Induced Spawning and Culture of Yellowfin Bream, *Acanthopagrus australis* (Günther, 1859) and Mangrove Jack, *Lutjanus argentimaculatus* (Forsskål, 1775)

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FRONTISPIECE: **Top**: A 6 kg male mangrove jack broodfish used in spawning induction trials.

Bottom: A pair of yellowfin bream broodfish (male upper, 240 g; female 310 g) used in spawning induction trials.

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Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Ken Cowden
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Ethics Statement

This research was conducted within the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Ethical clearance was granted by the James Cook University Experimentation Ethics Review Committee, approval number A167.

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Abstract

This study aimed to investigate the biological characteristics of the yellowfin bream, *Acanthopagrus australis*, and mangrove jack, *Lutjanus argentimaculatus*, relevant to their consideration for aquaculture. All stages of the production cycle were considered, except growout of mangrove jack owing to poor larval rearing success. The study was conducted on fish populations from the Townsville region of far north Queensland.

The yellowfin bream spawning season was found to extend for a period of at least 10 weeks, from mid June to early September. Data confirmed this species to be protandrous, maturing as males in the first year and changing to females at approximately 21-27 cm total length. Females are serial spawners with an 'asynchronous' ovary. The synthetic hormone LHRHa (des-gly^{10}, D-ala^{6}, pro^{9}-ethylamide) was capable of reliably inducing spawnings in mature females when administered in aqueous or pelletised form, whereas the hormonal preparation 'Ovaprim' was less effective. A minimum aqueous dose of 15-20 μg/kg LHRHa was necessary to reliably induce spawning, and at a dose of 40 μg/kg in an 85% cholesterol/15% cellulose pellet, multiple spawnings on consecutive nights were possible.

Yellowfin bream spawned in the late evening, after a latent period of approximately 45 h at 22°C. Single spawnings of over 100,000 eggs were observed from females of approximately 500 g, and a seasonal fecundity of at least 1.6 million eggs/kg female body weight was estimated. Eggs were spherical, transparent, pelagic and positively buoyant, and were apparently of good quality, generally showing high fertilisation and hatching rates. Mean egg and oil globule diameters were 786.8 ± 19.7 μm, and 186.2 ± 7.4 μm, respectively.

The incubation period ranged from 22.5-44.2 h over the temperature range 19.4-27.7°C. Mean larval total length and yolk volume at hatch were 2.03 mm and 0.116 mm^3, respectively. Total length at first feeding was 3.15 mm, and mouth width at this stage would indicate an optimum food width of 90-100 µm. The temperature and salinity optima for eggs and yolksac larvae, at which survival, growth and yolk utilisation efficiency were maximal, and the occurrence of deformities minimal, was 22.6-23.9°C and 35 ppt salinity. Light levels in the range of 0-2000 lux did not affect yolk utilisation efficiency.

Best larval rearing results, in terms of growth, survival and swimbladder inflation rate, were obtained using the 'greenwater' technique with rotifers, *Brachionus plicatilis*, followed by brine shrimp, *Artemia* sp., as the feeding protocol. Approximately 75% survival to metamorphosis, and 77% swimbladder inflation, were recorded using this method. Swimbladder inflation occurred between days 3-4, and the final inflation rate was unaffected by light levels in the range of 0-2000 lux. Larvae underwent metamorphosis between days 24-30, at which time their mean total length was 6.51 ± 0.8 mm. Weaning onto dry artificial food proceeded without difficulty. Juveniles showed some aggression in the form of 'tail-nipping' for a short period following metamorphosis.

Amino acid analyses for larval whole-body protein and rotifers, along with fatty acid analysis of fertilised eggs and rotifers, indicated that rotifers provided adequate essential amino acid nutrition, but were very low in HUFAs, particularly in DHA. The low HUFA content of bream eggs and high survival observed on HUFA-deficient rotifers are suggestive of the ability of this species to meet its HUFA requirements through bioconversion of shorter-chain fatty acids. This demands further investigation.

Juvenile yellowfin bream adapted well to netcage conditions, accepted artificial pellet food and showed high disease resistance. Survival through the first six months in netcages was estimated at over 85%, and for the following 18 months was 81%. Growth on pellet food formulated for barramundi (51.5% protein) was not rapid, with fish reaching 15 cm (85 g) in 12 months, and 22 cm (252 g) (approaching marketable size) in 25 months from hatch. Growth slowed considerably in winter months due to decreasing water temperatures and the onset of sexual maturity. There is, however, considerable scope for improvement in growth rate, and this is discussed. The food conversion ratio, gross growth efficiency and protein efficiency ratio were 1.72:1, 0.58, and 1.13:1 respectively.

The mangrove jack spawning season, as assessed from captive broodfish, extended for at least 6 months from mid-October to early April. Mature fish are dioecious, reaching sexual maturity at approximately 2.0-2.5 kg. Female mangrove jack are serial spawners with a 'group synchronous' ovary. The synthetic hormone LHRHa was capable of reliably inducing spawning of females with mean oocyte size over the threshold of approximately 400 µm, while the hormonal preparation 'Ovaprim' had far less efficacy. A priming and resolving aqueous dose of 25 µg/kg LHRHa, given 24 h apart, proved most satisfactory. Furthermore, at dosages of 50 µg/kg, LHRHa significantly increased male milt production within 12 h of injection. Manual strip-spawning was necessary following difficulties experienced in obtaining synchronised spontaneous male and female spawnings. While administration of LHRHa as a pellet in a cholesterol/cellulose matrix was capable of inducing final egg maturation and ovulation, difficulty in estimating strip-spawn timing favoured aqueous administration of hormone.

Fertilisation rates from strip-spawnings varied from 3.8-92.8%, with mean 51.0%. The latent period between injection and ovulation, at 29-30°C, was near 36 h 20 min. A very brief window of fertilisation, of approximately 10 min, ensued, during which fertilisation was maximal, illustrating the critical nature of strip-spawn timing if high fertilisation was to be achieved. Individual spawnings varied from 8,400-2.26 million eggs with a mean of approximately 0.5 million, and total seasonal fecundity of over 1.7 million eggs/kg was estimated. Eggs were spherical, transparent, pelagic and positively buoyant. Mean egg and oil globule diameters were 823.9 ± 22.7 µm, and 158.0 ± 3.9 µm, respectively.

Incubation time at 29.0°C was 18 h 10 min, and mean total lengths at hatch and at first-feeding were 2.12 mm and 3.17 mm, respectively. Yolk absorption was complete at approximately 36 h post-hatch, and the oil globule was fully utilised at approximately 70 h. At 42 h post-hatch, larvae had pigmented eyes, an open mouth and anus, and were apparently first capable of feeding. Mouth width at this stage would suggest an optimum initial food width of approximately 75 µm. Mangrove jack larvae exhibited a very brief window of initial feeding opportunity, rapidly succumbing to starvation. Maximum yolk utilisation efficiency and survival of yolksac larvae occurred at 22 ppt salinity and 30.5-34.0°C.

Swimbladder inflation was observed between days 2-4, and rates of over 70% were achieved under 'clearwater' conditions. Six larval rearing trials were conducted, differing in their use of clearwater and greenwater techniques, and first food items offered. Screened rotifers, oyster trochophores, and screened wild zooplankton were all tested. A similar pattern of mortality was observed in all trials, whereby over 95% of larvae died between days 3-6, corresponding with the transition to exogenous nutrition, and after which complete mortality was observed by day 12.

Possible reasons for this mortality pattern are discussed, and it is concluded that while the primary cause of mortality appears to be starvation and a failure to accept exogenous food, this may be a secondary consequence of sub-optimal egg quality, physical rearing conditions, and/or the use of inappropriate initial food items. Based on similar experiences by other groups researching mangrove jack aquaculture, this species would appear innately difficult to rear due to the small endogenous energy reserves and consequent brief window of initial feeding opportunity.

Based on the biological findings and existing economic relativities, the potential of the yellowfin bream and mangrove jack for commercial aquaculture is considered.

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CHAPTER 1

Introduction and Literature Review

Introduction

The families Sparidae (seabreams) and Lutjanidae (snappers) are among the most important as foodfish world-wide. Exceptional edible qualities and appearance have ensured high market acceptance, and intense commercial fishing efforts upon them. Declining natural stocks have prompted research into the aquaculture of many species from each family, both for restocking and farming purposes. This development has reached a more advanced stage among the sparids, with two species in particular now cultured successfully on a large scale. These are the gilthead seabream (*Sparus auratus*) of the Mediterranean and the red seabream or snapper (*Pagrus auratus*, formerly *P. major* or *Chrysophrys auratus*) of the cooler waters of the Indo-West Pacific. Both are examples of the highly successful and economically significant industries that can develop around species with suitable biological attributes. While no significant industries have as yet developed around hatchery-reared lutjanid species, research is underway on several species world-wide with this aim.

The gilthead seabream has been cultured in many Mediterranean and European countries for over a decade. It is the largest finfish mariculture product in that region, with production predicted to reach 35,000 metric tonnes (MT) by the year 1995 (Stephanis and Divanach, 1993). The red seabream was developed for aquaculture in Japan, where it has traditional significance, in the late 1960's, and now aquaculture production is second only to the yellowtail, *Seriola quinqueradiata*, and exceeded 40,000 MT in 1990

(Davy, 1990). Attempts are now underway to apply Japanese rearing technology to populations of this species inhabiting the Australian and New Zealand coastlines. Good market acceptance has kept prices of both *Sparus auratus* in the Mediterranean and *Pagrus auratus* in Japan high at about US $14/kg (Fukusho, 1991; Larrazabal, 1992).

Several other sparids, in various countries, are also being cultured commercially or are being investigated for this purpose. In Japan, *Acanthopagrus latus, A. schlegeli* and *Evynnis japonica* are all cultured commercially using techniques similar to those for red seabream (Foscarini, 1988), while research is currently underway to establish another industry for *Mylio macrocephalus*. In the Mediterranean, commercial quantities of *Diplodus puntazzo, D. sargus,* and *P. auratus* are being cultured, while research is continuing on *Dentex dentex, Diplodus vulgaris, P. pagrus* and on intergeneric hybrids (Kentouri and Divanach, 1982; Jug-Dujakovic and Glamuzina, 1988, 1990; Sweetman, 1992). In Taiwan commercial production of *A. schlegeli* and *A. latus* is in progress, while research continues on *S. sarba* and *A. berda* (Chen, 1990; Leu and Wu, 1990). In addition, work is underway in Hong Kong on *M. berda* (Mok, 1985), in Kuwait on *A. latus* and *A. cuvieri* (Hussain *et al.*, 1981; Jafri *et al.*, 1981), in France on *Diplodus sargus* (Divanach *et al.*, 1982), in South Africa on *Cheimerius nufar* (Garratt *et al.*, 1989), in North America on *Archosargus probatocephalus* (Tucker, 1987), and on *A. pagrus* and *P. auratus* in China (Tang *et al.*, 1979; Yen *et al.*, 1979).

Far fewer lutjanids have been examined for their aquaculture potential, and all that have belong to the genus *Lutjanus*. In several cases growout performance has been established from commercial production relying on juveniles sourced from the wild, and research has therefore concentrated on larval rearing aspects. In Singapore, studies on the aquaculture of John's snapper (*Lutjanus johni*) have been initiated (Lim *et al.*, 1985), and since the

late seventies, work has been undertaken in North America on red snapper (*L. campechanus*) (Arnold *et al.*, 1978; Rabalais *et al.*, 1980; Minton *et al.*, 1983), but commercial production has not been realised. Recently, work in North America has also targeted *L. analis*, *L. synagris* and *L. chrysurus* (Clarke, 1995). In the Philippines, Malaysia, Singapore, Thailand and Taiwan work has been underway on the mangrove jack or red snapper (*L. argentimaculatus*) since the mid 1980's, but reliable mass-production of juveniles has not yet been realised (Doi and Singhagraiwan, 1993; Emata *et al.*, 1994). Several other lutjanid species have been investigated from a biological rather than aquacultural perspective, and these findings are discussed below.

Research in Australia and project aims

Six species of sparids, including three of the genus *Acanthopagrus*, inhabit the waters of Australia, together with some 35 species of lujanids, of which 24 are in the genus *Lutjanus* (Rowland, 1984; Allen, 1985). Many of these are valued commercially, and are keenly sought after by recreational fishermen. Research on these species, with the aim of establishing commercial aquaculture industries, has been recent (since the late 1980's). In some cases the potential of breeding for stock enhancement purposes has added additional impetus to the research effort.

Of the sparids, the southern black bream, *A. butcheri*, has recently been induced to spawn in captivity at Taroona Marine Laboratories, Tasmania (Searle, 1992). A small percentage of larvae were reared through to metamorphosis. It was considered that the slow growth rate of this species would hinder its development for aquaculture, although a private company in Western Australia ('Aquaculture Products') now produces commercial quantities of juveniles (T. Graham, pers. comm., 1994).

Another sparid to attract interest for aquaculture in Australia is the snapper, *Pagrus auratus*. Recent research has demonstrated the close similarity between Australian populations of this species and those occurring off Japan (Battaglene and Talbot, 1992). As such, the Japanese culture technology appears to be applicable in Australia. The high market profile and price of this species should assure the development of a successful industry in Australia. Pilot-scale culture is now underway in New South Wales, southern Queensland, and Western Australia.

The only other sparid with important commercial and recreational significance to be studied, and one of the subjects of this report, is the yellowfin or silver bream, *Acanthopagrus australis*. This species has been studied from a purely biological perspective by Pollock in a series of papers (Pollock, 1982a, 1982b, 1984, 1985, Pollock and Williams, 1983, and Pollock *et al.*, 1983). Although some experimental hormone-induced spawnings and larval rearing trials of this species have previously been performed in Australia, these were of limited scope, and results were not published. They were performed at the N.S.W. Brackishwater Fish Culture Research Station in 1991 (S. Battaglene, pers. comm., 1991), at the Queensland University of Technology in 1991/92 (T. DeKluyver, pers. comm., 1993), and at a commercial fish hatchery ('Sea Harvest') at Mourilyan, Queensland in 1989 (S. Fielder, pers. comm., 1991). Only at the latter facility were larvae successfully reared through to metamorphosis. The remaining three sparids occurring in Australian waters, although having some recreational and/or commercial value, have not been investigated for aquaculture in Australia. However one of these, the tarwhine (*Rhabdosargus sarba*), is being investigated for aquaculture in Taiwan.

Of the 35 species of lutjanids inhabiting Australian waters, two are being investigated, both being tropical species in the genus *Lutjanus*. The John's or

golden snapper, *L. johnii*, is being researched by the Northern Territory Department of Primary Industries and Fisheries, Darwin. Work began in 1992, and at this stage broodfish have been induced to spawn successfully but larval rearing to metamorphosis has proven unsuccessful (C. Shelley, pers. comm., 1994). Similar larval rearing difficulties were experienced by researchers in Singapore when studying this species, with a mean survival to metamorphosis of only 1.0% (Lim *et al.*, 1985).

The second lutjanid to be studied for the purpose of aquaculture in Australia, and the other subject of this report, is the mangrove jack, *L. argentimaculatus*. Concurrent research on this species has been undertaken, and continues, at the Northern Fisheries Research Centre, Cairns, Queensland. Throughout Southeast Asia growout of juveniles caught from the wild demonstrated potential, but for large-scale aquaculture, reliable mass production of young soon proved necessary. Today, some hatchery-produced juveniles are supplementing those caught from the wild in Thailand and Taiwan, but survival rates to metamorphosis are still very poor and erratic. Good export markets exist in Southeast Asia for both snapper species which command a high price.

The economic evaluation of a species for aquaculture must involve a consideration of both marketing and biological aspects. The species chosen for the present study, the mangrove jack and yellowfin bream, have a proven market acceptance and demand. The suitability of their biological attributes, however, is unknown, and is the subject of this research. The aims of this project were therefore to develop effective and reliable techniques for broodstock spawning, larval rearing, and growout of juveniles, and, dependent on them, to assess the potential of these two species for aquaculture in Australia.

The known biology of yellowfin bream, *Acanthopagrus australis* (Günther, 1859)

Yellowfin bream are endemic to coastal and estuarine habitats between Townsville, Queensland, and the Gippsland Lakes, Victoria (Munro, 1945; Dunstan, 1965). The species is sought after by commercial and recreational fishermen throughout this range (Pollock and Williams, 1983). It is an attractive, firm white-fleshed fish with good market acceptance and value (wholesale ~ A $4.50-6.50/kg whole, 1995).

In a series of papers, Pollock studied some aspects of the biology of *A. australis* in the Moreton Bay area of Eastern Australia (Pollock, 1982a,b, 1984, 1985, Pollock and Williams, 1983, and Pollock *et al.*, 1983). It was found that yellowfin bream in Moreton Bay have a short spawning season from April - August with a peak period in July and August (Pollock, 1982b, 1983). Ripe adults migrate to surf bars to spawn, particularly at high tides on the full moon (Munro, 1944; Pollock, 1982a). Eggs and larval stages could be found around surf bars at these times. Metamorphosed juveniles (13-14 mm fork length) were found in littoral areas within the estuary two months after the spawning season (Pollock, 1983).

The yellowfin bream shows protandrous sex inversion and possesses a typical sparid ovotestis (Suparta *et al.*, 1984; Pollock, 1985), although there is some ontogenetic variation. Most juveniles become functional males by the age of two years, but a small proportion become primary females, while others remain functional males throughout their lives (Pollock, 1985).

Pollock (1982b), using tag-recapture data and length-frequency analysis of wild fish, found that bream grow to 14.5, 20.5 and a market size of 24.1 cm in their first, second and third years, respectively. Growth was quite linear to three years, after which the growth curve flattened towards the asymptotic

length (L_∞) of 29.5 cm. Maximum weight is at least 3 kg (Marshall, 1982). The diet is composed of small crustaceans, fish, molluscs, and polychaetes (Grant, 1982).

Little else is known of the biology of this species, although Rowland (1984) reported the occurrence of *A. australis* x *A. butcheri* hybrids from some estuaries along the coast of southern New South Wales, where the distributions of the two species overlap.

The known biology of mangrove jack, *Lutjanus argentimaculatus* (Forsskål, 1775)

Although a species prized for its recreational and edible quality, the mangrove jack, or red snapper, has been poorly studied. This is particularly true of its reproductive biology and early life history. Mangrove jack are distributed widely in the Indo-West Pacific, from Samoa to East Africa, and Australia to southern Japan. Juveniles and subadults occur in coastal estuaries and the lower reaches of freshwater streams, while adults migrate to offshore reefs and may occur at depths of 100 m or more (Marshall, 1982).

The mangrove jack is primarily a piscivorous carnivore, although crabs, prawns, various other crustaceans and cephalopods are also taken (Marshall, 1982; Allen, 1985). Spawning sites used are unknown, although ripe fish have been captured near middle and outer reefs in summer months (R. Garrett, pers. comm., 1993). Mangrove jack are dioecious, show no sexual dimorphism, and do not change sex during life (Allen, 1985; Grimes, 1987). Talbot (1960) found *L. argentimaculatus* off the East African coast to mature at approximately 35 cm standard length.

By analogy with other lutjanids, mangrove jack are likely to be nocturnal serial spawners (Carter and Perrine, 1994) with a high fecundity of at least 5-7 x 10^6 eggs from a large female (Grimes, 1987; Davis and West, 1993). The development of eggs and larvae to metamorphosis has been described in Thailand and the Philippines (Doi and Singhagraiwan, 1993; Emata *et al.*, 1994). Maximum reported size is 120cm.

Aquaculture of the Sparidae

The techniques employed in spawning induction, larval rearing and growout are similar for all species of bream grown commercially. Likewise, common problems have emerged, mostly in the areas of larval nutrition, swimbladder inflation, and disease. The following accounts of rearing practices employed in the culture of *S. auratus* and *P. auratus* serve to exemplify culture techniques used world-wide in the aquaculture of breams.

The gilthead seabream, *Sparus auratus*

The following account of farming procedures for the gilthead seabream is derived, except where stated otherwise, from reviews by Person-Le Ruyet and Verillaud (1980), Coll Mordles (1983), Barnabe (1990), and Sweetman (1992).

(a) Broodstock

Captive broodstock are most commonly maintained in indoor tanks 7-60 m^3 in size, using either closed recirculation or open flow-through systems. At some hatcheries using recirculation systems, temperature and photoperiod manipulations are used to extend the spawning season by several months. Broodfish are maintained at a density of approximately 5 kg/m^3, at a 1:2 ratio

(m:f) of fish 1-2 kg each. Feeding is five times per week with fresh foods (molluscs and crustaceans) supplemented with pellet food. *S. auratus* is a protandrous hermaphrodite and in captivity can transform to female by 16 months of age.

The natural spawning season is in autumn (November - January). Where hormone-induction is practiced, prospective females are chosen in late October based on the state of their ovarian development. Suitable females are anaesthetised and injected with Human Chorionic Gonadotropin (HCG), at the rate of 500-5,500 IU (mean 600 IU)/kg female. Males are also usually injected, but at the reduced rate of 300-1,500 IU (mean 300 IU) HCG/kg. Injected fish are subsequently placed into smaller spawning tanks of 3,000-4,000 l capacity, at a density of 2-5 kg/m^3, and at a 3:2 ratio (m:f). At other hatcheries fish are spawned naturally when temperature and photoperiod conditions are conducive, and handling is avoided.

The spawning tank is maintained at 33-35 ppt salinity and 18-20°C. Over the next three days the female swells, after which eggs begin to be shed daily for 5-30 days with no further interference. Occasionally, females are strip-spawned and the eggs artificially fertilised. From 300,000-3 million eggs/kg of female are obtained through the spawning season, with a mean fertilisation rate of 60%.

(b) Egg incubation

Eggs are 1 mm in diameter, transparent and pelagic. They are collected from spawning tanks and transferred to conical-shaped incubators at 2,000-5,000 eggs/l. Water is lightly aerated, and maintained at about 36 ppt salinity and 16-18°C. Eggs hatch in 45-50 h, and poor hatch rates of 40% are common.

