total length (a) and dry weight (b) at various intervals after first-feeding for *C. capensis* larvae offered either copepod nauplii and copepodites, L-strain rotifers, or an equal mixture of the two. Standard deviations are indicated by vertical bars in (a), n = 20 for all points.

6.3.3: Rearing trial #3

Water quality parameters measured did not differ significantly ($p>0.05$; t-test, Zar, 1984) between tanks during the experimental period. Temperature ranged from 16.4° to 18.0°C, dissolved oxygen from 7.95 to 9.80ppm, pH from 7.40 to 7.68 whilst salinity remained constant at 33ppt. At hatching, larval densities were 8.8, 30.1 and 46.7 per litre in the 10, 30 and 50 eggs/l tanks respectively (Figure 6.3).

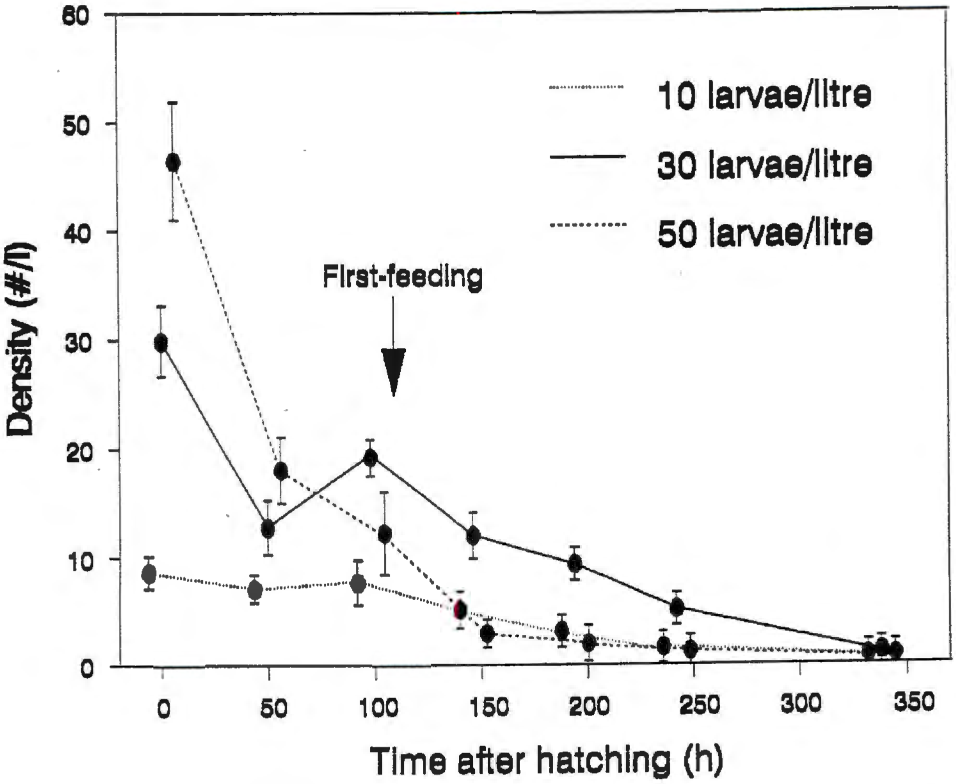


Figure 6.3: Larval density at various times after hatching for *C. capensis* maintained at three different densities. The mean and standard deviation of three samples from each tank is shown.

After 2 days densities in the 30 and 50 eggs/l tanks had decreased rapidly, with larval numbers in the latter dropping most dramatically. By 6 days post-hatching density in the 50 eggs/l tank was reduced to 3.3 larvae/litre, lower than that in the 10 eggs/litre tank at the same time. Density in the 30 eggs/litre tank decreased less rapidly up to first-feeding (4 days), after which time it decreased steadily. Larval density in the 10 eggs/l tank was virtually unchanged up to first-feeding at which time it was 7.9 larvae/litre. Thereafter density declined steadily until termination of the experiment.

Total length did not differ significantly (t-test; p>0.05; Zar, 1984) between larvae from the different tanks, although those from the 50/l tank were slightly smaller at first-feeding. Mean dry weight did not appear to differ between tanks, although statistical analysis could not be conducted due to the sampling technique used.