(c) Larval rearing

Larvae hatch at about 2 mm with a large yolksac and are transferred to larval rearing tanks of 7-15 m^3 size, maintained at 15-25 ppt and 20°C. Aeration is very gentle, and some form of oil skimmer is employed to remove any oily film on the water surface (discussed later). Tank shape is hemispherical or conical, with black sides and bottom to increase prey visibility to larvae. The larval density varies between hatcheries, but is in the range of 30-140 larvae/l. It is six days before larvae are ready to take exogenous food, by which time they measure 4.5 mm. The provision of suitable food is essential at this stage to avoid large losses.

First food offered is generally small-strain rotifers (approx. 140-270 μm lorica length), which are used in the first 20 days of rearing, at a density of 2-10/ml. Offering larvae small-strain rotifers at first feed, rather than large-strain, improves first feeding success, with consequent benefits to growth and survival (Polo *et al.*, 1992). During the rotifer-feeding stage it is common for unicellular algae (*Chlorella minutissima, Tetraselmis suecica, Nannochloris* spp., *Isochrysis tahitian, Pavlova lutheri,* or *Rhodomonas* spp.) to be added to the tank (approx. 5×10^4 cells/ml) for the purposes of providing a darker background and diffused light, which enable more efficient prey capture, and a continual nutritional enrichment of the rotifers. Despite these advantages, the industry intends to change from 'greenwater' to 'clearwater' techniques once the necessary technology becomes further refined. This will eliminate the costly production of unicellular algae.

Swimbladder inflation occurs between days 10-15, at a larval length of 4.0-5.0 mm (Chatain and Ounais-Guschemann, 1990). After swimbladder inflation, rotifer-enrichment with commercial products, such as Frippak Booster or Selco, is usually employed. Water exchange during the first 15

days varies from zero to a slow continual flow (about one tank exchange/ day), which is sometimes recirculated. Tank bottoms are not cleaned for the first two weeks, after which siphoning is conducted daily. Lighting varies from ambient light intensity and photoperiod to 250 lux and 24 h illumination by means of fluorescent lighting. Tandler and Helps (1985) found that continuous light supported the highest survival of larvae to metamorphosis, whereas growth was best under continuous light only up to an age of 20 days, thereafter being best under a 12 h photoperiod regime. Barnabe (1990) reports that swim bladder inflation can be inhibited at light intensities over 2000 lux.

From day 10 onwards, high-grade *Artemia* nauplii are introduced to the tank. These are gradually replaced by enriched *Artemia* metanauplii and inert weaning diets (such as "Sevbar") or ground crustacean and molluscan meat. Metamorphosis is reached at 55-65 days, at which time mean fish weight and size are 100 mg and 20 mm total length (TL), respectively, and weaning is near completion. Survival from hatch to metamorphosis is reportedly less than 15%. The price for 2 g fry in 1993 was US $1 each (Sorgeloos and Sweetman, 1993).

Once again some variations are observed at different hatcheries; for example, some use live or frozen copepods in the feeding schedule, others employ oyster trochophores or certain ciliates as first food, some add yeast to rearing tanks in addition to algae as rotifer food, and yet others do not attempt weaning until day 80, etc.

(d) Growout

Most commonly, weaned juveniles (1-2 g) are transferred to fine-mesh netcages (< 30 m^3) for further on-growing at an optimal temperature of 23-25°C. However in some areas (particularly in Spain) pond growout is also used, where juveniles are stocked at 3-4 fish/10 m^2. Being a euryhaline

species, growout of *S. auratus* can be in brackish water. On a diet of dry pellet food, a weight of 110-150 g is attained in the first year, 350-400 g in the second and 700 g in 2.5-3 years. A commercial size of 350 g is attained after approximately 18 months. A culture density in netcages of about 12 kg/m^3 is used initially, increasing to 20-30 kg/m^3 (in rare cases 60 kg/m^3) nearing harvest, depending on site. Mortality through growout is low at about 10%. After a size of 50 g, cage size is increased to 100 m^3.

(e) Culture problems

As with so many other aquaculture species, the principal bottleneck in the production cycle of *S. auratus* is the larval rearing component. In particular, the areas of swimbladder inflation, nutritional quality of live feeds, and disease require attention.

Initial larval rearing trials produced larvae with very low swimbladder inflation rates (0-25 %). Larvae without a functional swimbladder showed delayed growth (Chatain, 1987), lordotic skeletal deformities (Paperna, 1978), and higher mortality under stress (Chatain and Dewavrin, 1989). The slower growth is thought to result from a decrease in predatory efficiency and an increase in energetic needs, while the lordosis is hypothesised to result from continual swimming in an oblique position to avoid sinking (Chatain, 1989). Such problems have been experienced to varying degrees at most marine fish hatcheries world-wide. It has been shown (by placing liquid paraffin on the water surface) that gilthead seabream larvae require access to the water surface to inflate the swimbladder at approximately 10 days old, prior to the pneumatic duct closing (Chatain and Ounais-Guschemann, 1990).

In 1986 the development of a surface cleaner originated in Japan where similar swimbladder inflation problems had been experienced in the larval rearing of the red seabream, *Pagrus auratus*. The device consists of a U-shaped

trap floating on the water surface. Air is blown across the water surface from the open end into the trap. Any oil and debris on the water surface is blown into the trap and retained. The trap is cleaned by drawing absorbent paper over the water in the trap, or by scooping the film off. Through the use of this trap, inflation rates of over 80% are now possible (Chatain and Ounais-Guschemann, 1990).

The use of live food enrichment techniques exacerbated the problem, as the addition of enriched live food introduced with it significant amounts of oil. Even today it is common to not enrich live feeds until swimbladder inflation is complete.

The discovery of the essential nature of certain highly unsaturated fatty acids (HUFAs) to the growth and survival of marine fish larvae (Owen *et al.*, 1972; Yone and Fujii, 1975; Cowey *et al.*, 1976; Kanazawa *et al.*, 1979; Takeuchi *et al.*, 1990) brought about another large advance in larval rearing success world-wide. In particular, docosahexaenoic acid (DHA, 22:6n3) and eicosapentaenoic acid (EPA, 20:5n3) have been recognised as essential and unable to be synthesised by many, but not all, marine fish larvae.

Koven *et al.* (1989, 1990, 1992) reported that *S. auratus* also has the n-3 HUFA requirement in both rotifer and *Artemia* -fed stages, and that the larvae strongly conserve DHA, in particular, during starvation. Levels of n-3 HUFA over 5 mg/g DBW (dry body weight) rotifer and over 30 mg/g DBW *Artemia* appear necessary for optimal growth (Koven *et al.*, 1990, 1992). When levels of these HUFAs were raised from natural levels in the live food being provided to *S. auratus* larvae (through feeding of live food certain algal species, marine oil emulsions, or more recently commercial enrichment diets), growth and stress-resistance (but not survival) increased significantly (e.g. Koven *et al.*, 1992).

It is now common practice to enrich both rotifers and *Artemia* metanauplii before introducing them to larval rearing tanks, although the exact enrichment techniques used vary between hatcheries. Certain commercial products ("Selco", "Super Selco", and Frippak "Booster") are the most commonly used. The former two products tend to raise HUFA levels in live food more efficiently, but being emulsified oil, are also more likely to create an oily scum on the surface of the rearing tank. More recent products (for example "Culture Selco" and "Protein Selco") aim to replace the use of algae altogether, by supplying proteins, vitamins and minerals to the live feed as well as essential fatty acids.

Another problem associated with live food enrichment is the high bacterial load that may result in the enrichment tank. Unless well rinsed after enrichment, live foods can introduce harmful bacterial to the larval rearing tank, with associated disease problems. Although a long period of enrichment, for example 24 h, will generally raise the HUFA levels to a higher level in live feeds, enrichment is seldom carried out for longer than 8 h to minimise this problem.

In many hatcheries, disease-related mortality becomes apparent towards the end of the breeding season. Larvae in the first 30 days are far more susceptible than older fish. It is thought that this mortality is due to a build-up of pathogenic bacteria in the larval rearing tanks and live food culture and preparation areas. Several species of pathogenic bacteria have been implicated, including *Aeromonas hydrophila, Pseudomonas* spp., and *Vibrio* spp. (Sweetman, 1992). Hatcheries have taken different approaches to this problem. Some use antibiotics, usually sulfonamides or nitrofurans, in larval rearing tanks, although most accept the need for a shut-down and dry-out period, in conjunction with a disinfection of equipment. This batch-culture approach has proven successful in most hatcheries.

Other precautions taken by some hatcheries include the use of duplicated pipe systems, multiple isolated larval rearing areas, increased levels of water filtration and treatment prior to use, disinfection or decapsulation of *Artemia* cysts, rinsing of enriched live food with UV-sterilised seawater or freshwater, or an addition of antibiotics to enrichment tanks in an effort to reduce bacterial numbers.

The red seabream, *Pagrus auratus*

The following account of intensive farming procedures for red seabream in Japan is derived, except where stated otherwise, from reviews by Foscarini (1988) and Fukusho (1991).

(a) Broodstock

Broodfish are generally maintained in floating net cages, or less commonly in land-based concrete tanks. Water temperature sets a natural limit to where broodfish can be maintained, and juveniles grown out, as temperatures outside the range of 10-22°C are unsuitable. Land-based tanks are used when temperature/photoperiod manipulations are being used to extend the natural spawning season. Red seabream, like gilthead seabream, are euryhaline and tolerate salinities to 15 ppt.

Broodfish are fed a high quality artificial and fresh-food diet, but immediately before spawning only shrimps or krill are provided, at 8% body weight/day. The quality of the diet immediately before and during the spawning season is reflected strongly in egg quality, in terms of protein content, fatty acid (including HUFA) level and content of certain vitamins. Broodfish feed quality impacts on fecundity, egg buoyancy, hatch rate, and occurrence of larval deformities (discussed later).

The spawning season is in spring, from mid-April to early June, when water temperatures range from 12-25°C. Red seabream are capable of reproducing between the ages of 2-15 years, although 3-6 year-old broodfish are preferred due to their better gamete quality. The fecundity of broodfish of this age is approximately 400,000-1 million eggs/kg. Ripe fish (females distinguishable by the swollen abdomen; males by the discharge of sperm upon light abdominal pressure) are transferred to concrete land-based tanks of 50-100 m^3. Here they are stocked at 1-1.5 kg/m^3 at a sex ratio of 1:1, and held at a temperature of 18-20°C.

Where hormonal induction is used, only females with oocytes greater than 0.5 mm (assessed by cannular biopsy) are used. Dosages of 1000 IU/kg of either HCG or Puberogen will induce ovulation in 36-40 h. In the past, hormonal treatment of ripe broodfish has resulted in poor egg quality, thus natural spawnings are preferred today. An advantage of red seabream for aquaculture is that they will readily spawn naturally in captivity, at dusk and into the night, over a period of 60-80 nights. Fertilisation and hatch rates of 60-80% and 70-80%, respectively, are common.

(b) Egg incubation

Netted eggs are transferred first to separation tanks (30-50 l) where buoyant eggs are separated from those that sink. Buoyant eggs are transferred to 300 µm mesh incubation nets which are suspended in large concrete tanks. Eggs are stocked at 500/l and are gently aerated. Eggs are transparent, pelagic, and range in diameter from 0.85-1.1 mm, with an oil globule of 0.22-0.25 mm diameter. Eggs hatch in 50 h at 18°C.

(c) Larval rearing

Prior to hatching, buoyant eggs are weighed (to estimate number) and then transferred, at 12-70/l, to large rectangular or circular larval rearing tanks of 50-100 m^3 capacity. Newly hatched larvae measure 2.0-2.3 mm TL. By the completion of yolksac absorption (day 4), larvae measure 3.2 mm TL. From day 6, 10-30% of water is exchanged daily, increasing to 250-280% daily by day 50. Light levels are maintained below 10,000 lux. Unhatched eggs are removed as soon as possible, after which the tank bottom is cleaned every 3-4 days. An automatic wiper assists in this operation, the wiper rotating through 360° along the sloping tank bottom and pushing heavy debris toward the central drain.

Swimbladder inflation occurs between 5-10 days post-hatch when larvae are 3.5-4.0 mm TL. Previously, eggs and trochophore larvae of certain bivalve and urchin species were used as first food for *P. auratus* larvae. Today, for convenience, only the rotifer, *Brachionus plicatilis* is used (both L- and S-strains). These are provided from days 4-45 post-hatch at varying densities, initially at 3-5/ml. This is supplemented with frozen rotifers from days 16-40. From about 30 days post-hatch (larvae 8 mm TL), live and frozen *Artemia* nauplii, and frozen krill, are added until weaning commences on day 40. The mass culture of other suitable plankton organisms, such as *Trigropus japonicus*, *Acartia* spp., *Oitona* spp., and *Paracalanus* spp. is in an experimental stage.

High labour costs, and increasing prices of *Artemia* and krill, have raised interest in the use of artificial feeds as partial or whole replacements for live food. Although some progress has been made, the high cost of such feeds has limited their popularity.

Metamorphosis commences at a relatively small size of 7.5-8.5 mm (10 mg), and is complete at 12 mm and about 20-50 mg (age of 30-40 days). Mean

survival to this stage is 20-30% (Tandler *et al.*, 1989a). Following metamorphosis aggressiveness and cannibalism become apparent, and stocking density is reduced to 10-15 juveniles/l.

(d) Growout

At 12-17 mm TL, juveniles are moved to floating netcages (initially 45 m^3 is standard, at 1000-2000 fish/m^3) that hang from rafts in the sea. The juveniles are grown out in these cages, on mainly dry artificial feed. Some farms also still stock wild-caught juveniles. Temperatures of 20-28°C are preferred for growout, as feeding activity is reduced below 20°C. Weaning is carried out once juveniles have been transferred to seacages. Here they are fed at 250% body weight/day, as 10 feeds, on a mixture of fish meal and artificial food. A 100 W lamp is used at night to attract wild zooplankton into the netcages which helps the weaning process.

During the transition to seacages, considerable mortality (sometimes exceeding 50%) is reported to occur. Reasons for this are uncertain, but are thought to include cannibalism, dietary changes and disease. Netcage volume is progressively increased to 300 m^3 by harvest, when stocking densities of 10 kg/m^3 are common. In captivity, growth rate is over twice that in the wild, with juveniles reaching a weight of up to 600 g in 18 months, and 1.2 kg in 30 months. Food conversion, when fed exclusively a fresh fish diet, is about 5:1 in the first year, and 9.7:1 in the second year.

Besides intensive growout in cages, a semi-extensive method is also practised. Nets are used to enclose small shallow bays and inlets (50 ha for example). Growout is then carried out with or without the use of supplemental feeding. Furthermore, a recent research project is examining the use of sound waves of a particular frequency to attract released fish to the

supplemental feeding area. Once fish are trained it is hoped that enclosure nets will no longer be necessary thus reducing the costs of growout.

Almost all red seabream are marketed live, by way of water tanker trucks with attached oxygen supply. Prior to transport the fish are starved for 5-7 days in summer and 2-3 weeks in winter. The cost of production is US $10/kg in Japan, allowing a net profit of US $4/kg.

(e) Culture problems

The problems encountered in the culture of red seabream are again mostly in the area of larval rearing, and very similar to those of *S. auratus*. Survival rates following transfer and acclimatization of juveniles to netcages are generally good.

The same problems relating to correct swimbladder inflation as described previously for *S. auratus* have been experienced for *P. auratus*. The previously-mentioned oil skimming device, originally developed in Japan, is also standard equipment in Japanese hatcheries to remove any oily film on the water surface, greatly improving the rate of swimbladder inflation. Inflation rates of over 90% are now common. Lordotic deformities (affecting up to 40% of larvae in the past) have also been linked to swimbladder inflation failure of red seabream.

Similarly, HUFA deficiency in larvae was once a major cause of larval mortality. This was the result of feeding yeast-fed rotifers to the larvae. To overcome this problem, rotifers are now reared on the alga *Nannochloropsis oculata* and/or *Tetraselmis tetrathele* initially, and subsequently fed squid liver oil-enriched yeast (20 ml/100 l culture media) for one day prior to being used as food.

Disease-related deaths occur in both hatchery and growout areas, sometimes causing 100% mortality. The rotifer, *B. plicatilis*, has been implicated as a vector for bacterial diseases of larval stages. *Vibrio* spp. and *Alcaligenes cupidus* have been isolated from both moribund larvae and the rotifers they were fed. Such infections are minimised by rinsing rotifers for several hours in clean seawater before use, or by treating in a 10 ppm sodium nifurstyrenate bath for 30 min.

Infections of protozoa, such as *Ichthyophthirius* and *Oodinium*, occur in older fish, along with the viral disease *Lymphocystis*, the bacterial diseases *Vibrio* and *Edwardsiella*, and several external parasites.

A market-related problem in the growout stage is the darkening of body colouration during culture. This significantly reduces the commercial value from those showing a deep red colour. Today, fish within several weeks of harvest are fed intensively with krill and shrimps high in astaxanthin, which is responsible for the red colouration of the skin. Black covers are simultaneously placed over the netcages to decrease illumination and, hence, reduce black melanin pigments in the skin. The quality of cultured fish is still regarded as inferior to those wild-caught, and this problem is being addressed from the viewpoint of nutrition, genetics, and physical rearing conditions.

Aquaculture of the Lutjanidae

As stated previously, there are no aquaculture industries based on hatchery-reared lutjanid species. Small, localised industries based on wild-caught juveniles do exist in some countries, but supply severely limits their extent. Furthermore, the capture of wild juveniles is illegal in most first world

countries, prompting research into controlled hatchery rearing if significant industries are to become a reality. The following review summarises progress on the intensive culture of lutjanid species world-wide.

Attempts have been made to induce spawning and/or rear larvae of at least six species of lutjanid, all in the genus *Lutjanus*. These are the mangrove jack (*L. argentimaculatus*) in the Philippines, Thailand, Malaysia, Singapore, Taiwan and Australia (Doi and Singhagraiwan, 1993; Emata, 1994; Emata *et al.*, 1994; R. Garrett, pers. comm., 1994; and present study); the John's or Golden snapper (*L. johni*) in Singapore and Australia (Lim *et al.*, 1985; C. Shelley, pers. comm., 1994); the white-spotted snapper (*L. stellatus*) in Japan (Hamamoto *et al.*, 1992); the red snapper (*L. campechanus*) in North America and Panama (Arnold *et al.*, 1978; Rabalais *et al.*, 1980; Minton *et al.*, 1983); the gray snapper (*L. griseus*) in North America (Richards and Saksena, 1980) and the blue-and-yellow snapper (*L. kasmira*) in Japan and the Andaman Sea (Suzuki and Hioki, 1979).

Successful spawnings were obtained from all species, either through intramuscular hormone injection (generally HCG at 250-1500 IU/kg body weight) or by spontaneous occurrence, requiring no input by researchers. Where eggs were obtained from both sources, it was generally noted that better egg quality resulted from natural spawnings as reflected in hatch rates (often less than 50% after hormonal induction) and larval rearing success (Lim *et al.*, 1985; Doi and Singhagraiwan, 1993). Latent periods after hormonal injection were 27-37 h with *L. johni* and *L. argentimaculatus* (Lim *et al.*, 1985; Doi and Singhagraiwan, 1993; Emata *et al.*, 1994), and 42-56 h with *L. campechanus* (Minton *et al.*, 1983). All six species were observed to be serial spawners, shedding eggs over several nights, either in succession or more irregularly. Where stated, spawnings usually occurred between dusk and 9pm, although Doi and Singhagraiwan (1993) report a preferred spawning

time of 1-4 a.m. for the mangrove jack. Fecundities were generally high, at over 200,000 eggs/kg, although *L. campechanus* was an exception in this regard, yielding only about 3,500 eggs/kg (Minton *et al.*, 1983).

Eggs were transparent, spherical and pelagic, and in the range of 0.7-0.85 mm in diameter, with a small oil globule of 0.13-0.18 mm diameter. Larvae were also small, ranging in size from 1.5 mm TL (*L. campechanus*) to 2.5 mm (*L. stellatus*)

Attempts were made to rear the larvae of all species, except *L. stellatus*, and all met with similar difficulties. Only three species (*L. johni*, *L. argentimaculatus*, and *L. griseus*) could be reared to metamorphosis, all with very low survival. Without exception, heavy mortality was experienced to day 5, and a second mortality peak was noted for *L. johni* and *L. argentimaculatus* between days 18-20 (Lim *et al.*, 1985; Bonlipatanon, 1988; Emata *et al.*, 1994). Survival to metamorphosis in most trials was in the range of 1% or less. Exceptions did occur in the rearing of mangrove jack, where one trial reportedly achieved 16.6% survival (Wudthisin, 1984), another 10.3% (Bonlipatanon, 1988), and a third reported a rough estimation of 43% survival (Doi and Singhagraiwan, 1993). All three authors reported extremely erratic survival values. Emata *et al.* (1994) were unable to rear mangrove jack larvae beyond 28 days of age.

It appears that the very small physical size of larvae, and associated mouth size, have necessitated the use of very small live prey at first feeding. Where larvae have been successfully reared to metamorphosis, first food has been one of mussel larvae of about 60 µm (Lim *et al.*, 1985), oyster eggs of about 60 µm (Wudthisin, 1984), screened wild zooplankton of 35-73 µm (Richards and Saksena, 1980), copepod nauplii of 100 µm (Doi and Singhagraiwan, 1993), or sieved rotifers (Bonlipatanon, 1988). The problem of

obtaining sufficient quantities of these live food organisms has been a restraint. Other rearing attempts, using oyster larvae, however, have failed even though these were ingested in large numbers by larvae (Suzuki and Hioki, 1979; Minton *et al.*, 1983). This suggests nutritional and/or digestive causes of mortality.

In addition to the small mouth and larval size (and hence limited energy gathering potential (Chesney, 1995)), a small oil globule size has also been suggested as contributing to poor survival values (Doi and Singhagraiwan, 1993). The larvae have very little nutrition to sustain them through the critical period of commencement of exogenous feeding. These factors, coupled with the physiological disadvantages of development at high temperatures (Chesney, 1995), makes the availability of suitable live food organisms at first feeding most critical.

The intensive growout of wild-caught juveniles in netcages and brackish-water ponds in Southeast Asia has shown the viability of golden snapper and mangrove jack culture. Mangrove jack in Thailand reportedly reach a size of 400 g in six months, and 1 kg in 12 months (Doi and Singhagraiwan, 1993), and growth rates of the former species are similar (G. Schipp, pers. comm., 1994). Although cannibalism is somewhat of a problem among juveniles, this can be minimised through frequent grading and sufficient feeding. Large-scale culture of these lutjanids now depends on achieving reliable mass-production of juveniles.

SECTION A

INDUCED SPAWNING AND CULTURE OF YELLOWFIN BREAM, *Acanthopagrus australis*

CHAPTER 2

Induced Spawning

Introduction

Pollock (1982a, 1982b) noted that *Acanthopagrus australis* undertakes an annual spawning migration to surf bars at the mouths of estuaries in June-August, when water temperatures are at their lowest. Each female produces large numbers of planktonic eggs which are spawned primarily during high tide (Munro, 1944). Suparta *et al.* (1984) and Pollock (1985) confirmed yellowfin bream to be protandrous hermaphrodites, maturing as males at the age of two years when they first participate in spawning, and most fish inverting to female between years 2-5. This life history pattern is similar to that observed in two other *Acanthopagrus* species (*A. latus*, and *A. cuvieri*) (Abu-Hakima, 1984).

The ability to obtain reliable, quality spawnings from captive broodstock is fundamental to any aquaculture operation, and may be achieved by either environmental or hormonal manipulations. For many fish, such as yellowfin bream, the specific environmental cues that trigger ovulation and spawning have not been identified. For this reason hormone-induction is the option often taken. Variations of the synthetic peptide hormone LHRHa (luteinising hormone-releasing hormone analogue) have been shown to stimulate gonadotropin (GtH) secretion in teleosts, although the effects vary with the nature of the molecule and the species (Peter, 1983; Lin and Peter, 1986; Crim *et al.*, 1987). GtH is indirectly responsible for events leading to ovulation and spawning (Peter, 1982; Idler and Ng, 1983; Marte, 1989). Synthetic LHRHa is often termed

'superactive', due largely to a reduced enzymatic degradation compared with natural fish GtH-releasing hormone (Peter *et al.*, 1987), and has been successfully used to induce spawning in many species (reviewed by Crim *et al.*, 1987; Sherwood, 1987).

While hormone-induced spawnings of yellowfin bream have previously been achieved in captivity (S. Fielder, T. Dekluyver, S. Battaglene, pers. comm., 1992), these have not been described or documented. This chapter describes work on the most effective hormones and dosages, and methods of administration, for the induced spawning of yellowfin bream, *A. australis*. Also determined were the latent period, conditions conducive to spawning, female fecundity, the 'window of fertilisation', and the post-injection pattern of oocyte enlargement.

Materials and Methods

Broodstock collection and examination

Mature adult *A. australis* were caught by hook and line off Townsville, Australia (19° S; 147° E), from June to early September, 1992-1994. Captured fish were maintained in 100 l flow-through tanks (10 l/min exchange) for a maximum of 5 h before being anaesthetised with MS-222 at 70 mg/l. Spawners were then sexed and their length and weight noted. Males were examined for free milt, and females were cannulated with a fine plastic tube (1 mm I.D.) passed through the genital aperture. Oocytes drawn from the ovaries were subsequently viewed by microscope and eyepiece micrometer to determine the mean diameter of the largest size-class. Females were then given one of three hormone

treatments. Males were only treated (as detailed below) when milt production was considered sub-maximal (approx. 10% of males).

Hormonal induction

(i) aqueous LHRHa

A 40 µg/ml aqueous solution of LHRHa (des-gly^{10}, D-ala^{6}, pro^{9}-ethylamide) (Peptech, Sydney, Australia) was prepared by dissolving 1 mg of LHRHa in 25 ml of 0.9% saline. Fish were injected intramuscularly (in the epaxial region below the dorsal fin) with dosages of 10 µg LHRHa/kg for males, and varying from 5-20 µg/kg for females. A 1 ml syringe and 1.5 inch, 26 gauge needle were used. Trials were carried out on females at 5, 10, 15, and 20 µg/kg in triplicate to determine minimum dosage requirements for successful spawning. The volume of solution injected was maintained at 0.5 ml/kg or less to minimise back-flow losses.

(ii) pelletised LHRHa

Pellets were made using the methods of Sherwood *et al.* (1988), with some modifications. An 80% cholesterol/20% cellulose (or 85/15%) formulation was used to provide a more sustained release of hormone than by aqueous injection. The cholesterol (640 mg) and cellulose (160 mg) were well mixed (or 680 mg and 120 mg respectively for the 85/15% pellet). One milligram of LHRHa was dissolved in 0.4 ml of 80% ethanol, which was then added to the matrix powder and thoroughly mixed and allowed to dry. This was then pressed in a pelletiser to produce pellets approximately 5 mm x 2 mm, and weighing 5-10 mg each. Pellets were administered subcutaneously with a 12 gauge needle. Trials were carried out in triplicate at 20 µg LHRHa/kg in 80/20% pellets, and at 40 µg LHRHa/kg in 85/15% pellets.

(iii) Ovaprim

Another commercial hormonal preparation, "Ovaprim", was tested in triplicate for its effectiveness on yellowfin bream. This was administered as an aqueous injection at the manufacturer's recommended dose of 0.5 ml/kg (female body weight).

Serial spawning trial

As an adjunct to work with pelletised LHRHa, a single female bream (525 g) caught early in the spawning season was given a succession of hormone treatments (primarily in pellet form) to determine whether or not continuous serial spawning was possible, and if so, for how long. After each hormone treatment the female was allowed to spawn uninterrupted in a 1500 l tank with two un-injected males. When the female ceased to spawn for 2-3 nights after a spawning period, it was given another hormone treatment. This was continued until the female was considered to be nearly 'spent'. Data from each spawning were recorded (egg number and % fertilisation, egg and oil globule diameters, and spawning time), and regular samples of eggs were taken for fatty acid analysis as outlined below. The female was fed daily to satiation with prawn meat.

Fatty acid analysis of eggs

From every second or third spawning of the serial spawning trial, samples of 5,000-20,000 eggs in early cleavage were rinsed in a 3.2% ammonium formate solution to remove salts, and frozen at -80°C for fatty acid analysis. Samples were prepared and analysed for fatty acids by the method of Southgate and Lou (1995). A Hewlett Packard 5890 gas chromatograph, equipped with a fused silica capillary column was used to separate fatty acid methyl esters (FAMEs). The BPX70-coated (SGE,

Melbourne, Australia) capillary column was 12 m long with a 0.22 mm inside diameter. Helium was used as the carrier gas at 50 ml/min, and oven temperature was set to 150°C. FAME reference standards (Sigma) were used to identify individual fatty acids, with cod liver oil FAMEs used as secondary reference standards (Ackman and Burgher, 1965). Each analysis was performed in duplicate, however analytical problems precluded duplicate results for spawnings 1 and 9.

Spawning conditions

Mean maximum oocyte diameters for all females prior to injection are presented in Tables 1-3. Fish were allowed to recover before being placed in circular spawning tanks of 1000-2000 l capacity. Only one male was placed with each female, except for the serial spawning trial where two males were used, and disturbance was kept to a minimum until spawning occurred. Water was recirculated through an external biofilter at 7 l/min. Tanks were 90% covered with opaque black plastic or dense shadecloth and tank temperature and salinity ranges were 21-24°C and 33-39 ppt, respectively.

Spawning assessment

On the evening that spawning was anticipated, fine mesh nets were placed under the outlet flow and checked half-hourly for eggs. When a spawning was confirmed, time was noted and eggs were collected from the water surface by hand net, placed in 20 l of water, and the number estimated volumetrically by triplicate subsampling. When larval rearing was to be attempted, eggs were immersed in a 100 mg/l iodine bath for 10 min, at 4 h post-fertilisation, to reduce bacterial and/or fungal contamination.

Fertilisation rates were determined by examining 50 eggs at 1 h post-fertilisation. Mean egg and oil globule diameters of thirty eggs were recorded for each spawning by microscope and eyepiece micrometer.

From one spawning, a sample of rinsed eggs was separated into three groups of 10 eggs, placed briefly on absorbent paper to remove excess water, and then each lot placed on a pre-dried, pre-weighed filter paper and weighed to the nearest 0.1 µg on a Cahn model 21 microbalance. Each paper with lot of eggs was then placed in a drying oven at 60°C for 24 h before being weighed again for dry weight. Weights were divided by 10 to obtain mean individual egg weight.

Oocyte maturation post-injection and the 'window of fertilisation'

A mature female was injected with 20 µg aqueous LHRHa/kg, and then cannulated regularly from injection until oocytes reached a fully hydrated condition. The reaction of oocytes to the hormone application, as reflected in increasing diameter over time, was then plotted.

The window of fertilisation, or period of time in which fully hydrated eggs are able to be fertilised, was then determined by stripping and artificially fertilising eggs regularly from 30-40 h post-injection. Approximately 10,000 eggs were stripped per determination, to which one drop of milt was added, and fertilisation achieved using the 'dry' method (Huet, 1972). Triplicate samples of 30 eggs were observed microscopically from each of the 7 determinations after one hour to ascertain fertilisation rates.

Results and Discussion

Spawning season and reproductive biology

Although the exact bounds of the spawning season were not determined, mature fish of both sexes could be found from mid June to early September, indicating a spawning season of at least 10 weeks. This agrees with the findings of Kesteven and Serventy (1941), Munro (1949), and Pollock (1982b) from studies of southern populations of yellowfin bream. Fig. 1 shows the corresponding water temperatures and photoperiods during this period for Townsville. The spawning season coincides with minimum annual water temperatures and photoperiods, which are likely to have a controlling influence over the timing of spawning. Yellowfin bream appeared to feed actively during this time and were easily caught by hook and line.

During the spawning season, captured fish could be classified as either mature males in running ripe condition or mature females with yolk globule oocytes of 400 μm or greater in diameter. No immature, unripe, or completely spent fish of either sex were found. Running ripe males as small as 15 cm and 90 g were caught. This suggests that yellowfin bream become reproductively active in their first year, which was confirmed in the growout component of this work (Chapter 5). This disagrees with the findings of Pollock (1985) who showed bream to mature as males at the age of two years in Moreton Bay (27°S). This discrepancy may represent a genetic difference between isolated populations or may be a reflection of the higher water temperatures at lower latitudes.

Fig. 2 plots the lengths and corresponding sexes of all fish used in spawning trials, and clearly supports the suggestion put forward by Suparta *et al.* (1984), and later confirmed by Pollock (1985), that yellowfin

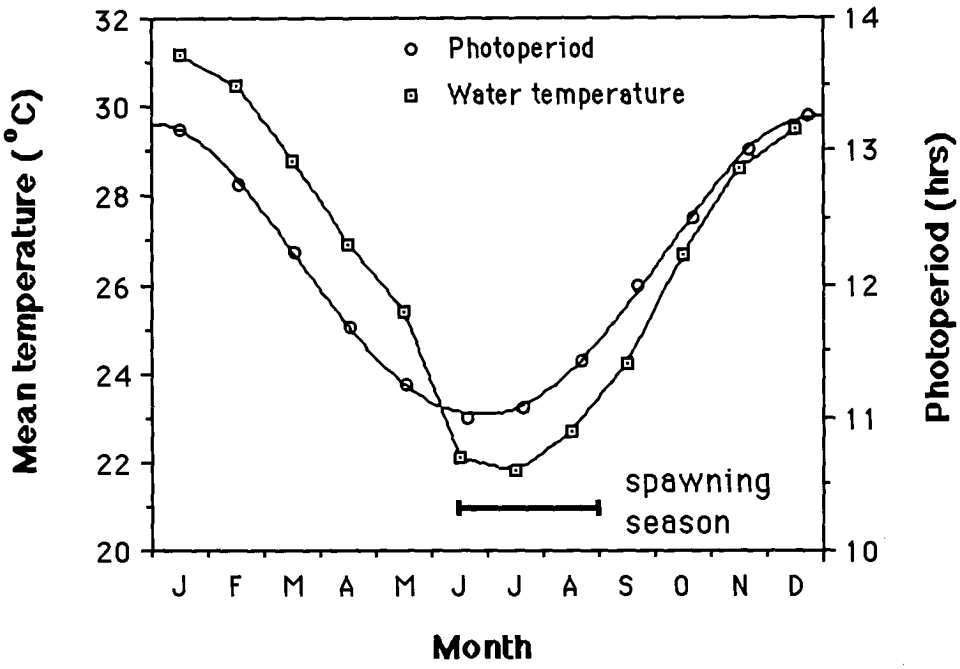


FIG. 1 - Mean monthly inshore water temperatures for Townsville (modified from Kenny, 1974), and corresponding photoperiod data. The spawning season for yellowfin bream, as observed in the present study, is seen to correspond with minimum water temperatures and photoperiods.

bream are protandrous hermaphrodites. Fig. 2 suggests an inversion when fish are in the size-range of 21-27 cm. It is possible, as suggested by Pollock (1985), that the largest males are 'primary' males which do not invert, and likewise that some females may never have been functional males. Confirmation of sex change was also possible from the growout component of this thesis (Chapter 5) where several fish (mean length 22.7 ± 1.4 cm) underwent a sex change from male to female in their second year. Pollock (1985) describes *A. australis* as having a typical sparid ovotestis.

On two occasions, during different years, running-ripe females were captured at the mouth of Townsville Harbour. On one such occasion the female was hand-stripped and the eggs artificially fertilised, yielding high quality eggs with excellent fertilisation (95%). Both instances occurred shortly after the new moon (2 nights and 5 nights after), and just before the top of big tides peaking near midnight. Munro (1944) reported most spawnings to occur near high tide during a full moon. In both lunar states spring tides are experienced, creating maximum water movement during the ebb tide into the ocean, where early larval development presumably occurs. Pollock *et al.* (1983) found the postlarvae (9-14 mm) to return into the estuary during the following full moon spring tides.

Pre-injection cannular biopsy

Fig. 3 is a frequency histogram of oocyte diameters from a wild-caught female bream early in the spawning season (mid-June). The sample is typical, except for the peak at 690 µm, which is present because this fish was preparing to spawn within hours of capture. Wallace and Selman (1981) classified ovaries into three types based on their oocyte size

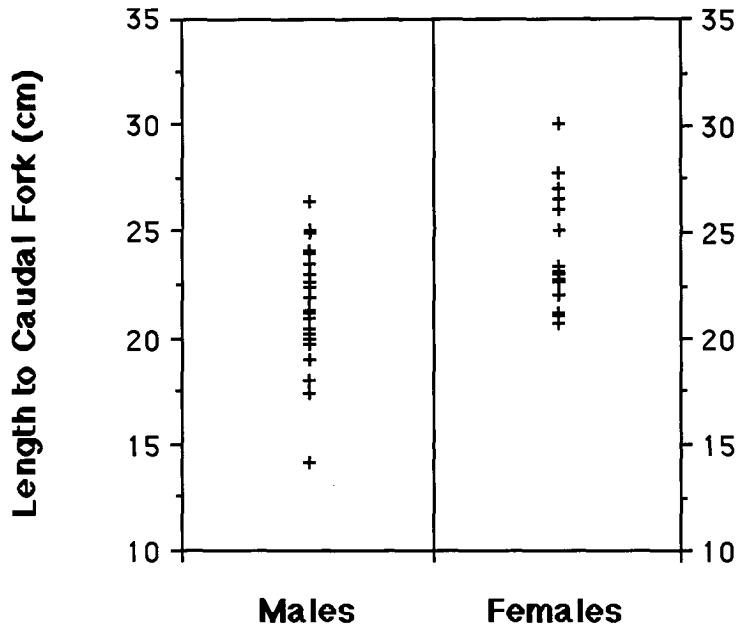


FIG. 2 - Lengths of all male and female spawners used in the present study, suggesting protandrous sex inversion.

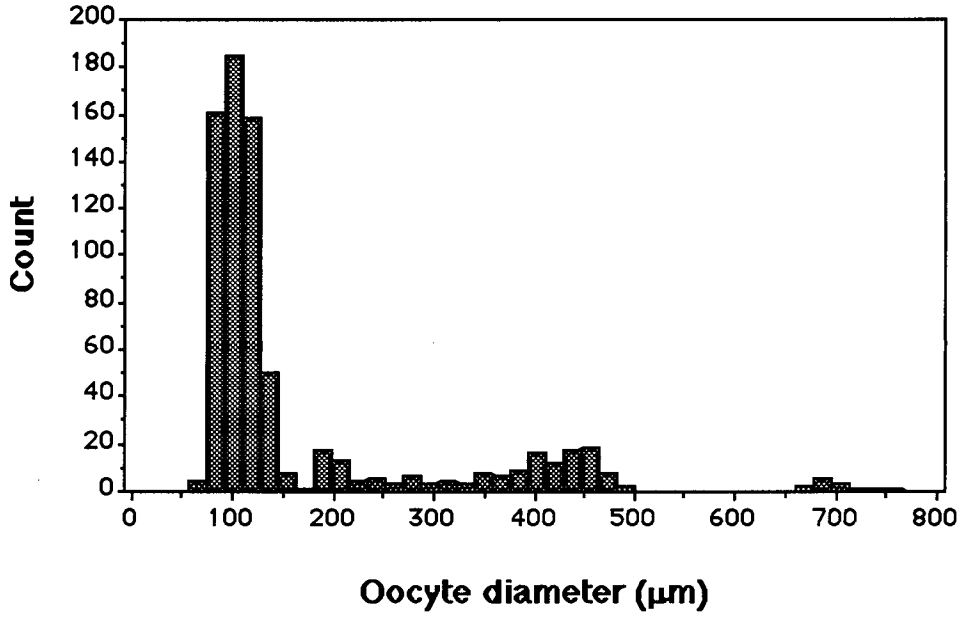


FIG. 3 - Frequency histogram of oocyte diameters appearing in a cannula sample from a wild-caught female bream in the spawning season. The peak at 690µm is unusual and indicates that this female was within hours of spawning when caught. The peak at 450µm will respond to appropriate hormonal stimulation.

distribution. According to this classification the yellowfin bream ovary is 'asynchronous', showing a range of smaller oocyte stages from which the current 'clutch' of eggs is derived. This indicates that yellowfin bream are serial (or 'partial' or 'multiple') spawners, spawning many times within the spawning season (DeVlaming, 1983). This is consistent with the known spawning habits of many other sparid species (Chen, 1990).

The peak at 450 µm is of yolk-globule stage oocytes, at the end of the vitellogenic stage of oocyte development, which respond to hormone treatment by advancing rapidly towards the final maturation (germinal vesicle break down, GVBD, and yolk granule coalescence), hydration and ovulation stages (West, 1989). All females caught in the spawning season had a similar peak. Apparently, oocytes mature to this stage, after which they remain dormant and do not advance further until appropriate hormonal stimulation in the form of a surge in circulating gonadotropin (GtH). This may occur either naturally in response to environmental cues or as a result of hormone injection.

Several studies have found LHRHa treatment to be successful in stimulating spawning only after fish had matured to a threshold reproductive stage (e.g. Carolsfeld *et al.*, 1988a). The minimum state of maturity required for yellowfin bream induction could not be determined in the present study, as even the least mature female caught, having a mean maximum oocyte diameter of 405 µm, was induced to spawn successfully.

Hormonal induction

(i) aqueous LHRHa

Goetz (1983) recognised that a minimum blood GtH level is required before the processes of final oocyte maturation will be induced to proceed.

Table 1 shows the result of triplicate spawning-induction trials done in the present study at dosages of 5, 10, 15, and 20 µg aqueous LHRHa/kg female body weight. A minimum dosage of 10 µg/kg was necessary to reliably initiate final oocyte maturation and full hydration, while a minimum dosage of 15 µg/kg was necessary for correct spawn timing (releasing eggs within their 'window of fertilisation'). Dosages of both 15 and 20 µg/kg most consistently produced good quality spawnings. The second trial at 15 µg/kg resulted in a low fertilisation rate (32%), but this is considered to be due to poor male performance rather than egg quality, as eggs microscopically appeared good according to criteria set out by Kjorsvik *et al.* (1990). The identification of a minimum effective dose for spawning induction is important as egg quality tends to deteriorate with increasing dosage (Kjorsvik *et al.*, 1990).

Carolsfeld *et al.* (1988a) stated that the response to a given LHRH analogue is species-specific. For example, the LHRHa used in the present study has very limited efficacy amongst the freshwater cyprinids (Peter *et al.*, 1987, 1988), and is required at dosages of up to 450 µg/kg to induce spawning in the mullet, *Mugil cephalus* (Lee *et al.*, 1987). Considering the low dosage requirements, and inferred high sensitivity of the yellowfin bream to the particular peptide used here (des-gly^{10}, D-ala^{6}, pro^{9}-ethylamide)LHRHa, this hormone must mimic closely the pituitary's natural gonadotropin releasing-factor. This responsiveness makes LHRHa an ideal agent for inducing spawnings in yellowfin bream.

Other publications detailing the induced spawning of sparids almost invariably referred to use of Human Chorionic Gonadotropin (HCG) at dosages ranging from 500-5,000 IU/kg (C. Mordles, 1983; Mok, 1985; Chen, 1990; Battaglene and Talbot, 1992). However, Chang *et al.* (1991)

LHRHa dose (µg/kg)	female size (g)	pre-injection oocyte diam. (µm)	oocyte hydration	spawning	egg number	% fert'n	comments
5	375	458.4	full	yes	13,500	< 1%	over-ripe
5	300	425.6	partial	no	-	-	-
5	375	474.8	full	yes	45,450	95%	good
10	475	468.5	full	yes	9,500	90%	good
10	300	470.3	full	yes	4,100	0%	over-ripe
10	475	461.5	full	yes	62,100	96%	good
15	300	405.0	full	yes	49,400	69.8%	good
15	275	447.0	full	yes	55,100	32%	poor male
15	425	435.1	full	yes	25,200	89%	good
20	525	446.4	full	yes	29,400	77.2%	good
20	225	515.1	full	yes	16,000	85%	good
20	375	412.1	full	yes	22,000	92%	good

TABLE 1 - Results of injecting mature female bream with aqueous LHRHa dosages ranging from 5 to 20 µg/kg, in triplicate. A minimum dosage of 15-20 µg/kg is required for consistent good quality spawnings.

successfully induced mature female black porgy (*A. schlegeli*) to spawn with LHRHa at dosages of 60 µg/kg, as well as with HCG at 3000 IU/kg.