6.3.4: Rearing trial #4

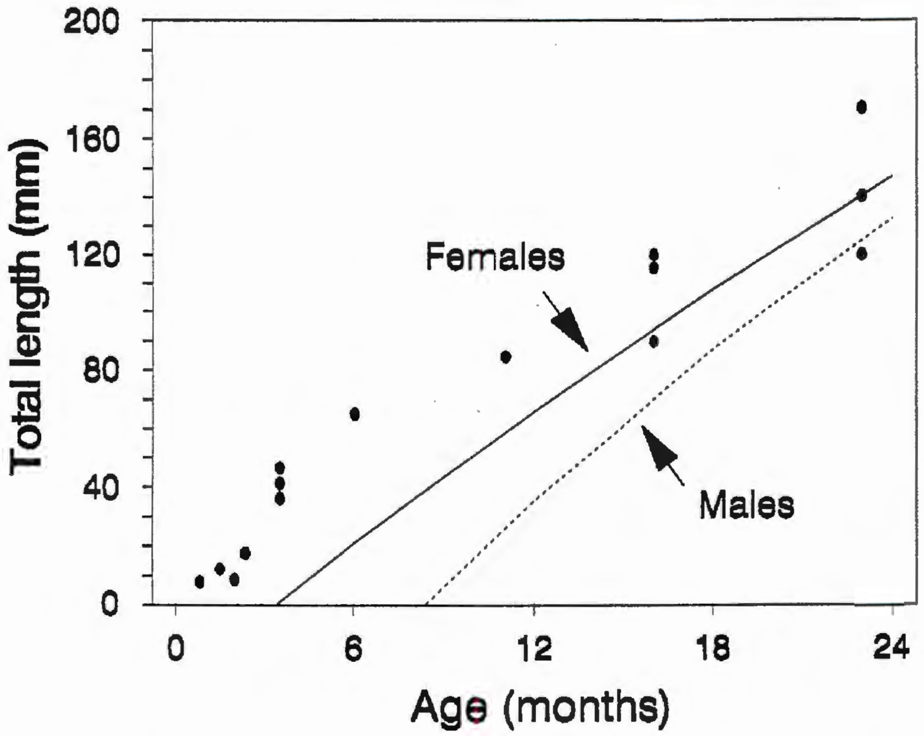
Attempts to rear larvae in containers having volumes of less than 60 litres invariably resulted in 100% mortality within 12 days after hatching. When reared in larger tanks, larvae survived for longer periods, with mortality being characterized by two distinct periods of mass mortality. The first mass mortality occurred 4-8 days after hatching and was undoubtedly linked to the failure of most of the larvae to successfully feed upon the offered organisms. After this first period larval numbers were much reduced, and estimates of larval survival indicated that only 10-30% of the original number remained. A second period of high mortality was observed 12-15 days after hatching, by which time percent survival was around 0-5%. It is probable that this second period of mortality was due to 'nutritional inadequacies' of the food organisms consumed by larvae which had successfully initiated feeding and survived the first period of mass mortality.

The survival of larvae fed w3-HUFA enriched food organisms did not appear to be improved compared to those fed other food organisms, since mass mortalities occurred at comparable times regardless of food type. In one experiment in trial #4 larvae survived for two days longer in static 5l beakers containing *Chlorella* than those in water without the algae, suggesting that the algae conferred some advantage to the larvae enabling them to endure slightly longer. In a few instances, larvae managed to survive for up to 30 days post hatch, although this was only in the 800l rearing tanks.

6.3.5: Rearing trial #5

Surprisingly, those few larvae which survived to metamorphosis (n = 7) did so in the uncontrolled and unmonitored portapools. Metamorphosis appeared to be completed by approximately 45 days post hatch. Post-metamorphosis galjoen adopted a semi-benthic lifestyle, remaining very close to the bottom and sides of the portapool and seldom venturing into the water column unless to feed upon just-added food. Juvenile galjoen also proved to be aggressive, with larger juveniles often observed attacking smaller ones. In two cases harassment by larger toward smaller juveniles resulted in death of the smaller fish. Juvenile galjoen fed well upon various assortments of live and frozen copepods and *Artemia* juveniles and adults of up to 8mm in length.

Approximately three months after metamorphosis, juveniles were easily weaned onto an artificial diet similiar to that used to maintain adult galjoen and described in Chapter 3. Estimates of total length of the four surviving juveniles at various ages were made by photographing the fish next to a ruler suspended in the rearing tank and are given in Figure 6.4, together with the growth equation for feral galjoen given by Bennett and Griffiths (1986).



<u>Figure 6.4</u>: Growth of juvenile *C. capensis* reared in the laboratory. Estimated total length for four individuals at various ages are plotted with the predicted growth curves for male and female galjoen given by Bennett and Griffiths (1986).

This predictive growth equation was derived using fish of 120mm TL and longer, and thus extrapolations to less than this size are inaccurate. By forcing Bennett and Griffiths (1986) equation through the hatching length of 2.04mm TL, the curve becomes aligned with the TL estimates given in Figure 6.4. Thus it would appear that galjoen grown in captivity exhibit a similiar growth rate to that of their feral counterparts. Unfortunately, mortality of the juvenile fish due to system failure and theft in the case of the last survivor, meant that further estimates of growth under culture conditions could not be determined.