(ii) pelletised LHRHa

A single 'slow-release' pellet implant was capable of inducing multiple spawnings of female bream on consecutive nights. Similar results were obtained by Almendras *et al.* (1988) when using pellet implants in barramundi. Whereas a single aqueous injection of LHRHa can raise plasma GtH over basal level for up to 48 h, a pellet implant can raise GtH levels for several weeks depending on the chosen cholesterol/cellulose matrix (Crim *et al.*, 1988). Table 2 shows the results of pellet implant spawning induction trials. It can be seen that a pellet containing 20 µg LHRHa/kg in an 80% cholesterol/20% cellulose matrix was not sufficient to reliably induce even single spawnings. However, by altering the matrix mix to 85% cholesterol/15% cellulose, and doubling the hormone dose to nearly 40 µg LHRHa/kg, there was a significant improvement with two females producing consecutive good-quality spawnings.

This result is in accordance with the findings of Sherwood *et al.* (1988), who measured the release of LHRHa from various pellet formulations using a perfusion chamber. These authors found that approximately 90% of the LHRHa content was released in 24 h from a 75% cholesterol/25% cellulose pellet; whereas only 21% was released in the same period from a 95/5% pellet. Together, the higher hormone content and more sustained release from the 85/15% pellets used in the present study (containing 40 µg LHRHa/kg) were most effective. Continuous nightly spawnings could likely be achieved through use of, for example, a 90/10% composition, containing up to 100 µg LHRHa/kg. Alternatively,

LHRHa dose (µg/kg)	female size (g)	pellet composition (chol./cell.)	pre-injection oocyte diam. (µm)	1st spawning egg #/% fert'n	2nd spawning egg #/% fert'n	comments
19.1	425	80/20%	459.3	58,400/98%	-	good spawning.
20.9	275	80/20%	465.4	-	-	partial hydration.
19.7	475	80/20%	470.1	-	-	full hydration, no spawning.
39.7	475	85/15%	453.3	22,500/97%	51,750/92%	good spawnings.
38.5	325	85/15%	465.4	14,900/<1%	-	over-ripe.
39.2	300	85/15%	478.4	36,700/90%	28,150/96%	good spawnings.

TABLE 2 - Results of spawning induction trials using various pellet compositions and LHRHa dosages. The slower-release pellets (85/15%) at dosages near 40 µg/kg were most successful, inducing multiple consecutive spawnings.

both an aqueous and slower-release (e.g. 90/10%) pellet hormone application could be given simultaneously.

(iii) Ovaprim

At the recommended dosage of 0.5 ml/kg, Ovaprim was capable of reliably initiating oocyte final maturation and hydration, ovulation and spawning (Table 3). However, in only one of the three trials did the female spawn while eggs were in their window of fertilisation to yield good quality eggs with high fertilisation rate. Eggs from the other two spawnings were classically over-ripe, showing a wide periviteline space, wrinkled surface, irregular shape and partially opaque yolk. This permitted only low fertilisation rates of 17% and < 1% (Table 3). To improve these results, a higher dosage would appear necessary. Back-flow loss could then become significant unless administered intraperitoneally. The high viscosity of Ovaprim makes it more difficult to work with than aqueous LHRHa.

Ovaprim consists of the fish gonadotropin-releasing hormone (D-arg^6, pro^9-NEt)sGnRH together with the dopamine antagonist domperidone, in a propylene glycol solvent. The exact concentrations of active agents could not be ascertained due to proprietary confidentiality. This hormone molecule is different from the LHRHa molecule used successfully previously. Peter (1986) and Carolsfeld *et al.* (1988a) stress the specificity of peptide hormones and their varied efficacy on different fish species. The molecule used in Ovaprim is apparently less potent on yellowfin bream than (des-gly^{10}, D-ala^6, pro^9-ethylamide) LHRHa, or the manufacturer's recommended dosage is lower.

The dopamine antagonist domperidone is present in Ovaprim to suppress the pituitary's gonadotropin release-inhibitory system

Ovaprim dose (ml/kg)	fish size (g)	pre-injection oocyte diam. (μm)	oocyte hydration	spawning	egg number	% fert'n	comments
0.5	375	476.9	full	yes	83,000	17.2	over-ripe
0.5	500	437.1	full	yes	58,960	97	good
0.5	300	481.8	full	yes	45,780	< 1	over-ripe

TABLE 3 - Results of spawning trials using the hormonal preparation 'Ovaprim' at the suggested dosage. Only one female spawned while eggs were in their 'window of fertilisation'.

(Donaldson and Hunter, 1983). In yellowfin bream this system appears to be weak, as females could be easily induced to spawn with relatively low LHRHa dosages, and without a dopamine antagonist.

In conclusion, LHRHa was the most effective hormone tested, requiring a minimum aqueous dose of 15-20 µg/kg to reliably induce single spawnings, and at a dose of 40 µg/kg in an 85% cholesterol/15% cellulose pellet, permited multiple spawnings on consecutive nights. However, clearly the response of individual fish to similar or identical treatments is quite variable (Tables 1-3), a result not surprising given the physiological complexity of processes involved in teleost reproduction. It is therefore acknowledged that additional replications would be useful in further refining minimum effective dosages.

Serial spawning trial

Spawning details from this trial are summarised in Figs 4-9. This 525g female spawned successfully on 27 nights in the 42-day trial, with only four additional hormone treatments after the initial pellet implants. A total of 862,000 eggs were spawned, with mean fertilisation rate of 84.7% (range 25.9-98.7%). Figs. 5-9 show graphically the variations throughout the spawning series in spawning time, spawning size, percent fertilisation, and egg and oil globule diameter.

Based on the results from the 'stand-alone' pellet implant trials, the results from this trial were unexpected. The same pellet that produced unreliable results previously (80/20% matrix; 20 µg LHRHa/kg) was capable of inducing up to nine consecutive spawnings in this trial (Fig. 4). This apparent increased sensitivity to the hormone may be explained by the self-potentiation effect of LHRHa after repeated treatment of a fish (Peter, 1980; Carolsfeld *et al.*, 1988a), and the likely persistence of low

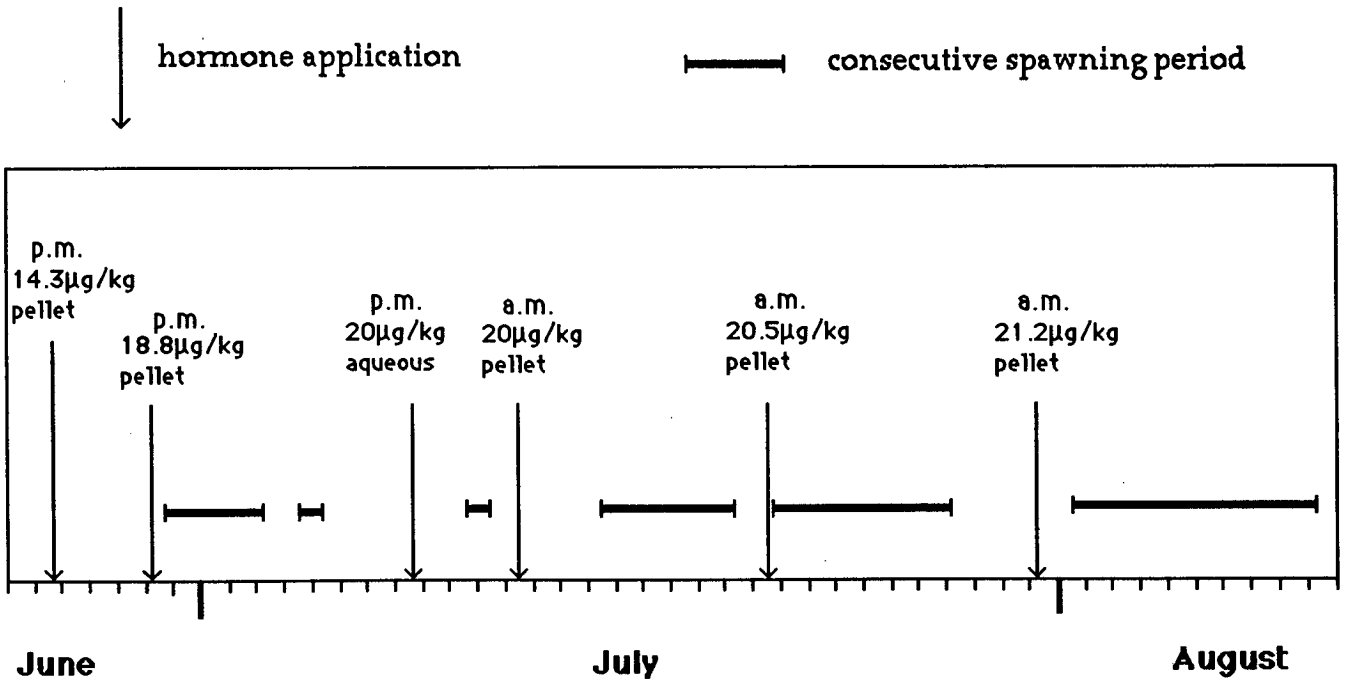


FIG. 4 - Results of the serial spawning trial, indicating hormone applications, times and resultant spawning periods. Female size was 525g.

levels of LHRHa in the circulation from previous pellets. Hormone release from a pellet is asymptotic, releasing diminishing amounts of hormone over very long periods (Carolsfeld *et al.*, 1988b; Sherwood *et al.*, 1988). Pellets of any composition have been shown to still release small amounts of hormone into circulation 28 days post-implant (Sherwood *et al.*, 1988). Prior to the last pellet implant, after which nine consecutive spawnings were observed, the female had received a total of four pellet implants. It follows that the residual hormone from these together could maintain elevated blood LHRHa levels, explaining the prolonged periods of spawning. Additional support for this explanation comes from the observation that spawning blocks became longer as the series progressed. First, four consecutive spawnings were observed, then five, then seven, then nine. This would be expected if each additional pellet implant further raised hormone levels in the circulation. Of course replication of this preliminary trial is necessary to confirm its results, particularly given the individual variations in physiological response to hormone injection.

Spawning time was generally in the early evening after dark, between 6 p.m. and 10 p.m. (Fig. 5). This is a characteristic common to most pelagic egg-producers (Thresher, 1984). However, there was a trend for spawnings to get later over time. Noticeable exceptions occurred for the first spawning following hormone treatments administered in the early morning, when spawning time was significantly later than usual (Fig. 5). This appears to be a reflection of the time of hormone injection, which forced the female to spawn outside the desired spawning time. After the first spawning in a block, the female apparently regained control over spawning time. No such abnormal spawning times were observed when hormone administration was carried out at 10-11 p.m. The first three spawnings of the entire series were the largest, each exceeding 67,000

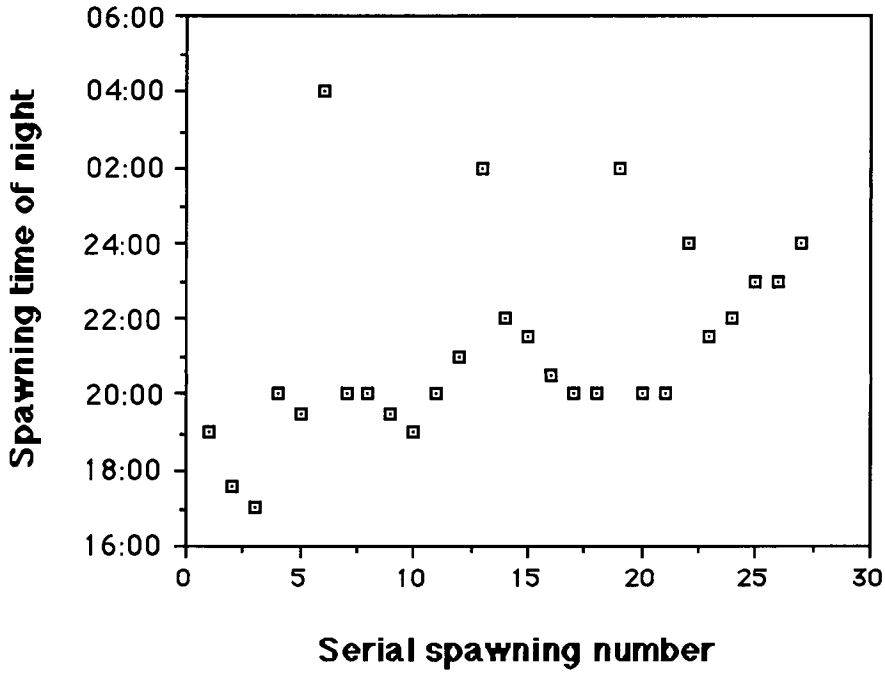


FIG. 5 - Spawning times recorded from the female bream used in the serial spawning trial. Most spawnings occurred between 7 p.m. and 10 p.m.

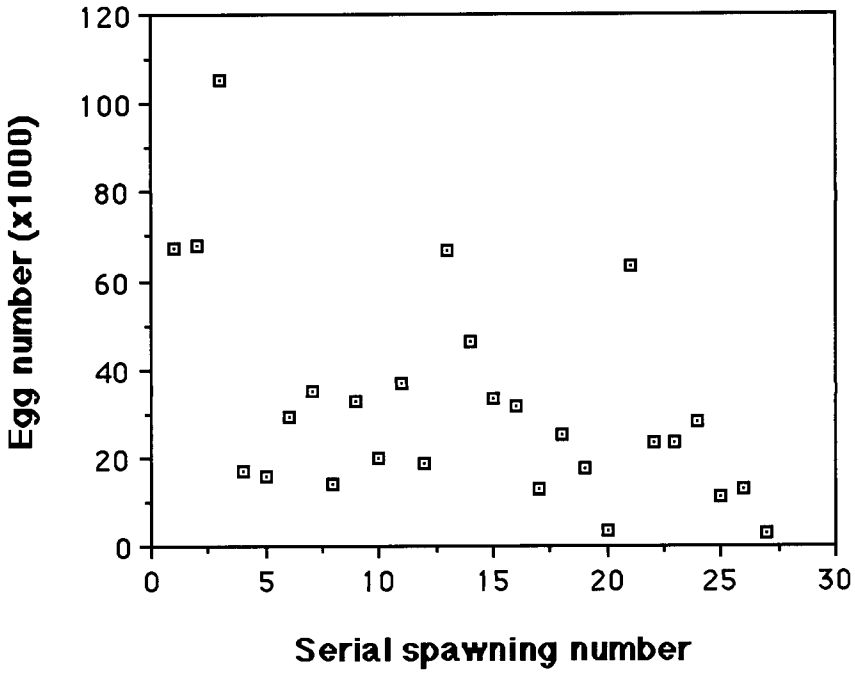


FIG. 6 - The number of eggs spawned at each spawning in the serial spawning trial. The majority of spawnings fall in the range of 10,000 to 40,000 eggs.

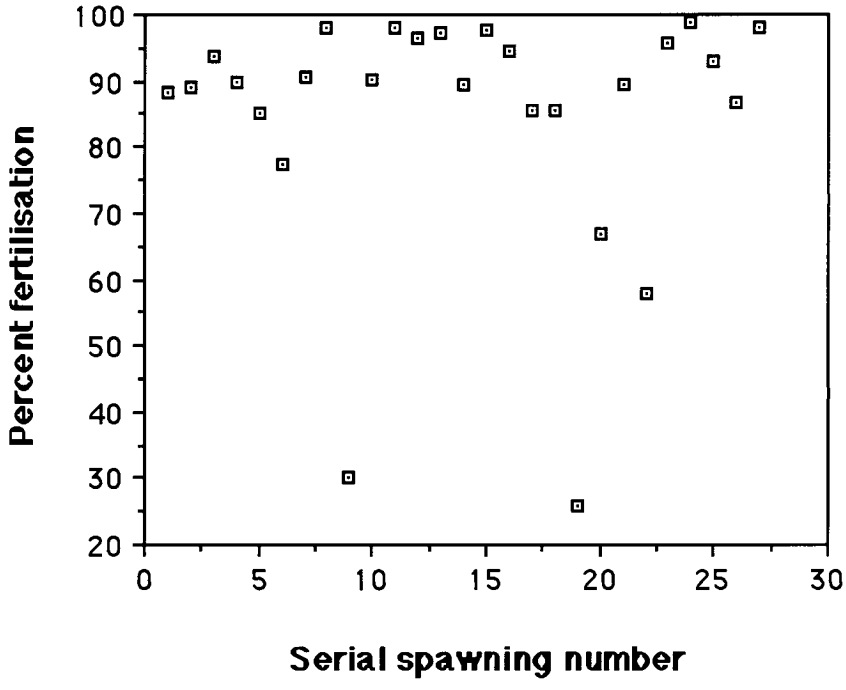


FIG. 7 - Percent fertilisation achieved in each spawning of the serial spawning trial. Generally fertilisation is high (>85%). Lower rates appeared to be due to male deficiency rather than poor egg quality.

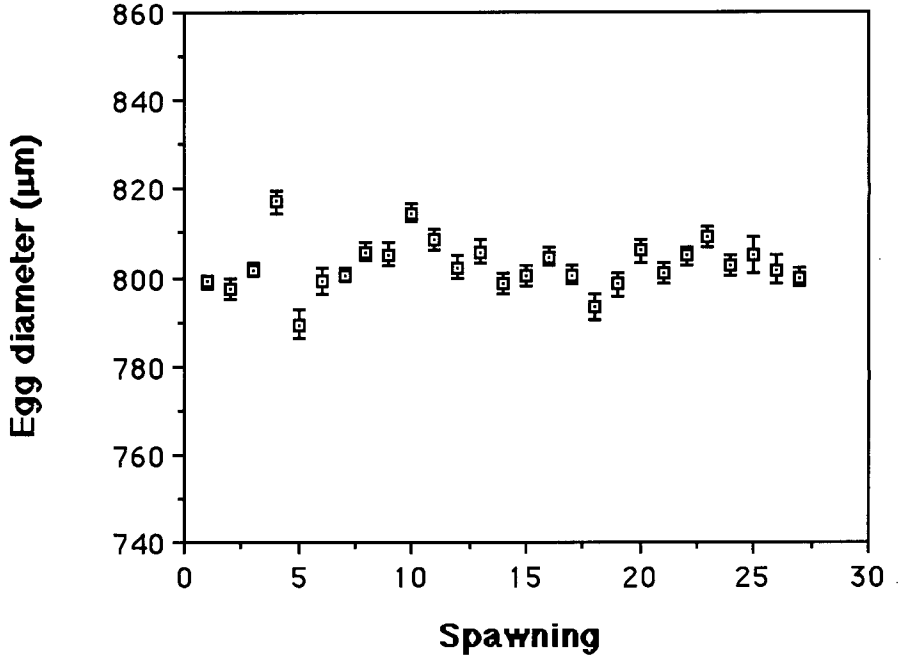


FIG. 8 - Mean egg diameters from spawnings throughout the serial spawning trial. Egg diameters are consistently near 800µm. Error bars represent standard errors (n=30).

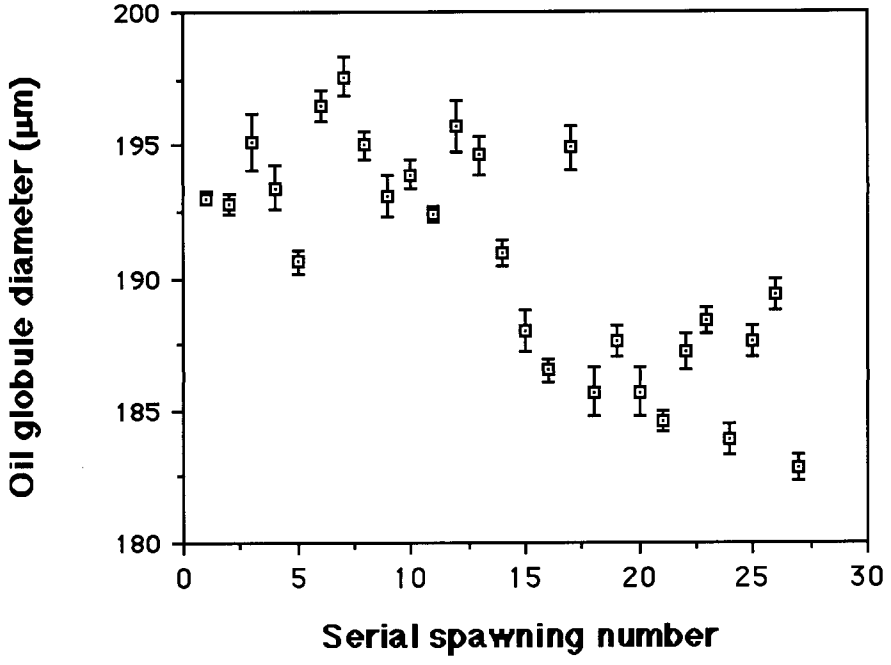


FIG. 9 - Mean oil globule diameters from spawnings throughout the serial spawning trial. The diameters tended to decrease as the spawning series progressed. Error bars represent standard errors (n=30).

eggs. Most subsequent spawnings were in the range of 10,000-40,000 eggs (Fig. 6). At the very end of the series there was a trend towards smaller spawning sizes.

The fertilisation rate was generally very high, with 22 of the 27 spawnings exceeding 85%. Two spawnings, however, were below 30% (Fig. 7). Both cases are thought to be due to poor male performance in fertilising, as eggs physically appeared to be of good quality according to criteria set out by Kjorsvik *et al.* (1990).

Egg diameters were quite consistently near 800 μm, although some apparently random variation (up to 3.5%) did occur (Fig. 8). As egg and larval size are known to correlate well (Kjorsvik *et al.*, 1990) this would appear to represent a means for small differences in survival potential to arise between spawnings, if the size differences are carried through to first-feeding. Kjorsvik *et al.* (1990) state, however, that published data do not indicate egg diameter to be a good criterion for egg quality.

Oil globule diameters tended to decrease in size as the spawning series progressed over time (Fig. 9). The mean oil globule diameter was significantly larger at 194.1 ± 2.0 μm (± s.d.) for the first ten spawnings, than at 186.3 ± 2.1 μm for the last ten ($t = 8.48$; d.f. = 18; $p < 0.001$). As the volume of a sphere varies with the cube of the radius, this difference translates to a large 11.6 % decrease in mean oil globule volume. The oil globule sustains the larva while making the transition from endogenous to exogenous nutrition. It therefore should play a critical role in minimising the mortality peak often observed at this time. Eggs showing smaller oil globules may therefore be considered to be inferior in quality, and circumstantial evidence would suggest that species whose larvae have

relatively small oil globules are more difficult to rear artificially (Doi and Singhagraiwan, 1993).

Fatty acid analysis of eggs

The fatty acid composition of eggs from every second or third spawning from the serial spawning trial is presented in Table 4. Due to their recognised importance to marine fish larvae, variations in the % of EPA (20:5n3) and DHA (22:6n3) between spawnings have also been plotted graphically in Fig. 10. It can be seen that the fatty acid composition of eggs from different spawnings fluctuates to a considerable extent, and that the four fatty acids oleic (18:1n9), linoleic (18:2n6), eicosapentaenoic (20:5n3), and docosahexaenoic (22:6n3), are particularly variable. Above all, levels of DHA were most unstable. Given the documented importance of n-3 HUFAs (highly unsaturated fatty acids) to marine larval fish, and the established link between egg n-3 HUFA level and survival potential in numerous species (e.g. Kraul *et al.*, 1992; Tuncer and Harrell, 1992; Rainuzzo, 1993; Watanabe, 1993), the fluctuations observed here in EPA, and DHA in particular, may represent true variations in egg quality. Levels of EPA and DHA have been suggested as criteria in egg quality assessment for gilthead seabream (Mourente and Odriozola, 1990b). These variations, in turn, could be expected to impact on larval survivorship as they are carried over to the larva (Mourente and Odriozola, 1990a). This, however, this was not investigated in the present study.

Contrary to expectations, initial spawnings (up to the sixth) were inferior in quality, from the viewpoint of % DHA content, than later ones. Spawnings 8-18 were consistently higher in % DHA, after which levels became less stable and tended to decline (Fig. 10). It should be borne in

Spawn #	Fatty Acid										
	14:0	16:0	16:1n7	18:0	18:1n9	18:1n6	18:2n6	20:4n6	20:5n3	22:5n3	22:6n3
1	**1.86**	**17.77**	**4.91**	**7.87**	**17.39**	**1.50**	**4.09**	**4.00**	**1.35**	**4.16**	**10.88**
	-	-	-	-	-	-	-	-	-	-	-
2	**2.76** (2.71-2.81)	**20.40** (19.92-20.88)	**6.11** (4.50-7.72)	**7.27** (6.91-7.74)	**21.86** (19.2-24.52)	**2.75** (2.70-2.80)	**5.69** (5.47-5.91)	**4.03** (3.60-4.45)	**5.57** (4.94-6.20)	**5.03** (4.83-5.23)	**11.71** (11.46-11.96)
4	**2.63** (2.53-2.73)	**23.54** (23.24-23.83)	**5.24** (5.05-5.42)	**7.78** (7.41-8.14)	**18.64** (17.57-19.71)	**2.29** (2.03-2.54)	**4.89** (4.37-5.41)	**3.60** (3.51-3.69)	**2.98** (1.89-4.07)	**4.79** (4.72-4.87)	**11.89** (11.82-11.96)
6	**2.53** (2.48-2.58)	**22.51** (21.32-23.69)	**6.58** (5.09-8.07)	**6.54** (6.14-6.93)	**21.48** (18.71-24.24)	**2.36** (2.30-2.41)	**5.40** (5.09-5.71)	**3.33** (3.21-3.45)	**4.93** (4.11-5.75)	**4.38** (4.10-4.66)	**12.41** (12.24-12.58)
8	**2.81** (2.41-3.20)	**24.11** (24.11-24.12)	**7.81** (6.70-8.91)	**6.39** (6.06-6.72)	**12.83** (12.05-13.60)	**3.19** (2.78-3.61)	**1.60** (1.31-1.89)	**3.73** (3.61-3.85)	**3.91** (2.15-5.67)	**4.56** (4.06-5.05)	**19.25** (19.23-19.27)
9	**3.47**	**22.79**	**8.13**	**5.72**	**16.03**	**3.66**	**2.36**	**3.63**	**3.70**	**4.28**	**18.44**
	-	-	-	-	-	-	-	-	-	-	-
10	**1.82** (1.08-2.56)	**24.01** (21.95-26.07)	**7.55** (5.96-9.13)	**7.35** (6.75-7.94)	**13.98** (12.85-15.10)	**3.15** (2.78-3.52)	**1.43** (1.41-1.45)	**3.89** (3.73-4.06)	**4.37** (4.08-4.66)	**4.28** (4.12-4.45)	**18.59** (17.40-19.77)
13	**2.67** (2.16-3.17)	**21.11** (17.91-24.32)	**6.88** (5.78-7.97)	**6.05** (5.56-6.54)	**13.06** (13.01-13.10)	**2.86** (2.74-2.98)	**1.25** (1.14-1.36)	**3.99** (3.91-4.06)	**2.70** (1.26-4.14)	**4.59** (4.44-4.74)	**18.12** (17.49-18.75)
15	**1.83** (1.14-2.51)	**24.14** (22.18-26.10)	**7.30** (5.80-8.85)	**7.16** (6.67-7.66)	**15.47** (14.22-16.72)	**3.08** (2.71-3.44)	**1.44** (1.33-1.54)	**4.62** (4.53-4.70)	**4.87** (4.61-5.13)	**4.75** (4.68-4.82)	**17.10** (16.57-17.62)
18	**3.10** (2.83-3.37)	**24.11** (22.88-25.32)	**8.12** (7.16-9.07)	**7.19** (6.71-7.68)	**15.74** (15.62-15.86)	**3.29** (2.94-3.65)	**1.59** (1.30-1.88)	**4.60** (4.09-5.10)	**4.75** (4.27-5.22)	**4.66** (4.14-5.17)	**17.22** (16.76-17.67)
21	**2.06** (1.12-3.00)	**22.93** (21.49-24.36)	**8.60** (6.96-10.24)	**6.46** (6.19-6.73)	**17.29** (16.19-18.39)	**3.37** (2.84-3.89)	**1.72** (1.54-1.89)	**4.19** (3.93-4.46)	**5.15** (5.09-5.20)	**4.43** (4.26-4.60)	**14.2** (13.47-14.93)
24	**3.06** (3.04-3.07)	**22.38** (21.87-22.89)	**6.73** (6.12-7.34)	**6.70** (6.58-6.81)	**16.89** (15.67-18.10)	**3.56** (3.44-3.67)	**1.53** (1.46-1.59)	**5.03** (4.94-5.11)	**4.57** (4.44-4.67)	**5.35** (4.97-5.72)	**16.85** (16.82-16.88)
26	**2.65** (2.60-2.70)	**22.55** (20.52-24.58)	**7.79** (6.40-9.19)	**6.47** (5.80-7.15)	**17.37** (15.52-19.22)	**3.53** (3.43-3.63)	**1.83** (1.81-1.85)	**4.75** (4.28-5.22)	**5.82** (5.42-6.20)	**4.78** (4.71-4.84)	**15.19** (14.20-16.17)

TABLE 4 - Fatty acid profiles of eggs from the serial spawning trial. Figures in bold represent the mean of duplicate determinations (range below), except spawnings 1 and 9 which were not replicated due to an analytical error.

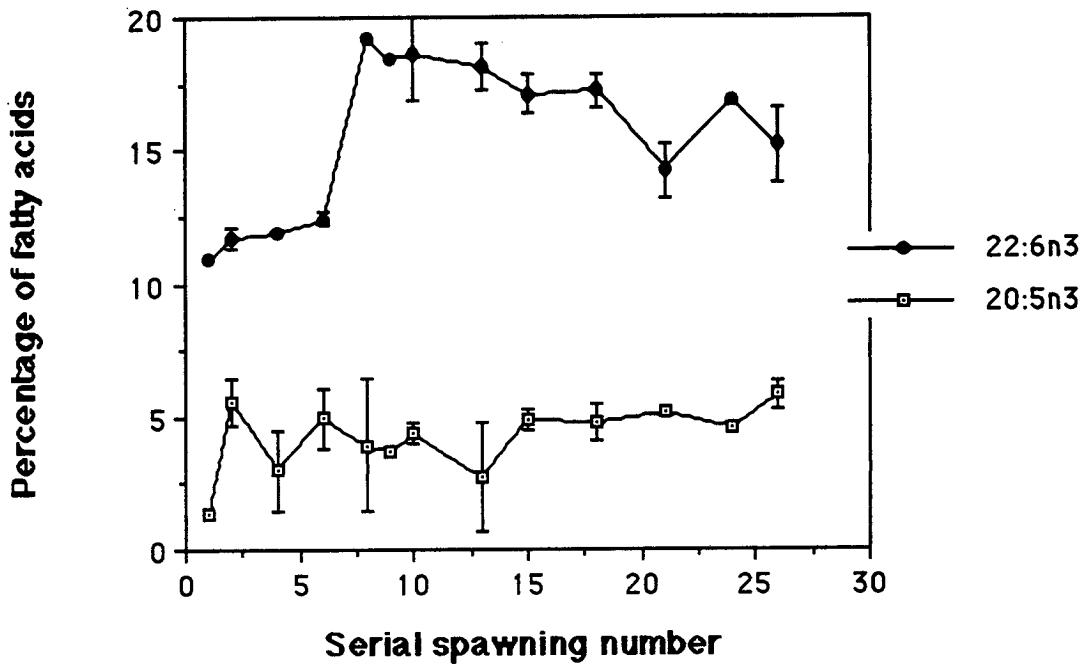


FIG. 10 - Variations in EPA (20:5n3) and DHA (22:6n3) content of eggs from different spawnings of the serial spawning trial. Data points represent means (± s.d.) of duplicate fatty acid determinations.

mind that these data represent % fatty acid composition, not absolute quantities per egg. As oil globule size also varied throughout the spawning progression (Fig. 9), absolute fatty acid levels will be a reflection of both this and % fatty acid composition. The highest quality eggs are likely to be those with the biggest oil globule and highest % DHA content, and this combination is seen within the range of spawnings 6-15 (Figs. 9 and 10).

The reasons for such variability are unclear. Few studies have investigated biochemical variability between egg batches from a single female, although variation between different females of a species has been demonstrated several times (e.g. Craik and Harvey, 1984b; Ulvund and Grahl-Nielsen, 1988; Kraul *et al.*, 1992), and similarly differences between individual eggs from a single spawning have been found (Ulvund and Grahl-Nielsen, 1988).

The fatty acid composition of the broodfish diet is well known to influence the fatty acid profile of spawned eggs (Watanabe *et al.*, 1984a-d, 1989; Mourente and Odriozola, 1990b; Rainuzzo, 1993). While the diet of the female prior to capture in the present study is unknown, it consisted solely of prawn meat throughout the serial spawning trial. Prawn meat is high in EPA and DHA (Clarke and Wickins, 1980), which at first appears to explain the large increase in DHA from spawning 8 onward. However, the natural diet is usually also considered to be high in essential fatty acids (Rainuzzo, 1993), so the prawn diet in captivity is unlikely to be superior. Furthermore, the eighth spawning occurred some 22 days after this female was brought into captivity, and the effects of a change in dietary HUFA level would be expected to have been manifested earlier. Watanabe *et al.* (1984c), studying the red seabream, found that changes in the nutritional composition of broodfish diets were remarkably reflected in the eggs in as

little as several hours, and that fat-soluble materials, such as fatty acids, were incorporated particularly quickly.

Also of interest is the apparent inverse relationship between levels of linoleic acid (18:2n6) and DHA (22:6n3) in the eggs (Table 4). This provides circumstantial evidence of a biochemical link between these two fatty acids. Such a link has been described (Brenner, 1974; Yu and Sinnhuber, 1976; Watanabe *et al.*, 1978; Leger *et al.*, 1981), whereby n-6 fatty acids such as 18:2n6 have an inhibitory effect upon the n-3 fatty acid bioconversion, such that elevated levels of 18:2n6 are accompanied by reduced levels of n-3 HUFA. The inhibitory effect occurs through competition between n-6 and n-3 precursors for the Δ6-desaturase enzyme. This interaction, then, may also to some extent explain variations in HUFA levels between spawnings.

Variations in maternal stress level may also contribute to the observed trend. Kraul *et al.* (1992) reported a correlation between broodstock stress levels and egg DHA content and larval survival. Further research is required to uncover reasons for the low DHA levels found in the initial spawnings, but, if replicated, this would imply that the best quality eggs from this species may come not from initial spawnings, but from those well into a spawning series.

Spawning assessment

(i) spawning behaviour

The spawning act was not witnessed as it occurred after dark, however the behaviour of broodfish from several hours after injection until a couple of hours before ovulation was observed on several occasions. During this time the male would frequently swim alongside the

female, often at considerable speed. When the female stopped, the male would often place itself in front of the female, angling its head down at 45° and simultaneously erecting its dorsal fin as a display. The male also frequently nudged the belly and vent of the female with its snout. Good spawning behaviour appears to be a prerequisite for successful spawnings, as on two occasions in which such behaviour was not observed the spawnings failed. The only other published account of spawning behaviour in a sparid is for *Chrysoblephus laticeps*, which showed similarities to the above (Buxton, 1990).

(ii) egg characteristics

Yellowfin bream eggs are spherical, transparent, pelagic, and positively buoyant, with a narrow perivitelline space. The mean size of fertilised water-hardened eggs was 786.8 ± 19.7 µm (range 751-817 µm). This represents a mean egg volume of 0.255 mm^3.

A single oil globule is present of mean diameter 186.2 ± 7.4 µm (range 167.0-197.7 µm). This represents a mean oil globule volume of 0.00338 mm^3. Mean egg wet and dry weights were 295.4 ± 3.3 µg and 38.7 ± 5.0 µg respectively, indicating a water content of 86.9%.

These eggs are quite typical of those described for other sparids, and consistent with the descriptions of *A. australis* eggs reported by Kesteven and Serventy (1941). However, they are amongst the smallest, in terms of both egg and oil globule diameters, in the family. Other species in the genus *Acanthopagrus* have eggs that are most similar to those of *A. australis*, while species in the genera *Diplodus*, *Sparus*, and *Pagrus* tend to have larger eggs and oil globules (Houde, 1974; Hussain *et al.*, 1981; Divanach *et al.*, 1982; Foscarini, 1988; Jug-Dujakovic and Glamuzina, 1988; Chen, 1990).

(iii) fecundity

Fig. 11 illustrates the relationship between female size and number of eggs shed in the first spawning after a hormone treatment. All 20 spawnings showing high egg quality and fertilisation rate are included; results from the serial spawning trial are not. Differences in spawning size could not be attributed to female size. Neither could they be attributed to pre-injection oocyte diameter, or to the of hormone treatment (dosage, hormone type or method of administration). First spawning size appeared to be quite random within the range of 9,000-83,000 eggs. Given that all females were wild-caught, and that at least some are likely to have spawned one or more times prior to being caught, they would be at various stages in their seasonal spawning progression and a randomness is not unexpected, even if larger females have greater fecundity.

As yellowfin bream are serial spawners, an estimate of true fecundity comes only from the serial spawning trial in which the female was repeatedly induced to spawn until nearly spent. This female weighed 525 g and yielded 862,000 eggs, representing a fecundity of 1.64 million eggs/kg. This should be regarded as a minimum fecundity as, again, the female may already have spawned prior to capture, although this possibility is low as it was caught early in the spawning season (June 25th). A fecundity of 1.64 million eggs/kg is amongst the highest reported in the family, which is not surprising when we consider the well-established inverse relationship between egg diameter and fecundity.

(iv) latent period, time of injection, and preferred spawning time

The latent period varied somewhat between females, but showed no clear correlation with temperature, time of day of injection, or the type or method of hormone administration. Weak correlations were found with

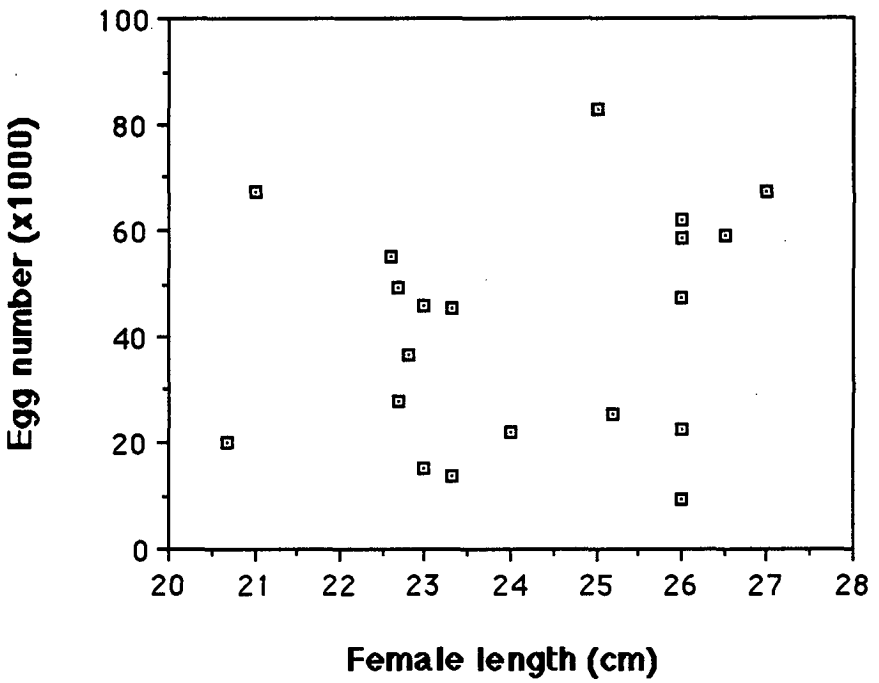


FIG. 11 - Number of eggs shed from females in the first spawning after hormone treatment, relative to female size. There is no trend for larger females to spawn more eggs in their first spawning.

the hormone dosage applied (Fig. 12), and with the state of ovarian development prior to injection (Fig. 13). Multiple regression was used to separate the effects of hormone dosage and oocyte size on latent period, but the model showed no significant effects ($F=4.65$, d.f.=2, adj. $R^2=0.55$, $p=0.091$). Partial R^2 values for hormone dosage and oocyte size were 0.64 ($p=0.08$) and 0.16 ($p=0.43$) respectively, showing hormone dosage to correlate best with latent period. However, eggs from the two females with a mean pre-injection oocyte diameter greater than 500 µm already showed signs of commencing hydration, and appear to be responding with shorter latency than other females (Fig. 13).

It appears, therefore, that females with oocyte diameters less than approximately 500 µm may be categorised together for the purposes of spawning induction, being expected to spawn approximately 45 hours after injection (range 40-53.3 h in the present study) at temperatures in the 21-24°C range. Any such females which spawned beyond this range without exception spawned over-ripe eggs which showed poor fertilisation rate. Females with pre-injection oocytes larger than approximately 500 µm seem to have already undergone some form of triggering, and will spawn in a shorter time (~36-37 h for fish with oocytes near 525 µm).

The preferred spawning time became apparent during the repeat serial spawning trial when the female consistently spawned between 6-10 p.m., except where the time of hormone application forced the female to spawn differently. Best spawning results were obtained when a female was injected at such a time as to enable the estimated latent period of 45 hours to coincide with its preferred spawning time (approximately 8 p.m.). As an example to the contrary, in one instance a female was injected at 6 p.m., which, assuming a latent period of 45 hours, was targeting the

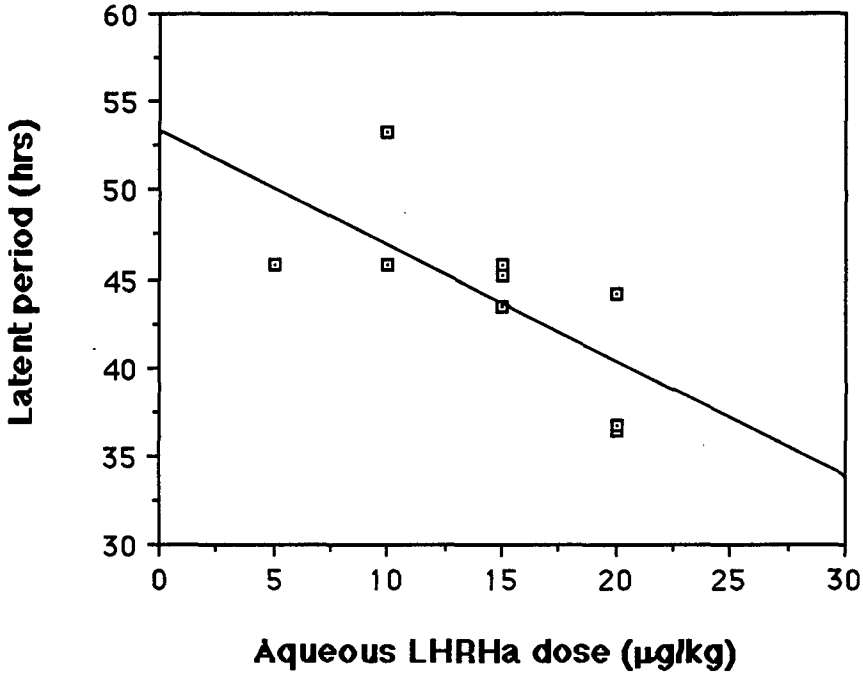


FIG. 12 - Effect of LHRHa dosage on observed latent period. Only successful spawning trials using aqueous LHRHa are included. There is a weak trend for larger hormone dosages to result in shorter latent periods (r=0.68).