6.4: Discussion

According to Struhsaker *et. al.* (1973), the presence of pathological bacteria in culture systems is equally important as nutrition in affecting early larval survival. Reduction of bacterial populations through the use of various antibiotics or ultra-violet sterilization resulted in increased hatching success (Oppenheimer, 1955) and larval survival (Struhsaker *et. al.*, 1973; Shelbourne, 1975) for several marine species. Ultra-violet light is reported to be totally bacteriocidal when seawater is filtered and passed through a UV-light unit (Struhsaker *et. al.*, 1973; Uys, 1986). Gatesoupe (1982) found that the survival of larval turbot, *Scopthalmus maximus*, was markedly improved when they were fed rotifers that had ingested a sulphadiazine antibiotic. At a recent workshop on water quality maintenance in intensive aquaculture, Brownell (1986) highlighted the importance of microbes (bacteria, fungi, viruses and some algae) in modifying the culture environment through the release of ectocrines and hence adversely affecting the growth and survival of cultured organisms. Bacteria and other biofouling organisms were considered by Walmsley *et. al.* (1986) to be the two most important biological factors influencing a culture environment.

The results of trial #1 would seem to indicate that either galjoen eggs and larvae are remarkably resistant to bacterial infection, or that the culture system used in this instance did not contain significant numbers of bacterial pathogens. However no attempt was made to count the numbers of bacteria present. The antibiotic employed, oxytetracycline hydrochloride, has been successfully used to combat *Aeromonas* infections (Christensen and Larsen, 1983; James *et. al.*, 1988), was found to significantly suppress a mixed bacterial community consisting mainly of *Pseudomonas* (Struhsaker *et. al.*, 1973) and is considered one of the most effective and frequently used antibiotics in fish farming (Alderman, 1988). However Struhsaker *et. al.* (1973) found that this antibiotic was toxic to larval *Caranx mate* at a concentration of 50 ppm. The dosage of 1.6mg/l used in this experiment would be equivalent to an active ingredient dose of <0.1 ppm; no signs of toxic effects were exhibited by the galjoen larvae.

Since larval nutrition is crucial to survival, optimization of feeding regimes is of the utmost importance for successful rearing. As was discussed in the Chapter 5, the timing of the provision of live food for first-feeding marine larvae is critical. This is especially true for galjoen larvae which are highly vulnerable to starvation and show a short time to the point of no return. A second factor which is equally crucial to larval survival is that of food type. At present, most marine larvae are cultured on live foods, at least for the first few weeks after hatching (May, 1973; Girin, 1979). Typically, live food organisms should be easy to capture, small enough to be successfully ingested, fulfill the nutritional requirements of the larvae and be present in sufficient numbers to ensure high availability to the larvae. Due to the enormous numbers required, live food organisms should also be simple to culture and mass produce.

Two organisms, in particular, are used worldwide as live food for first-feeding marine larvae; the rotifer *Brachionus plicatilis*, and nauplii of the brine shrimp *Artemia franciscana*. Both fulfill most of the required criteria, being slow moving, relatively small and easy to mass produce (Theilacker and McMaster, 1971; Sorgeloos, 1980). Other live food organisms used include wild plankton assembleges consisting mainly of copepods (Checkley, 1982), the protozoan *Fabrea salina* (De Winter *et. al.*, 1975), the copepod *Tigriopus japonicus* (Lee *et. al.*, 1981) and oyster larvae (Garratt *et. al*, 1990). A complete list of the most commonly used organisms for larval feeding has been given by May (1970).

The results obtained in trial #2 demonstrate that first-feeding galjoen larvae were able to successfully capture and ingest all the live food organisms offered to them, with the exception of *Artemia* nauplii. However, the decrease in both total length and larval dry weight, and the fact that few larvae survived longer than 9 days after first-feeding, suggest that these food organisms may have been nutritionally inadequate. Larvae exhibited characteristics typical of starvation despite being able to ingest the various food organisms. The larvae of several local species including hottentot, *Pachymetopon blochii*, zebra, *Diplodus cervinus*, blacktail, *Diplodus sargus capensis*, the Cape sole, *Heteromycteris capensis*, and the lace sole, *Synaptura kleini*, have been successfully reared to metamorphosis using *Brachionus* as the single food source at first-feeding (Brownell, 1979). Thus the failure of galjoen larvae to survive on *Brachionus* or any of the other food organisms offered indicates that this species may have more stringent nutritional requirements than those listed above.