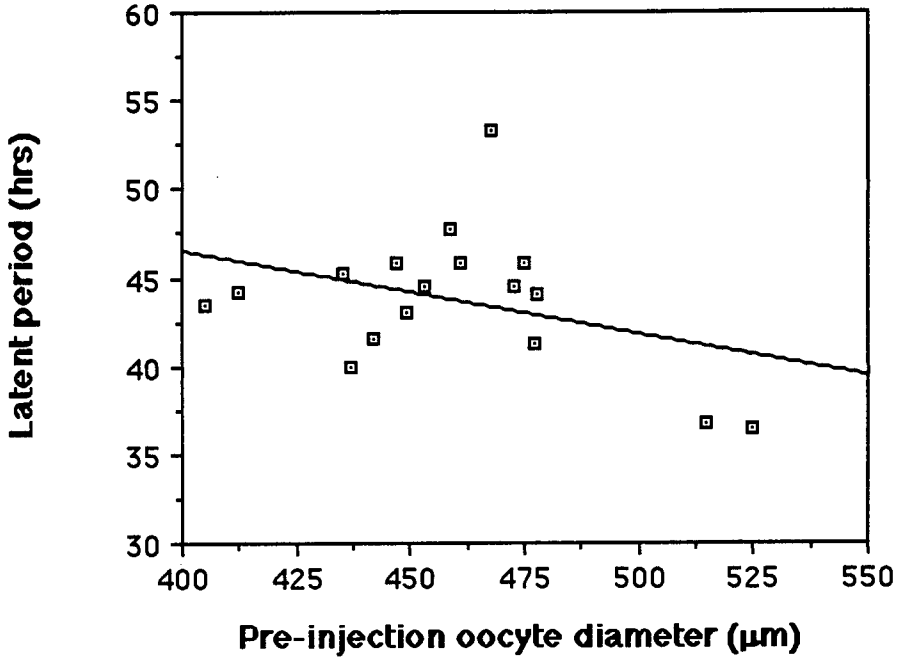


FIG. 13 - Observed latent period for all successful spawnings, relative to oocyte diameter prior to hormone injection. No significant correlation exists, although the two females with oocyte diameters over 500 µm appear to spawn with shorter latency. R=0.37.

female to spawn at 3 p.m. The female refrained from spawning until after dark by which time the eggs were clearly over-ripe. The best time for hormone administration, then, was approximately 10-11 p.m.

These observations, and others showing the clear importance of correct hormone injection time (e.g. Zohar (1988) while spawning the gilthead seabream), may also be explained by the hypotheses put forward by Alvarino *et al.* (1992) that daily changes occur in sensitivity either of the pituitary to LHRHa or of the ovary to GtH; or that injected hormones interact with daily patterns of endogenous hormone secretion, as shown to exist in the red seabream, *P. major* (Matsuyama *et al.*, 1988).

Oocyte maturation post-injection and the 'window of fertilisation'

Fig. 14 depicts both the enlargement of oocytes over time post-hormone treatment, and the period of time, or 'window', in which fully hydrated eggs are fertile for a female of 300 g and 22 cm. This particular female had a very short latent period of approximately 35 hours 30 min. It may be seen that in the first 24 hours post-injection the oocytes enlarge minimally, but then enlarge rapidly to a fully-hydrated condition by 30 hours. At this stage the eggs are still not able to be fertilised, but reach maximum fertility in the vicinity of 35 h 30 min. From here fertility again declines sharply. A percentage of eggs, therefore, could be fertilised for at least 5 hours, but maximal fertility occurred for a period of only approximately one hour. Although resulting larval condition was not investigated in the present study, Fortuny *et al.* (1988) found that sabalo eggs fertilised towards the end of their window of fertilisation showed elevated proportions of malformed larvae at hatch.

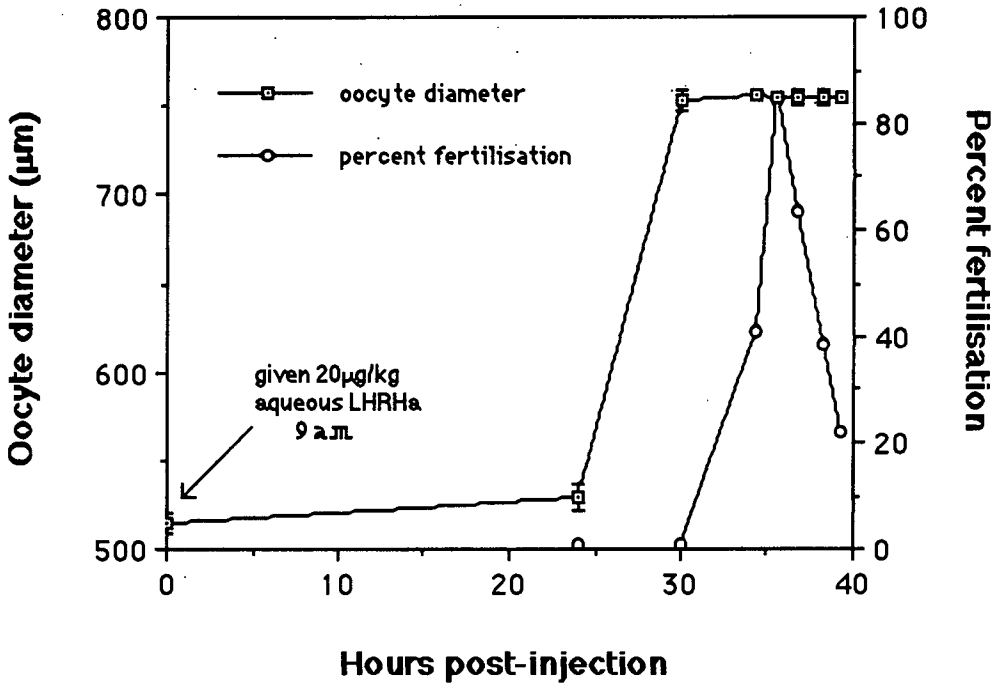


FIG. 14 - Path of oocyte final maturation and hydration of a 300 g female after hormonal treatment, as reflected in increasing oocyte diameter. Also the 'window' in which eggs are able to be fertilised is indicated. It may be seen that maximum fertilisation is achievable for only a short period of time, in this case near 36 hours. Error bars represent standard errors (n=30).

CHAPTER 3

Egg Incubation and Yolk Absorption

Introduction

Fish hatcheries aim to maximise the survival and growth of early life stages. The extent to which this is achieved is dependent on numerous factors, amongst which the physical rearing environment is of fundamental importance. Maintaining optimum temperature, salinity and lighting conditions will ensure maximal hatch rates, yolk utilisation efficiency (and hence first-feeding size), and survival, with lowest levels of deformity-related mortality (Blaxter, 1969, 1988; Alderdice, 1988). Further influences of temperature alone on growth, feeding rate, digestion, gut evacuation rates, behaviour, swimming speed, and indirect effects on the oxygen capacity and viscosity of water are also recognised (Blaxter, 1988). Hatchery considerations include the desirability to minimise the duration of larval rearing, and hence hatchery running costs.

In most marine fish hatcheries the largest mortality peak occurs through starvation, when larvae are undergoing the change from endogenous to exogenous nutrition (i.e. soon after yolk absorption). This has been termed the 'critical period' by fisheries biologists who have suggested that this stage may have a controlling influence on year-class strength of wild fish (Blaxter and Hempel, 1963; May, 1971,1974; Ehrlich, 1974). This mortality peak has generally been explained by a failure of larvae to capture first prey items, insufficient suitable prey items, and/or a low resistance to starvation (May, 1971,1974; Miller *et al.*, 1988). Maximising predation efficiency and resistance to starvation appear to be obvious ways of improving survival rates, and can

be achieved through maximising larval size at first-feeding (e.g. Blaxter and Hempel, 1963; Ryland and Nichols, 1967; Blaxter, 1969). First-feeding size, in turn, depends on such factors as egg size and yolk utilisation efficiency (Blaxter and Hempel, 1963; May, 1974; Hunter, 1981). While egg size may be maximised to some extent by careful selection and treatment of broodstock, yolk utilisation efficiency is largely dependent on the physical environment through yolk absorption (Ryland and Nichols, 1967).

Identification of optimum larval rearing conditions is particularly important amongst many marine fish, due to their relatively small size and limited energy stores and consequent short 'window' of initial feeding opportunity. This is in stark contrast with larvae of salmonids, such as the Atlantic salmon, *Salmo salar*, which have a 'window' of initial feeding opportunity lasting several weeks, and for which initial feeding can be delayed by 1-2 weeks without adversely affecting subsequent survival or growth (Koss and Bromage, 1990). Miller *et al.* (1988), in a review of 72 species of marine and freshwater teleosts, established the highly size-dependent susceptibility of first-feeding larvae to starvation. Furthermore, temperature may be more critical in the culture of warmwater marine fish than for temperate species, as the early life stages of warmwater species are often stenothermal (Brett, 1970; Saksena *et al.*, 1972; May, 1975; Santerre, 1976).

Blaxter and Hempel (1963) found that first-feeding larvae of the herring, *Clupea harengus*, survived longer before starvation when maintained in darkness and presumed this to be due to reduced activity and hence metabolic demand. Marr (1965, cited in Blaxter, 1969) found that the efficiency of yolk utilisation in the alevin of salmon, *S. salar*, and hence size at first-feeding, was dependent on light level. From these studies it appears that activity and metabolic rate can be influenced by light level, providing another

parameter which should be investigated before maximum performance can be gained from eggs and larvae.

The aims of this component of the thesis were to briefly describe the developmental sequences of eggs and early larval stages, and to determine optimum temperature and salinity conditions, in terms of growth, survival and incidence of deformity, through egg incubation and yolk absorption in yellowfin bream. Also, the notion that larger first-feeding larvae result when yolksac absorption is allowed to proceed in darkness, was investigated.

Materials and Methods

Egg and larval developmental sequences

Approximately 2000 newly-spawned eggs, obtained through hormonal induction of wild-caught broodfish, were placed in a 10 l incubator receiving light aeration. Water temperature was maintained at 23.0 ± 0.5°C, salinity at 34 ppt, and lighting at approximately 200 lux (12 h photoperiod). Times were noted, and black and white photographs taken, of major developmental events. This was continued through hatch and yolk absorption until the oil globule was absorbed.

Optimum temperature/salinity conditions for eggs and yolksac larvae

An experiment was designed to investigate the effects of incubating eggs and maintaining yolksac larvae at 5 different temperatures and 3 different salinities. A 5 x 3 factorial design was chosen to allow the investigation of all combinations thereof. The specific temperatures used were: 19.4, 22.6, 23.9, 25.4 and 27.7°C. These temperatures represent means of measurements taken throughout the experimental period. Temperatures did not vary in any bath

more than ± 0.6°C from these. The salinities chosen were 23, 29, and 35 ppt. This range was selected in order to determine whether yellowfin bream are restricted to full-strength seawater during spawning, as juveniles less than 2 months of age are known to tolerate salinities below 10ppt (pers. obs.). Salinities were checked by refractometer at the start, at hatch and at the completion of the trial, and remained essentially constant throughout the experiment, except in the 27.7°C bath where salinities increased up to 2 ppt over reference levels. Three replicates of each combination were provided, amounting to 45 experimental tubs.

The one litre experimental tubs used were circular and flat-bottomed in shape (12 cm diameter), and made of opaque food-grade plastic. Lids were fitted to minimise evaporation. Tubs were two-thirds immersed in temperature-controlled water baths, 9 tubs being placed randomly per bath. Each tub was equipped with an adjustable air supply in the form of an airstone. Temperature was measured twice daily throughout the trial. An opaque black cover was placed permanently over each bath.

Eggs were obtained through hormone-induced spawning of wild-caught broodfish, and all were derived from the same spawning. Comparison of egg appearance and dimensions, hatch rates and larval sizes with other spawnings suggest that egg quality was typical for the species. Eggs were incubated for 2 h to allow unfertilised eggs to sink and be removed. Remaining fertilised eggs were disinfected in a 100 mg/l iodine bath for 10 min before the commencement of the experiment. From the iodine bath eggs were rinsed and lots of 59 eggs in early cleavage randomly allocated to each of the 45 experimental tubs (i.e. stocking density 59 eggs/l). Tubs to contain 35 ppt salinity were filled up to one litre with 1 µm-filtered seawater, eggs added, then placed in the water baths to equilibrate to bath temperature. Tubs to contain lower salinities had eggs added to an appropriate volume of

35ppt seawater to which distilled water was slowly added. Equilibration rates did not exceed 2.6°C or 9 ppt/h.

Egg incubation time was noted for each tub based on the time taken for approximately 50% of eggs to hatch (determined by direct count). The number of eggs to hatch in each tub was also noted. A sample of 10 larvae was taken from each tub after hatch, and the notochord length measured, using an ocular micrometer, and the percentage of larvae carrying obvious physical deformities noted at this time. These most commonly were spinal curvatures, or deformities of the finfold, caudal region, or skeleton.

Similar measurements were taken upon near completion of yolksac absorption - time to 95% yolksac absorption, percentage survival relative to the number of hatched larvae, and, using an ocular micrometer, notochord length was again noted.

Significant differences in hatch rate and survival to completion of yolk absorption were detected using two-way analysis of variance (ANOVA; $p < 0.05$), although survival data were first subjected to arcsin transformation (Bishop, 1983). Unfortunately, low hatch and survival rates, and high numbers of deformed larvae, at combinations of high temperature and low salinity precluded full 5 x 3 two-way ANOVA from being carried out on lengths at hatch and at yolk absorption, and on hatch deformity data. Instead, hatch deformity data were analysed by log-linear analysis ($p < 0.05$) (Zar, 1984); a 3 x 3 two-way ANOVA using all salinities but only the three lower temperatures was used to detect significant hatch size differences; and a one-way ANOVA was used to detect significant size differences at yolk absorption between all temperatures at 35 ppt salinity. Homogeneity of variance was tested for sets of data by examining residuals, and the Tukey multiple comparison test was used to separate effects.

Effect of light intensity on yolk absorption efficiency

To investigate the effect of light intensity on the efficiency of yolk absorption, larvae were allowed to absorb their yolksacs at three different light levels, under otherwise equivalent conditions. Three 10 l plastic containers were used for each treatment, which included complete darkness, low light (250 lux), and high light (2000 lux). Fluorescent lighting ('cool white' tubes) was used to minimise heating effects. The three containers in darkness were completely covered with opaque black plastic. Each container was filled to the 8 l mark with 34 ppt salinity water, which was maintained at 23°C ±1°C. To this, 400 newly-hatched larvae were added by volumetric estimation (i.e. 50 larvae/l).

At regular intervals (0 h, 10 h, 20 h, 30 h), and at 95% yolk absorption (39 h), samples of 15 larvae were removed from each container, anaesthetised in 70 mg/l MS-222, and preserved in buffered 2.5% gluteraldehyde (Oozeki and Hirano, 1988). These were later measured for total length, and yolksac length and width, enabling an estimation of yolk volume to be made using the formula below (Alderdice *et al.*, 1979). Thirty larvae were measured before preservation to allow an estimate of preservation shrinkage effects to be made.

$$\text{yolk volume} = \frac{4}{3}\pi\left(\frac{R_1}{4}\right)^2 \times \left(\frac{R_2}{2}\right)^2$$

where R_1 = radius of yolksac length
R_2 = radius of yolksac width

Plots of yolk disappearance and larval growth over time were then made for each treatment. One-way ANOVA ($p < 0.05$) and the Tukey multiple comparison test were used to compare yolk usage and growth patterns between treatments at each time interval, and most importantly to compare the final lengths of larvae after yolk absorption from each of the three light treatments. Homogeneity of variance was tested for sets of data by examining residuals.

Results and Discussion

Egg and larval developmental sequences

Egg development was rapid, reaching hatch by 33.5 h after fertilisation, at 23°C. Mean total length at hatch was 2.03 mm (range 1.82-2.14 mm), with a mean yolk volume of 0.116 ± 0.027 mm^3 (from larvae of mean length 2.13 mm). The yolksac does not extend anteriorly to the head, and an oil droplet, of volume 0.00338 mm^3, is situated in the posterior part of the yolk (Plate 16). Numerous pigment spots are present over the body surface at hatch. These all appear to be characteristics common to sparid larvae (Hussain *et al.*, 1981; Divanach *et al.*, 1982; Jug-Dujakovic and Glamuzina, 1988; Fukusho, 1991; Pankhurst *et al.*, 1991). Larvae were distributed evenly throughout the water column while the yolksac was being absorbed, after which the mean total length of larvae was 3.15 mm.

The development of yellowfin bream eggs was similar to that of other sparids and of pelagic eggs in general. The timing of major developmental events at 23°C was as follows:

fertilisation	0 min
blastodisc	10 min
first cell division	35 min
second cell division	45 min
morula stage	2 h 20 min
blastula stage	4 h 30 min
gastrula stage	8 h
late gastrula stage	11 h 30 min
neurula stage	14 h
early embryonic body	17 h 30 min
somatic segmentation, Kupffer's vesicle, melanophores, appearance of optic vesicles	18 h 30 min
disappearance of Kupffer's vesicle	22 h 30 min

heart starts to beat	24 h 20 min
HATCH	33 h 30 min
yolk half absorbed	10 h 40 min post-hatch
yolk fully absorbed, eyes pigmented, mouth and anus open	39 h post-hatch
oil globule absorbed	85 h post-hatch

Plates 1-19 depict most of these developmental events.

With a hatching size of 2.03 mm, yellowfin bream larvae are amongst the smallest of the Sparidae. This is apparently a reflection of the relatively small egg size, which is the smallest reported for any sparid. Only two species, *A. cuvieri* and *Archosargus probatocephalus*, have smaller reported hatch sizes of 1.87 mm (Hussain *et al.*, 1981) and 1.65 mm (Tucker, 1987), respectively. Similarly, size at first feeding is relatively small at 3.15 mm, which is the same as that reported for *A. cuvieri.* At the other extreme is *Diplodus sargus*, with a mean hatching size of 3.0 mm, and a first-feeding size of 4.3 mm (Divanach *et al.*, 1982). In general, the recorded hatch size of sparid larvae has fallen in the range 2.0-2.8 mm, and first-feeding size in the range 3.15-3.8 mm (Faranda *et al.*, 1985; Mok, 1985; Jug-Dujakovic and Glamuzina, 1988; Chen, 1990; Fukusho, 1991; Pankhurst *et al.*, 1991).

Optimum temperature/salinity conditions for eggs and yolksac larvae

Both temperature and salinity, within the ranges of the experiment, had important effects on egg and early larval development of yellowfin bream. Statistical analyses showed that effects of temperature and salinity on percent hatch, percent hatch deformities, and percent survival to yolksac absorption were all significant (see below). Also, temperature had a significant effect on larval size at completion of yolk absorption. Most of these factors have obvious relevance in an aquaculture situation. Eggs and larvae could tolerate

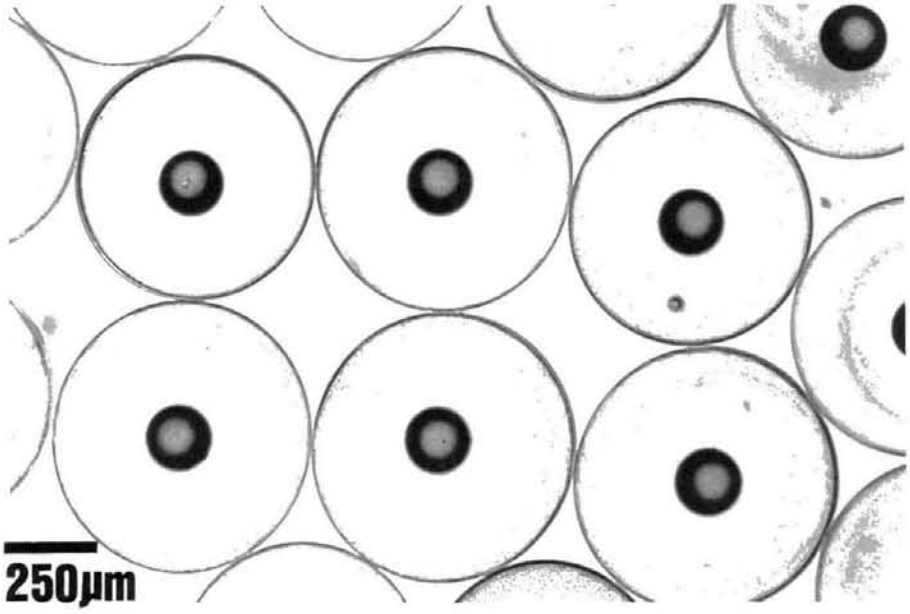


PLATE 1 - Formation of the blastodisc, 10 min. Scale applies to Plates 1-11 and 13-15.

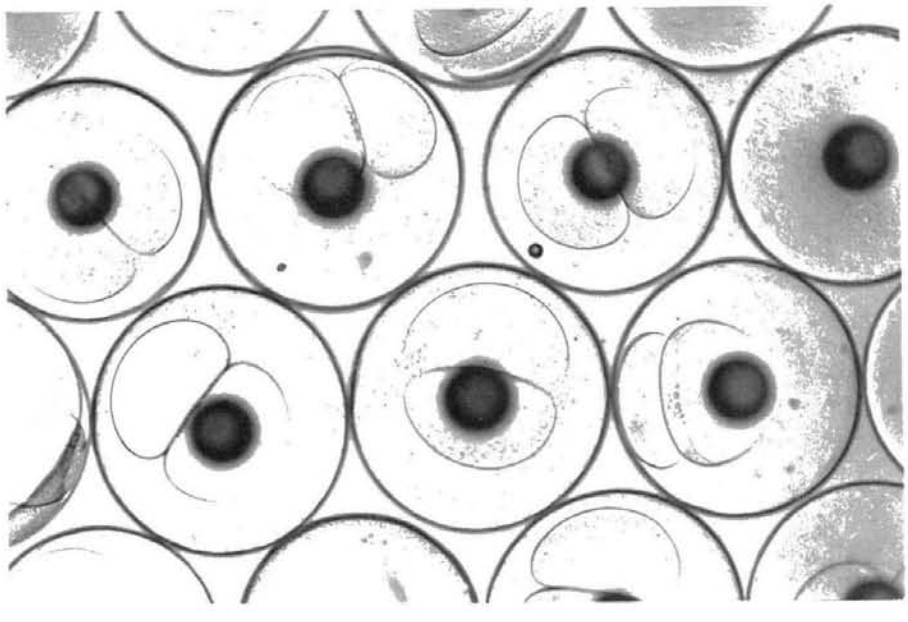


PLATE 2 - First cell division, 35 min.

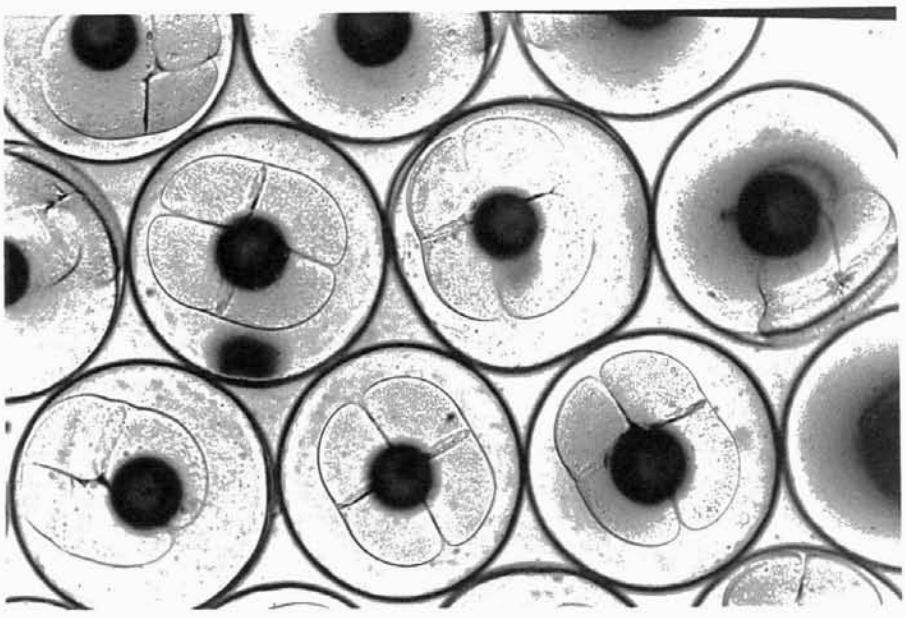


PLATE 3 - Second cell division, 45 min.

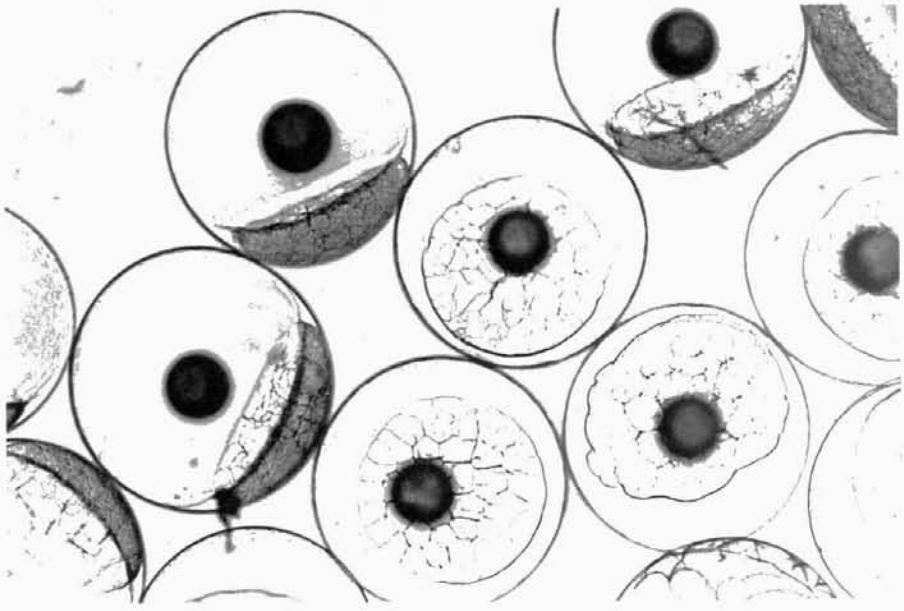


PLATE 4 - Early morula stage, 2 h 20 min.

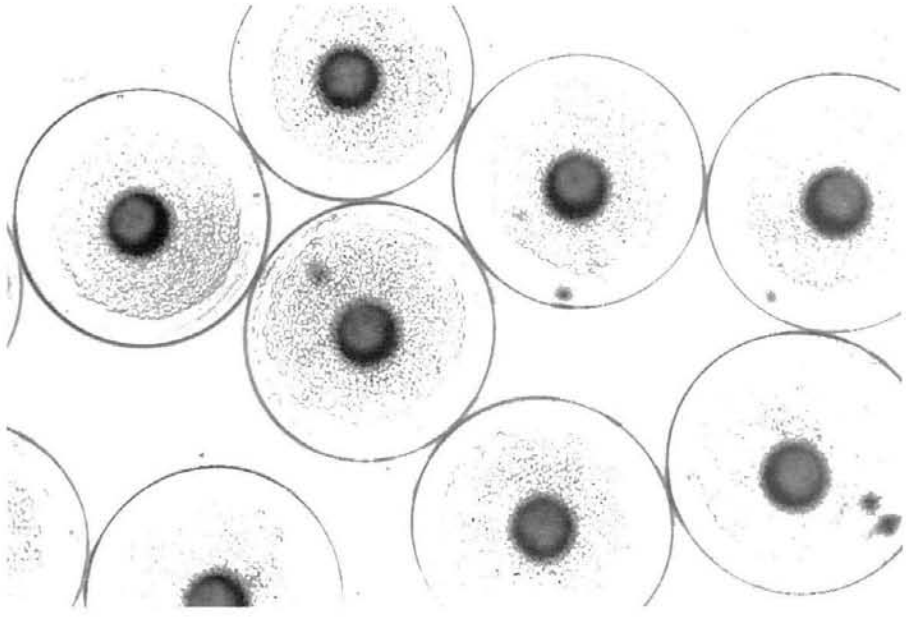


PLATE 5 - Blastula stage, 4 h 30 min.

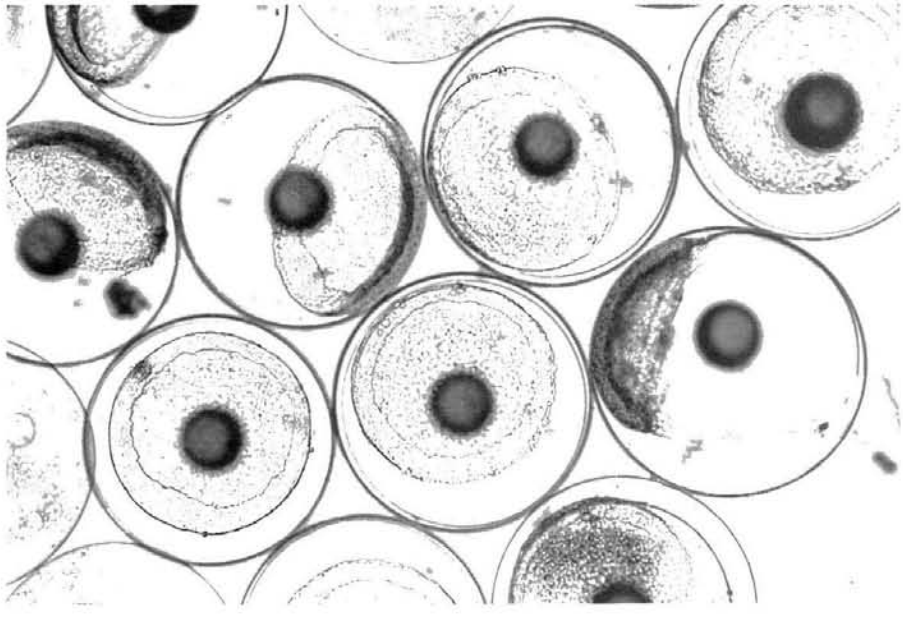


PLATE 6 - Gastrula stage, 8 h.

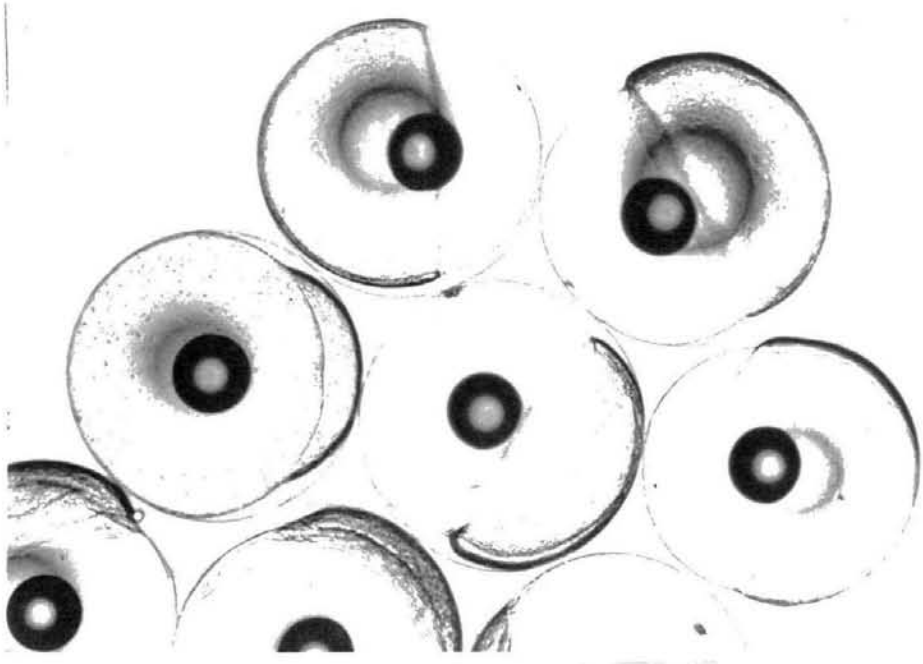


PLATE 7 - Late gastrula stage, 11 h 30 min.

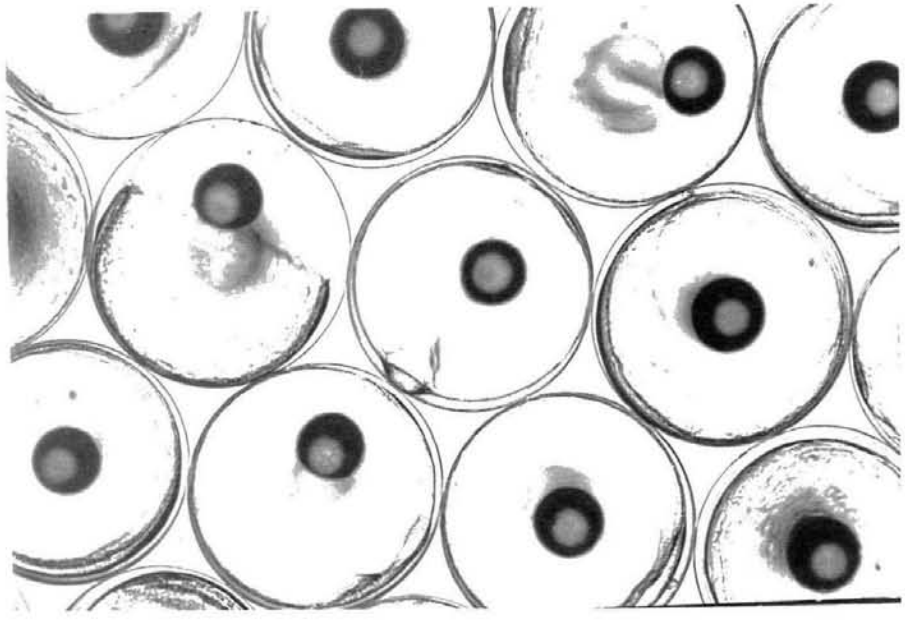


PLATE 8 - Neurula stage, 14 h.

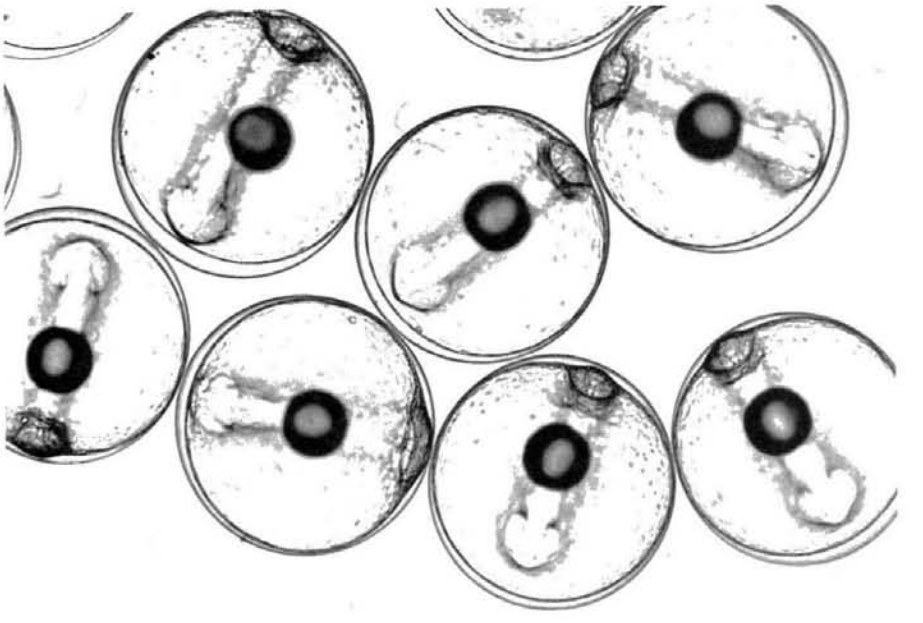


PLATE 9 - Early embryo, 17 h 30 min.

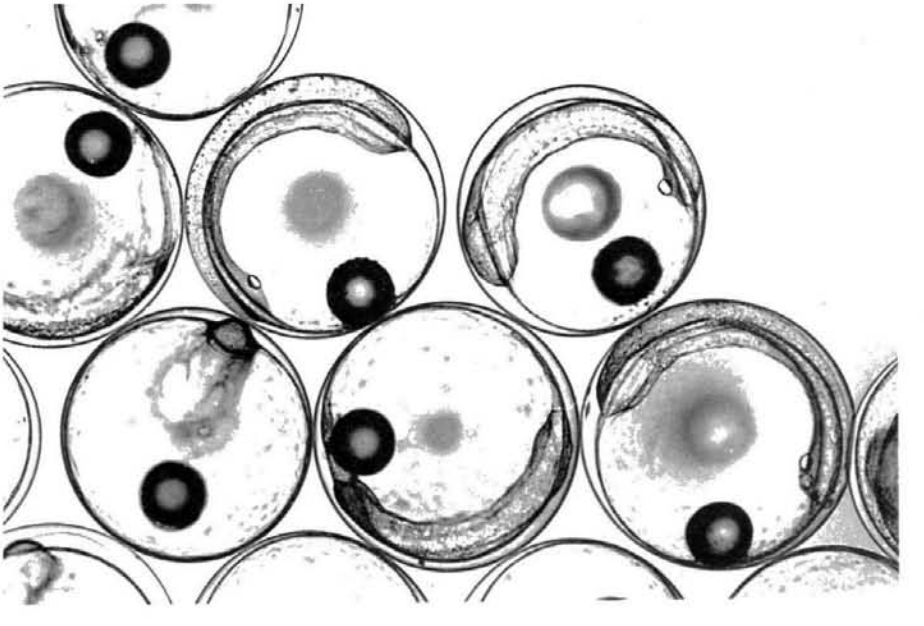


PLATE 10 - Formation of optic and Kupffer's vesicles, somites and first melanophores, 18 h 30 min.

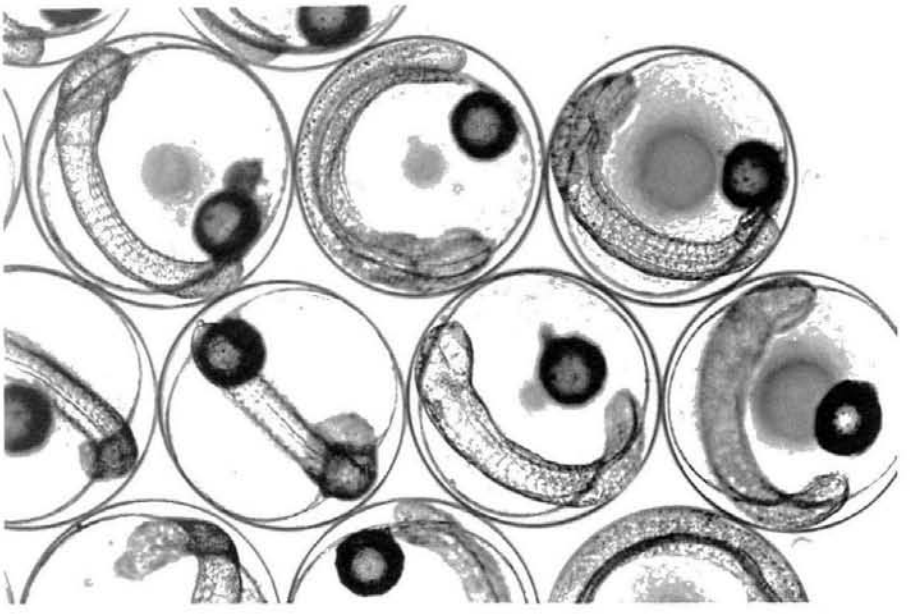


PLATE 11 - Disappearance of Kupffer's vesicle, further pigmentation and somite division, 22 h 30 min.

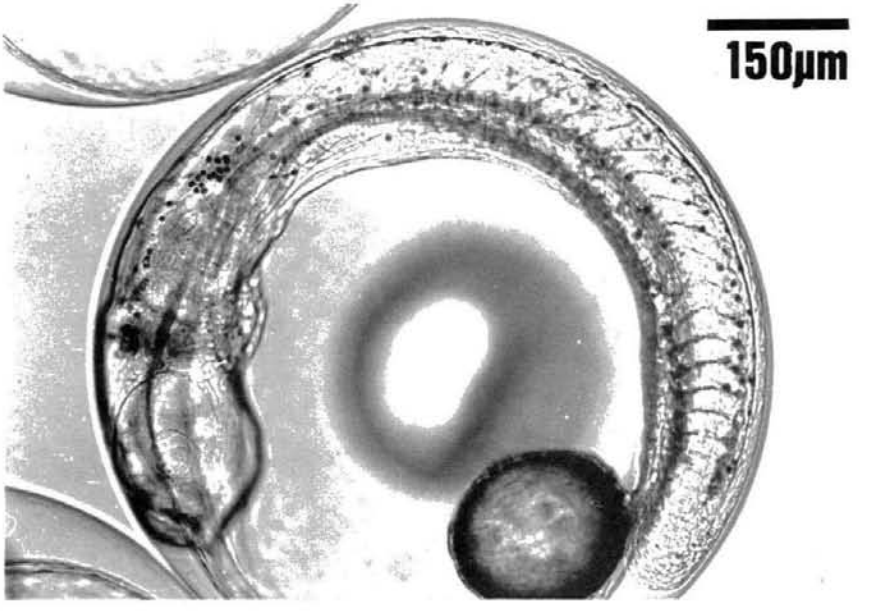


PLATE 12 - Advanced embryo with heartbeat at high magnification, 28 h.

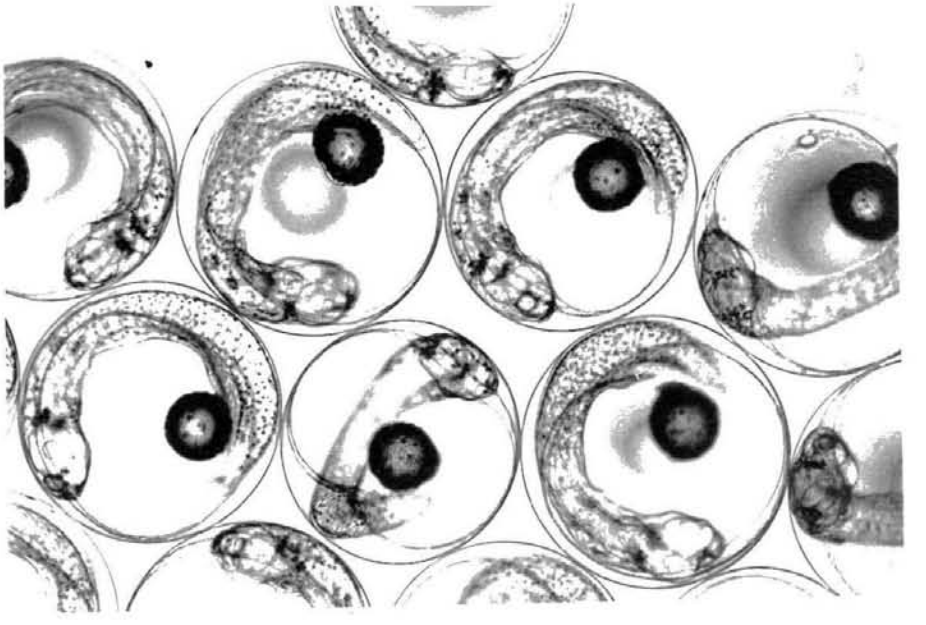


PLATE 13 - Pre-hatch embryo showing frequent movement, 30 h 10 min.

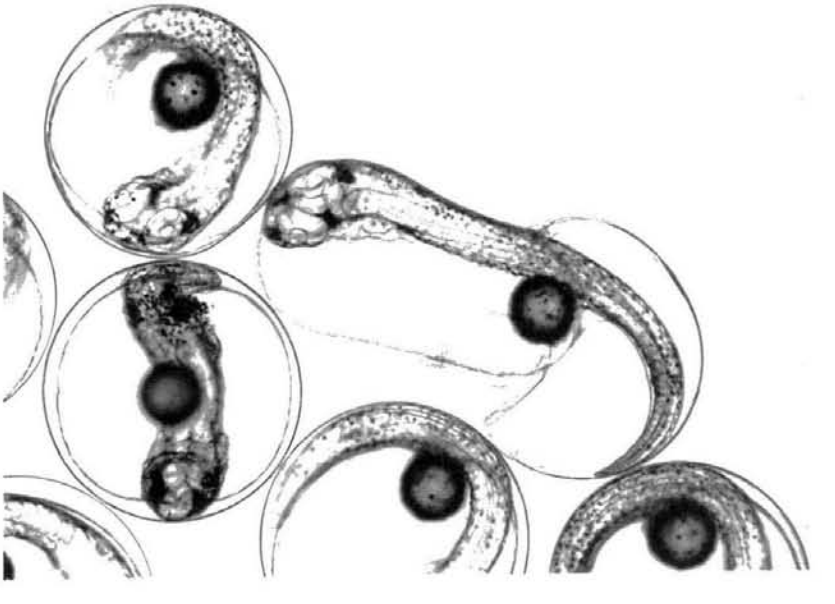


PLATE 14 - Hatch, 33 h 30 min. Note large yolksac, posterior oil globule and lack of eye pigmentation.