Recent work to assess the nutritional quality live food organisms used in larval production of marine fish has shown that the class of fatty acid contained in the food organism is the principal factor in determining dietary value (Watanabe *et. al.*, 1978a; 1978b). Fujita *et. al.* (1980) demonstrated that *Artemia* could be classified into two types on the basis of fatty acid composition. The first type, known as the freshwater type, contained large amounts of linolenic acid (18:3w3), an essential fatty acid (EFA) for freshwater fish (Watanabe *et. al.*, 1978b). The second *Artemia* type (termed the marine type) contained large amounts of highly unsaturated fatty acids (HUFA), mainly eicosapentaenoic acid (20:5w3) and docosahexaenoic acid (22:6w3), both of which have been identified as EFAs for marine fish (Fujii and Yone, 1976; Watanabe *et. al.*, 1978b). Survival of red seabream, *Pagrus major*, larvae fed *Artemia* nauplii of the marine type was markedly higher than those fed nauplii of the freshwater type, which exhibited lethargy leading to mass mortality approximately 1 week after initiation of feeding (Fujita *et. al.*, 1980). It has been suggested that reports of mass mortalities of various marine larvae fed brine shrimp nauplii only have been due to a w3-HUFA deficiency in the food organisms (Watanabe *et. al.*, 1983).

Techniques to increase the EFA content of live food organisms used for marine larvae have recently been developed, and include indirect and direct methods of EFA enrichment. In the indirect methods, live food organisms are grown using a culture medium rich in w3-HUFAs such as enriched yeast or marine *Chlorella*, before being offered to marine larvae (eg. Imada *et. al.*, 1979; Kitajima *et. al.*, 1979, 1980; Fukusho *et. al.*, 1980). A further indirect EFA-enrichment technique involves the secondary feeding of live food organisms with microcapsules containing a high percentage of total lipids (eg. Teshima *et. al.*, 1981; Sakamoto *et. al.*, 1982; Walford and Lam, 1987). Direct methods of EFA enrichment involves the application of lipids containing w3-HUFA to live food organisms (eg. Watanabe *et. al.*, 1982; 1983b).

Cuttlefish liver oil, the lipid used in trial #4 to directly enrich live food organisms, has been successfully used in the past (Imada *et. al.*, 1979; Kitajima *et. al.*, 1980; Watanabe *et. al.*, 1982). In this instance direct enrichment did not increase the survival of galjoen larvae. However, the fatty acid composition of the lipid was not determined, and it is possible that eicosapentaenoic and docosahexaenoic acids were either lacking completely, or were present in too low concentrations to allow the food organisms to take up sufficient to be of benefit to the larvae. It is also possible that galjoen larvae have a specific requirement for another type of fatty acid altogether. Similiarly, indirect enrichment attained by culturing rotifers on *Chlorella* did not markedly increase larval survival. Despite the widespread use of algal-fed *Brachionus* and *Artemia*, Dendrinos and Thorpe (1987) have suggested that these are far from ideal for the purpose of commercial rearing of marine fish larvae, and probably lead to serious dietary deficiencies.

Attempts to rear marine larvae using microcapsules as the sole food source have revealed that first-feeding larvae are unable to digest the microcapsules (Kanawaza *et. al.*, 1982). It has been suggested (Dabrowski, 1979 <u>in</u> Walford and Lam, 1987; Vu, 1983) that the initial digestion in marine larvae proceeds by the action of enzymes present in the natural food ingested, i.e. by way of autolytic proteolysis. Thus EFAs contained in the microcapsules must be conveyed to the larvae via live food organisms which are able to digest the capsules before being digested in turn by the larvae. The results of Kanawaza *et. al.* (1982) notwithstanding, some marine larvae have been reared on artificial diets directly from first-feeding. These include sea bass, *Epinephalus tauvina* (Barnabe, 1976 <u>in</u> Girin, 1979), sole, *Solea solea* (Gatesoupe *et. al.*, 1977 <u>in</u> Girin, 1979) and plaice, *Pleuronectes platessa* (Kirk and Howell, 1972; Adron *et. al.*, 1974). However, these authors found that using artificial diets resulted in lower survival and growth compared to larvae reared on live foods.

Recent studies on marine larval nutrition have suggested that biochemical analysis of the nutrients utilized in endogenous feeding, ie the yolk, will identify the probable nutrients required for exogenous feeding (Dendrinos and Thorpe, 1987; Fyhn, 1989). Fyhn (1989) has further suggested that free amino acids (FAA) are an important energy source during embryonic development of marine fishes, and that an exogenous supply of FAA's is necessary when larval reserves are depleted. Since the digestive tract of marine larvae is often morphologically and functionally incomplete when they initiate exogenous feeding (Govoni *et. al.*, 1986 in Fyhn, 1989; Ferraris *et. al.*, 1987), FAA's from ingested food could provide an indispensible source of energy. Characterization of the fatty acid and amino acid composition of the yolk of developing larvae should be the first step in determining the suitability of various food particles as live food for first-feeding marine larvae.

Several studies to determine the effects of food organism concentration on the survival and growth of marine larvae have been conducted (Houde, 1975, 1978; Roberts *et. al.*, 1978; Werner and Blaxter, 1980; Tandler and Sherman, 1981; Tandler and Mason, 1983). A positive asymptotic response of both survival and growth with increasing food concentrations was reported for for larval sea bream, *Archosargus rhomboidalis* (Houde, 1975), and for larval herring, *Clupea harengus* (Werner and Blaxter, 1980). A different response was reported for larval gilthead bream, *Sparus aurata*, which showed highest survival and growth at mid-range food concentrations of 10-15 organisms/ml (Tandler and Sherman, 1981; Tandler and Mason, 1983). These authors suggested that low survival and growth at high food concentrations was due to the accumulation of metabolites excreted by the food organisms in the semi-closed systems used. In culture systems with a rapid turnover rate gilthead bream survival was proportional to food concentration (Tandler and Sherman, 1981). Food organism densities of 10-15/ml used in the galjoen larvae rearing trials described here were deemed appropriate, since although the culture systems used were flow-through, the water turnover rate was relatively low. In addition, the vast amounts of live food organisms required to maintain the required densities was often difficult to achieve. Production of sufficient food organisms to maintain densities in larval tanks of above 20/ml would have been virtually impossible given the culture facilities used.

Experiments examining the effects of stocking density on larval growth and survival have produced inconsistent results. High survival at initial densities of 60 eggs/l was reported for plaice, *Pleuronectes platessa* (Shelbourne, 1975) and bream, *Acanthopagrus cuvieri* (Teng *et. al.*, 1982). Houde (1975) observed that survival and growth of larval sea bream, *Archosargus rhomboidalis*, decreased with increasing stocking densities from 2 to 32 larvae/l over a range of food densities. Similiarly, Roberts *et. al.* (1978) found growth of larval red drum, *Scianeops ocellata*, to be greatest at a density of 2 larvae/l. Houde (1975) reported that larval survival in laboratory rearing experiments was seldom high at densities of more than 10 larvae/litre. He further suggested that inhibition of larval growth and survival at high densities was not due to increased metabolite levels, but was primarily due to competitive interactions between larvae. At high densities larvae appeared to spend more time avoiding each other than feeding. Shelbourne (1975) proposed that the establishment of hierarchial patterns amongst larvae at high densities could significantly increase mortality. The results from trial #3, where high initial larval densities were followed by a drastic reduction in numbers even before first-feeding, contradicts the suggestion of Houde (1975), since mortality in the elevated larval density tanks was high before first-feeding had begun. In addition, prior to first-feeding, larvae were generally passive and did not exhibit any competitive interactions. These results suggest that increased metabolite levels were responsible for reduced larval survival.

Brownell (1980a; 1980b) described the water quality requirements for successful first-feeding in marine larvae, and considered that un-ionized ammonia was potentially the most hazardous water quality parameter in a culture situation. Ammonia is the principal nitrogenous end product excreted by fish (Forster and Goldstein, 1969), and under culture conditions of high fish density can accumulate to produce changes in water chemistry rarely encountered in the natural habitat. Ammonia toxicity is increased by elevated pH and temperature, and by lowered dissolved oxygen concentrations and salinities above and below blood isotonicity (Holt and Arnold, 1983). Total ammonia concentrations of 1.4 to 3.4 mg/l Nitrogen were found to decrease feeding success in first-feeding marine larvae; levels of this magnitude can be rapidly attained in overloaded culture systems

(Brownell, 1980a).

Of the other water quality parameters, nitrite and nitrate have been considered non-toxic at levels likely to be encountered in marine larval culture systems (Brownell, 1980a; Holt and Arnold, 1983). Dissolved oxygen concentrations of less than 5.5 mg/l and pH levels of below 6.0 and above 8.4 were found to reduce larval first-feeding success (Brownell, 1980b). Survival of mullet, *Mugil cephalus*, larvae was significantly reduced at dissolved oxygen concentrations of less than 5.4 ppm (Sylvester and Nash, 1975). In trial #3 the pH in all tanks was within the range determined by Brownell (1980b) as being suitable for first-feeding marine larvae, whilst dissolved oxygen levels did not fall below 7.95 ppm.

Salinity and temperature are key environmental factors influencing the survival and growth of marine fish larvae (Akatsu *et. al.*, 1982). In a culture situation, salinity is generally the most unregulated and uncontrolled environmental parameter (Nash and Koningsberger, 1981). After reviewing the effects of salinity on teleost eggs and larvae, Holliday (1969) concluded that larval survival in many species would be increased at salinities iso-osmotic with larval body fluids, generally 10-16 ppt. Studies by Liao (1971 in Nash and Shehadeh, 1980) and Freddi *et. al.* (1981) have supported this view, reporting a higher larval survival at reduced salinities for mullet, *Mugil cephalus* and gilthead seabream, *Sparus aurata* respectively. Akatsu *et. al.* (1983) found that starved early larvae of brown spotted grouper, *Epinephalus* *tauvina* exposed to salinities of 8-24 ppt survived longer than those at higher salinities. Although no experiments were performed to investigate the effects of either salinity or temperature upon survival and growth of galjoen larvae, observations from the various rearing trials revealed that larval survival decreased fairly rapidly at temperatures of 22°C and above. Thus it would appear that early larvae are less tolerant of raised temperatures than developing embryos, since embryonic survival was high at temperatures up to 26°C (see Chapter 5). In a culture situation temperature must be kept within the temperature tolerance range of the species. Although the practice of maintaining raised temperatures to promote higher growth rates appears appealing, it may prove to be cost-ineffective.

The effect of tank size on the success of rearing marine larvae has been noted by many workers, who have reported that large culture tanks generally increase growth and survival (Popper *et. al.*, 1973; Jones *et. al.*, 1974; May *et. al.*, 1974; Hussain and Higuchi, 1980). This has been ascribed to the conservative characteristics of large water volumes, which reduce fluctuations in temperature, salinity, pH and dissolved oxygen (Popper *et. al.*, 1973). In the various experiments comprising trial #4 this definitely appeared to be the case, since no galjoen larvae survived for longer than 12 days when reared in small-volume containers, but survived for longer periods of time when reared in larger containers. Although it is probable that rapid water quality fluctuations do occur in small rearing tanks and are the major cause of lowered survival, it is possible that an increased incidence of mechanical damage due to the larvae more frequently coming into contact with the container walls may also be responsible for high mortality. The poor survival of larvae in trial #2, where mortality was 100% by 9 days after hatching in all tanks, is probably due in part to the small size of the experimental containers used. However, this does not necessarily invalidate the results obtained, since the experiment was primarily designed to assess which food organisms could be successfully captured by first-feeding galjoen larvae.

The increasingly widespread practice of rearing marine larvae in "green water" (water with a high concentration of planktonic microalgae such as *Chlorella* or *Dunaliella*) has been supported by a number of authors (Harada, 1970; Nash and Kuo, 1975; Alderson and Howell, 1983; Olesen and Minck, 1983) who reported increased survival and growth of cultured larvae. Reasons for this increase have been attributed to the following mechanisms;

1) phytoplankton release oxygen and therefore maintain high DO levels,

2) phytoplankton remove metabolites (eg ammonia) which otherwise might reach toxic levels,

3) some phytoplankton species release antibiotics which inhibit bacterial growth,

4) live food organisms can feed on the phytoplankton and are therefore more nutritionally valuable to larvae, and

5) larvae may be able to feed directly on the phytoplankton.

In rearing trial #4 the presence of algae in the rearing tanks did not increase larval survival. Furthermore, the algae tended to settle in the water column fairly quickly despite gentle aeration, resulting in a build-up of detritus at the bottom of the tank. Algae was also found to clog the outlet filters, resulting in reduced water turnover rates until such time as the filters were changed.

6.5: Conclusion

The results of the rearing trials have shown that galjoen larvae are exceptionally difficult to rear. Despite showing a high success at ingesting most of the food types offered at first-feeding, few larvae survived beyond two weeks after hatching. This could indicate that galjoen larvae have exceptionally stringent nutritional requirements, which were obviously not met by any of the food organisms offered.

CHAPTER 7: CONCLUSIONS

The results obtained from this study can be summarized as follows:

1) Galjoen is a widely known table fish regarded as excellent eating. A particular market for fresh galjoen has been identified as the restuarant trade in the southwestern Cape. Although not accurately determined, demand for galjoen is high, and would require approximately 50 tonnes per year to supply 25 restaurants. The estimated value of this demand is R380 000 per anum. In terms of economic criteria, galjoen ranks as a high potential candidate species.

2) Adult galjoen are easily maintained in captivity. Although occasional aggressive interactions between adults were observed, this did not happen often enough to be considered a problem. A method allowing the sex of live galjoen to be determined has been elucidated, and can be successfully applied both during, and from several months prior to the natural spawning season.

3) Natural spawning of galjoen occurs readily in captivity when conditions approximate natural spawning-season conditions. Galjoen are serial spawners, with an average time between spawns of approximately 2 weeks. In addition, galjoen are an extremely fecund species, a single female being capable of producing in excess of 2 million eggs per spawning season. Galjoen do not respond well to either of the two gonadotropins tested (HCG and PMSG) to induce spawning; spawn products generally being unviable due to too rapid development of the oocytes. The efficiacy of

second generation techniques using various gonadotropin releasing hormones or steroids to induce spawning needs to be examined.

4) The development of fertilized galjoen eggs is temperature dependent, developmental rate increasing with increasing temperature. Eggs were found to be tolerant of temperatures ranging from 16° to 26°C; at temperatures of 14°C and less however, 100% mortality was encountered. Newly-hatched larvae exhaust their endogenous food reserves rapidly, and show a short period to time of irreversible starvation. Based on the sequence of development, larvae begin to feed from 110h (at 18.0°C) after hatching. Because of their small mouth size at first-feeding, larvae require very small live food organisms.

5) Galjoen larvae were found to be extremely difficult to rear. Trials using a variety of live food organisms, culture containers of varying volumes, UV sterilized and antibiotic treated water and "green water" culture systems were unsuccessful in rearing larvae to metamorphosis. Mass mortalities of larvae were observed at first feeding and again at approximately 1 week after the introduction of food. On several occasions, dead larvae with food in their stomachs were found. Larvae that grew to metamorphosis and beyond did so in culture tanks that were not controlled or monitored. Growth rates of the few larvae that survived beyond metamorphosis suggested that growth under culture conditions was similiar to that in the natural environment.

The fact that galjoen larvae were found to be difficult to rear greatly reduces the potential of this species for culture, since commercial culture of any organism is dependent upon a reliable and sufficient supply of seed (Hecht, 1985). Most finfish mariculture systems have found successful larval rearing to be the most difficult culture aspect to achieve (Shepherd, 1988).

The results of this study suggest that larval nutrition is the single most important factor in successful rearing of galjoen larvae, since these larvae appear to have extremely stringent nutritional requirements. The food organisms offered to galjoen larvae were most probably lacking in some essential nutrient, which could be w3-HUFAs.

A second factor that substantially reduces the suitability of this species for culture is the slow growth rate exhibited by those juveniles that survived under culture conditions to metamorphosis and beyond. The results presented in Chapter 2 indicate that restaurateurs would prefer to receive fish of approximately 400mm TL. In the natural environment, female galjoen require 6.5 years whilst males take 8 years to achieve this length (Bennett and Griffiths, 1986). Since the growth rate of cultured galjoen was no different to that shown by their feral counterparts, a period of at least 5-6 years would be required to rear fish to this size, assuming that the difficult problems encountered in larval rearing trials were solved to allow sufficient numbers of larvae to be reared beyond metamorphosis. Even if the market size was reduced to 300mm or 200mm TL, a culture period of 4 or 3 years respectively would be required.

Obviously, the cost of maintaining fish for 3-4 years before marketing would be prohibitive. In addition, the culture facility would have to be run for the first 3 years without any financial return, and this would drastically reduce the economic viability of such a venture.

The conclusion that can be drawn from this study is that galjoen is unsuitable as a mariculture species. This is primarily due to the difficulties experienced in larval rearing, although it is probable that these could be overcome using sophisticated techniques of food enrichment. However, these techniques would increase running expenses and this, together with the fact that producing market-size galjoen would require an unacceptably long ongrowing phase, would certainly increase operational costs to the point where production would be economically unviable.

CHAPTER 8: MARICULTURE IN SOUTH AFRICA; A PROGNOSIS

The mariculture industry in South Africa lags behind that of other technologically developed nations, especially with regard to finfish culture. The historical abundance of cheap fish from the sea is no doubt the primary reason, but the relatively low emphasis on fundamental aquacultural research until the last decade has probably been a contributing factor. In addition, complex and poorly-known laws and regulations pertaining to the use of the sea, inadequate information on the long-term demand for mariculture products and a lack of information on suitable culture sites have hampered the full development of mariculture locally (Bruton, 1986).

It is possible that galjoen larvae could be successfully reared once their nutritional requirements were accurately determined using the biochemical analysis of endogenous nutrients suggested by Dendrinos and Thorpe (1987) and Fyhn (1989). Assuming these requirements could be met under culture conditions, survival rates would no doubt improve since nutrition is perceived to be of critical importance to early survival, possibly more so than for other species. However, at this early stage of development of finfish mariculture in South Africa, it is felt that a species which would not require such a high level of technological input would be better suited to being the pioneer endemic finfish species produced on a commercial scale. This is suggested to allow the fledgling finfish mariculture industry that much more of a chance of commercial success, since an expensive first mistake in choice of species would undoubtly prejudice the industry against similiar attempts with other species. In addition, high-technology operations invariably have expensive production costs, although this would be partially offset in this case by the high economic returns that could be realized by large-scale production of galjoen.

The development of finfish mariculture in South Africa requires the comprehensive assessment of several candidate species for culture. Whilst this study has indicated that galjoen does not show a high potential for culture, more promising results have been obtained by Garratt *et. al.* (1989) working with santer seabream (*Cheimerius nufar*). Santer larvae were reared through to juveniles of 5+ months from naturally spawned eggs of captive fish. Although the best survival obtained was only 0.5%, Garratt *et. al.* (1989) concluded that *C. nufar* was a potentially valuable species for culture.

Other local species which have been assessed for their suitability as mariculture candidates include white steenbras (*Lithognathus lithognathus*) and leervis (*Lichia amia*). Harris (1991) found that white steenbras showed efficient utilization of a commercial trout pellet diet, exhibiting absorption efficiencies of 86-98%, food conversion of 21.3-32.6% and specific growth rates ranging from 0.19 to 0.60% body weight per day. Based on a bioenergetic model derived from these results, Harris (1991) concluded that white steenbras was a suitable mariculture species provided that growth rates could be improved. Loubser (1990) reported absorption efficiencies of 79-94% and a high scope for growth for leervis fed a commercial diet, and suggested that these results demonstrated that this species may also be suitable for mariculture. Both white steenbras and leervis thus show good potential for growth under culture conditions. However, further research aimed at determining the feasability of production of larvae and consequent mass rearing needs to be conducted in order to fully assess the suitability of these species for mariculture.

Another locally-occurring species that is percieved to show a very high potential for mariculture in South Africa is the blacktail, *Diplodus sargus capensis*. Like santer, blacktail belong to the Family Sparidae, several members of which are currently being cultured in various parts of the world (Girin, 1983). Although one of the more common inshore fishes along South Africa's coastline, blacktail is virtually unexploited commercially, with negligible amounts being taken by line, speargun or beachseine. Whilst as a commodity blacktail would be competing with products from the linefish industry, continual production of this species would ensure a year-round supply. This would be especially advantageous during the winter months, when linefish catches are markedly reduced. In addition, the recent rapid increase in linefish prices means that alternative sources of fresh fish must be found in order to meet the growing demand for this product.

Blacktail have been reared both locally (Brownell, 1979) and elsewhere, including France (Kentouri and Divanach, 1982) and Italy (Mazzola *et. al.*, 1983). Brownell (1979) reported rearing at least 10 batches of larvae through metamorphosis up to an age of 6 months, and found that first-feeding larvae fed readily and showed high survival on both *Brachionus* and *Proales*, without requiring w3-HUFA enrichment. Successful induced spawning using HCG and resulting in a 60% hatch rate was reported by Mazzola *et. al.* (1983), who reared juveniles to a weight of 51g and length of 135mm in 9 months with a survival of 5.4%. In addition to being relatively easy to rear, the biology of local populations of blacktail is well documented. Estimates of growth rate have been reported by Smale and Buxton (1989) who found that juvenile blacktail attained 100-120mm in their first year. Sexual maturity is attained at a length of approximately 160mm (van der Elst, 1981) with spawning occurring all year round, peaking from October to December (Coetzee, 1986). Blacktail exhibit protandrous hermaphroditism, functioning first as males and later in life as females, although adult fish generally possess both testicular and ovarian material in their gonads (Micale *et. al.*, 1985; Coetzee, 1986).

Blacktail thus fulfill most of the biological criteria required for culture, being easy to rear, showing rapid growth rates and being amenable to sophisticated techniques such as hormone-induced spawning. Since a well-developed Mediterranean market for blacktail exists, where prices of at least US $10/kg are attained (Girin, 1983), the potential for export of locally-produced blacktail is extremely high. Given South Africa's current economic state with its exceptionally poor exchange rate, any enterprise producing exportable goods is virtually assured of financial success. Culture of blacktail in South Africa would therefore appear to be extremely feasible, since production could be exclusively destined for foreign markets. In view of these facts, it is felt that pilot-scale production of blacktail should be undertaken immediately. The results obtained from such a study could lead to blacktail becoming South Africa's first large-scale commercially cultured marine fish.

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