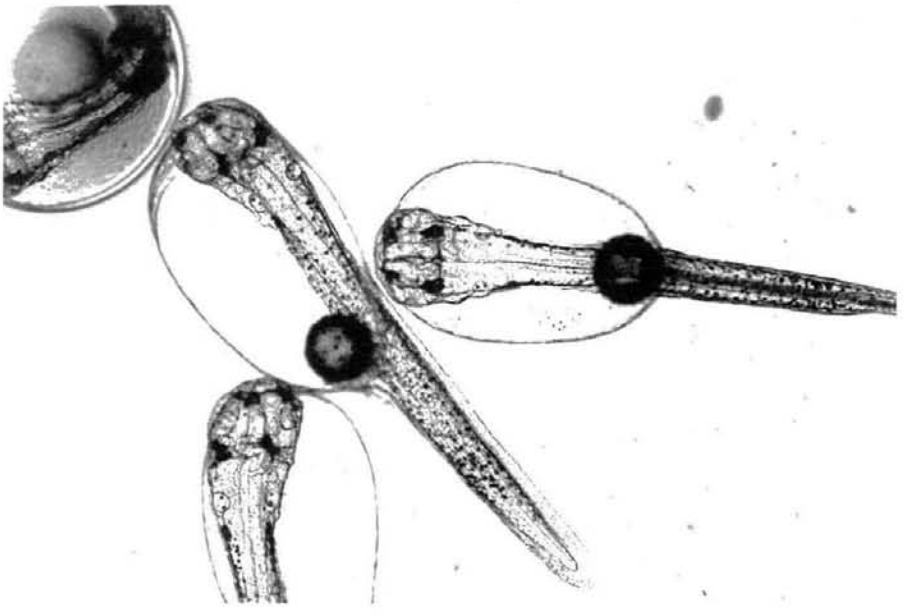


PLATE 15 - Newly hatched and straightened larvae, T.L. 2.0 mm, 34 h.

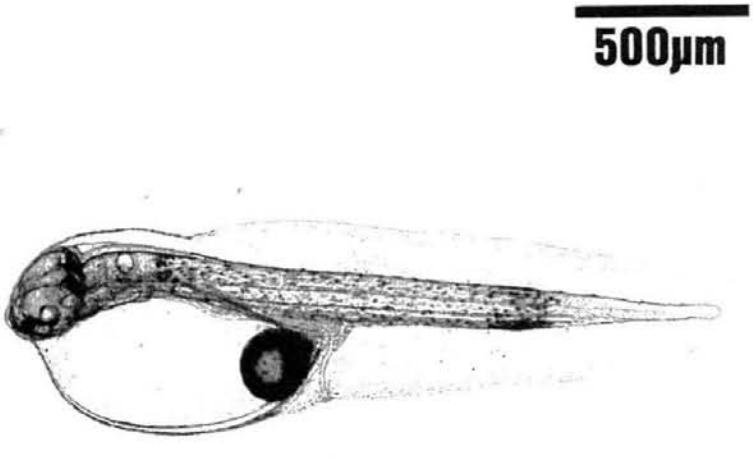


PLATE 16 - One-third yolk absorption, 7 h post-hatch.

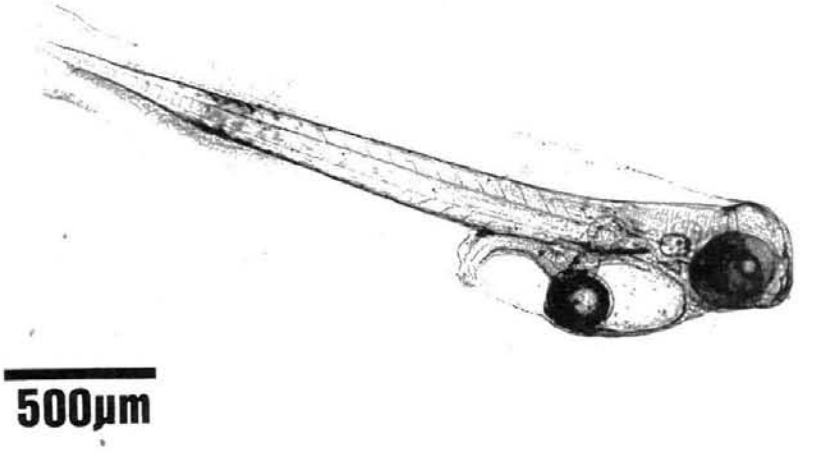


PLATE 17 - Approaching complete yolk absorption, 31 h post-hatch. Note appearance of eye pigmentation.

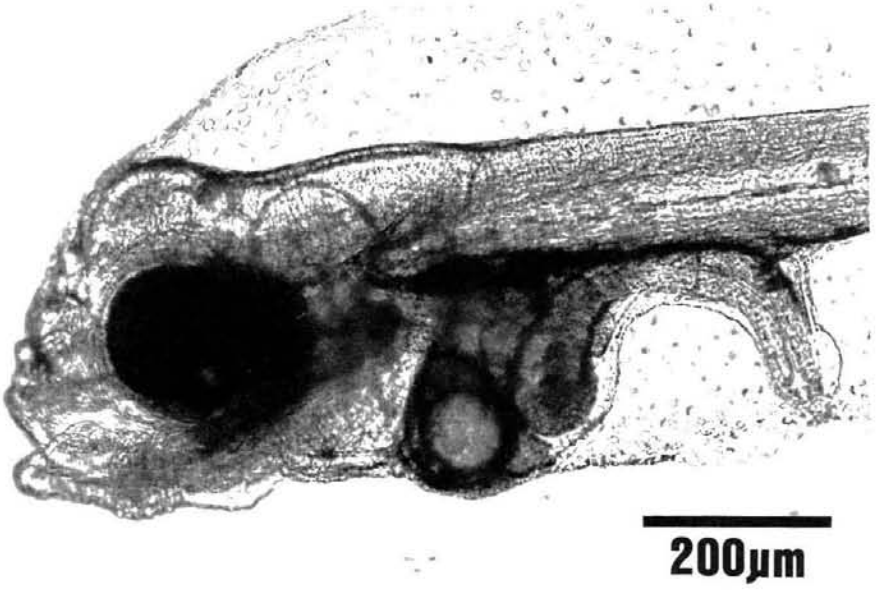


PLATE 18 - Larva after yolk absorption at high magnification, 45 h post-hatch. Note fully pigmented eyes, open mouth and anus, and remaining oil globule.

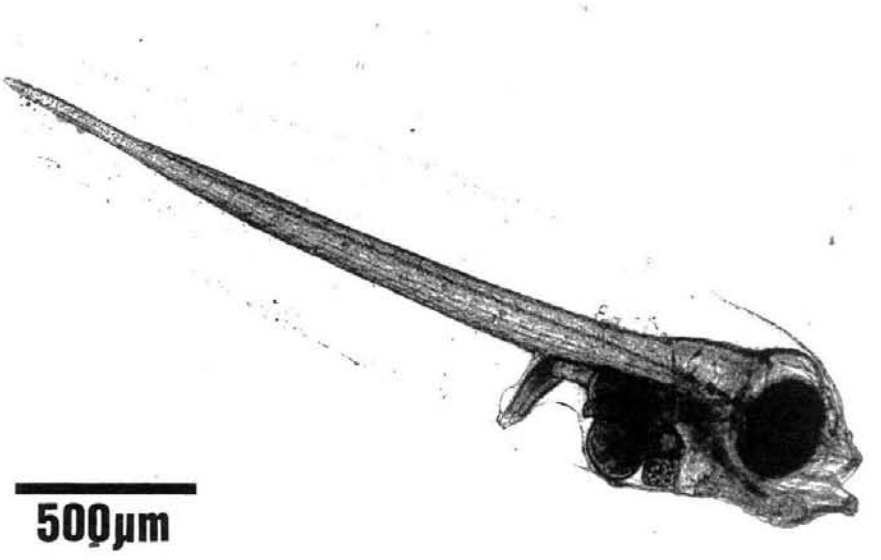


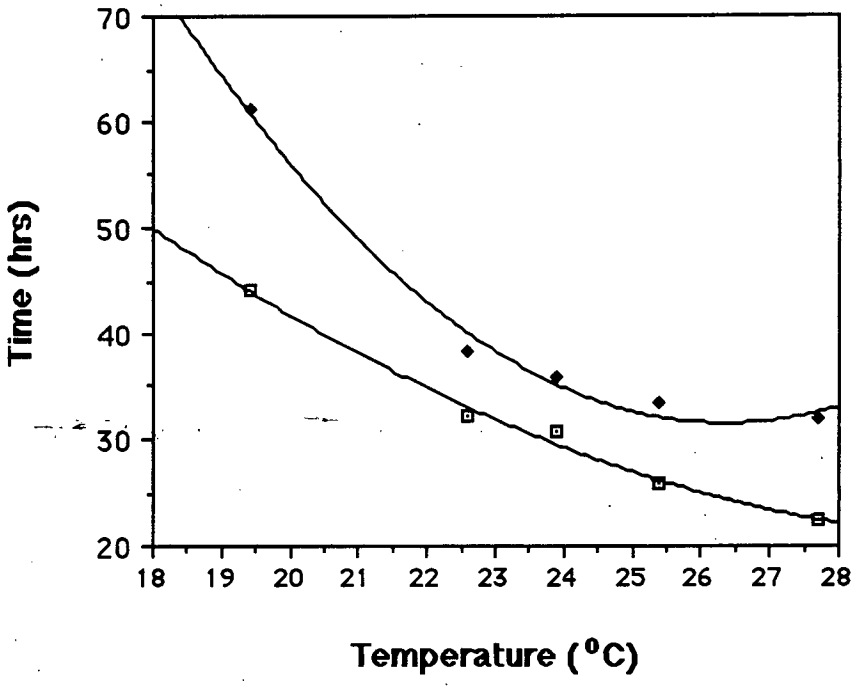
PLATE 19 - Feeding larva, 85 h post-hatch.

all temperatures and salinities investigated, but not all combinations thereof. The proportions of abnormal larvae increased at temperature and salinity extremes. Distinct optimum conditions were observed, as reflected in survival, growth and deformity data.

(i) temperature

Fig. 1 illustrates the accelerating effect of increasing temperatures on egg development and larval yolk absorption. This is expected from the recognised Q_{10} coefficients for metabolic processes (Blaxter, 1969). Egg incubation time varied from 44 h 45 min at 19.4°C, to 22 h 30 min at 27.7°C. Likewise, time for yolk absorption varied from 61 h 15 min to 32 h over the same temperature range.

Figs. 2-5 illustrate the significant effect temperature has on percent hatch ($F=18.7$, d.f.=4, $p < 0.001$), percent survival to yolk absorption ($F=6.4$, d.f.=4, $p < 0.001$), notochord length at yolk absorption ($F=5.52$, d.f.=4, $p < 0.001$) and the percentage of hatch deformities (Chi-sq.=16.1, d.f.=8, $p < 0.05$). Figs. 4 and 5 depict this influence on yolk absorption size and the percentage of hatch deformities at a salinity of 35 ppt, the only salinity for which survival provided replicate data (allowing comparison by ANOVA). The size of larvae at complete yolk absorption is of particular interest (Fig. 4). At this stage larvae are preparing to commence feeding, and it is now widely recognised that larger larvae are more efficient predators, have a greater swimming and searching ability, a larger mouth size, and greater resistance to starvation (Blaxter and Hempel, 1963; Ryland and Nichols, 1967; Blaxter, 1969), and are hence more likely to feed successfully. The ability to capture larger prey items is advantageous by virtue of their rapidly increasing calorific values (Hunter, 1981).



▫ egg incubation time (hrs) ($y = 184.35 - 10.46x + 0.167x^2$ $r = 0.99$)

◆ yolk absorption time (hrs) ($y = 451.65 - 31.88x + 0.605x^2$ $r = 0.99$)

FIG. 1 - Effect of temperature on egg incubation and yolk absorption times, at near optimal salinity conditions (35ppt). Quadratic equations have been fitted to the data and their equations and 'r' values included.

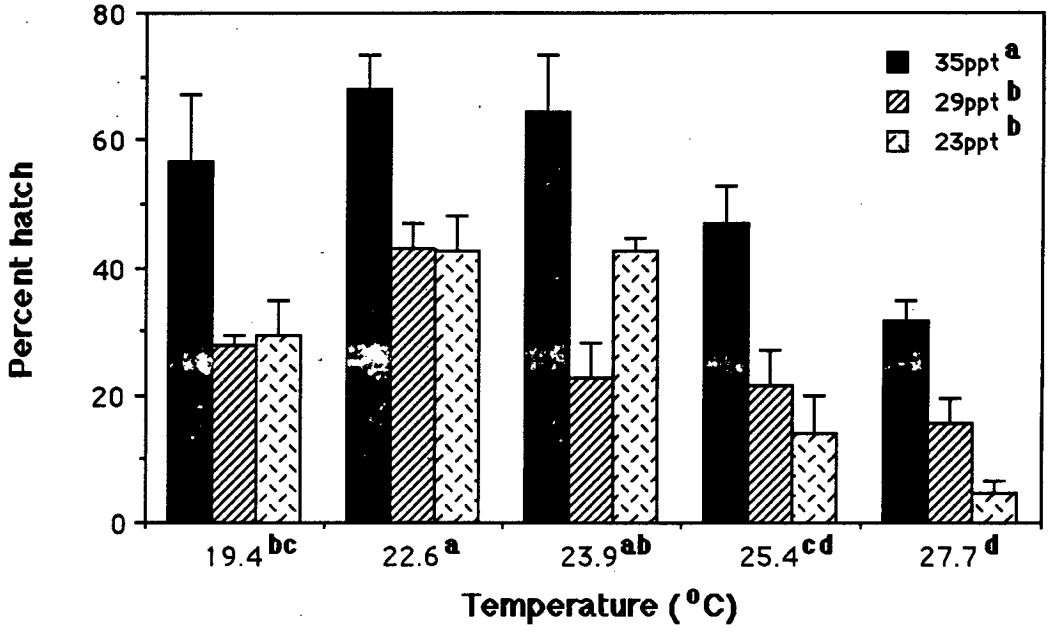


FIG. 2 - Influence of temperature and salinity on observed hatch rate. Data bars represent means (± s.e) of three replicates of 59 eggs each. Temperatures or salinities showing dissimilar superscripts represent a statistically significant difference (2-way ANOVA, $p < 0.05$).

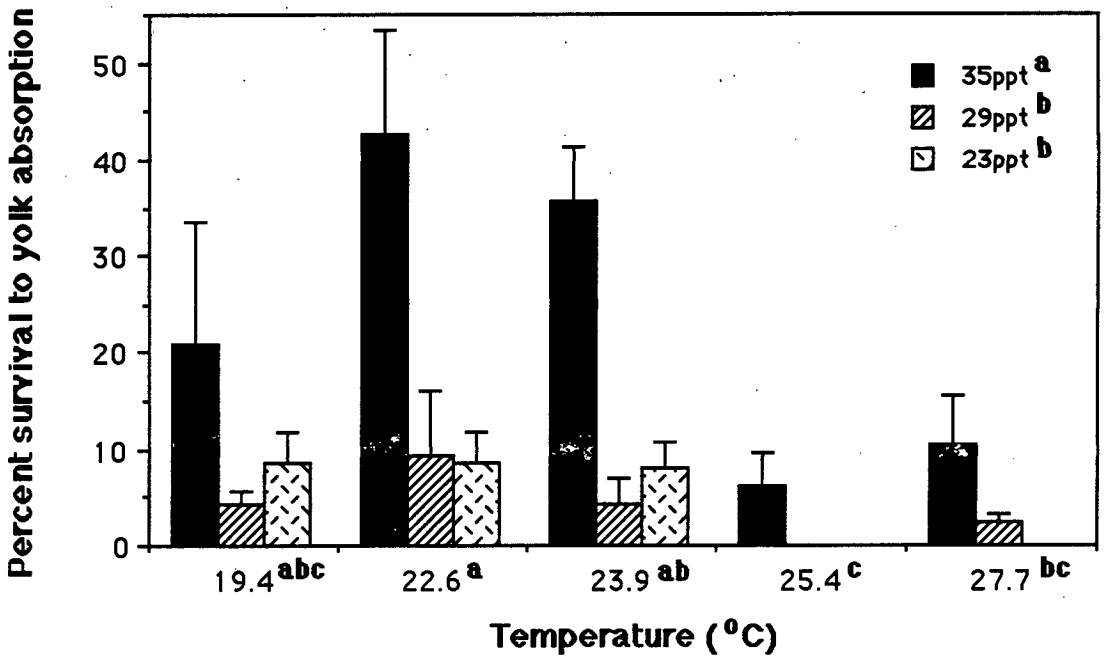


FIG. 3 - Influence of temperature and salinity on survival from hatch to the completion of yolk absorption. Data bars represent means (± s.e.) of three replicate containers each initially containing 59 eggs. Temperatures or salinities showing dissimilar superscripts represent statistically significant differences after arcsin transformation (2-way ANOVA, $p < 0.05$).

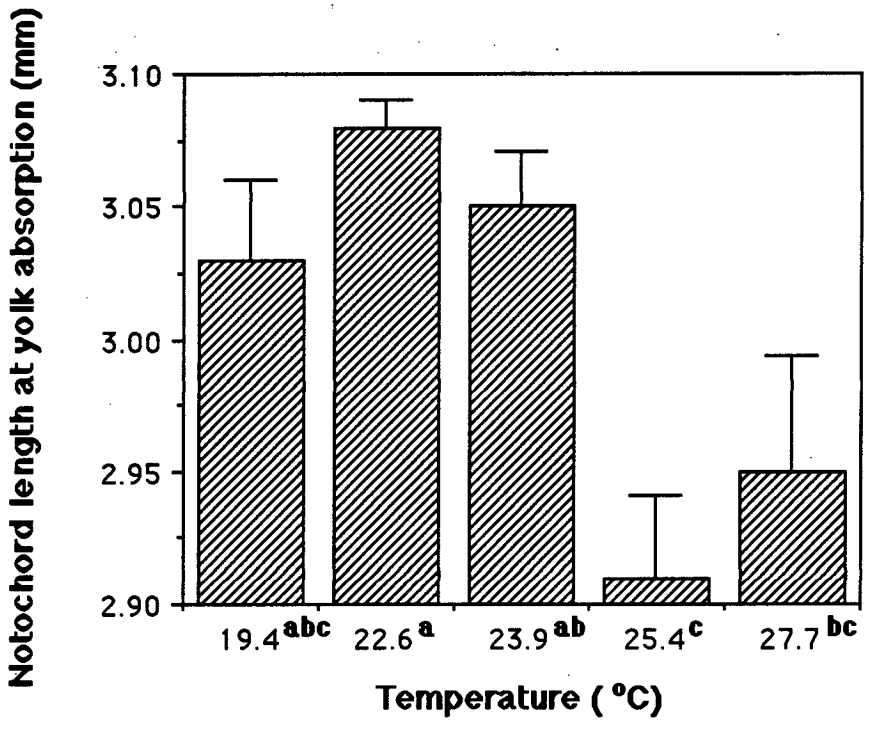


FIG. 4 - Influence of temperature on notochord length of larvae at the completion of yolk absorption at a near-optimal salinity of 35 ppt. Data bars represent means (± s.e.) of three replicates of 15 larvae each. Temperatures showing dissimilar superscripts represent statistically significant differences (ANOVA, $p < 0.05$).

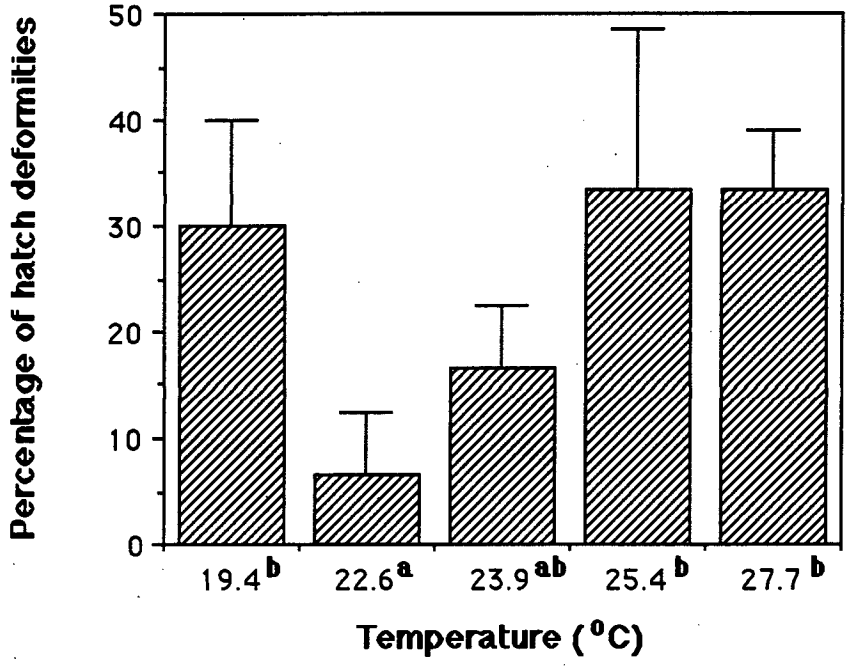


FIG. 5 - Percentage of deformities seen at hatch over the temperature range investigated, at a near-optimal salinity of 35 ppt. Data bars represent the means (± s.d.) of three replicates of 10 larvae each. Temperatures showing dissimilar superscripts represent statistically significant differences (ANOVA, $p < 0.05$).

Using any of these four measures as the criterion, a distinct thermal optimum exists at 22.6-23.9°C (Figs. 2-5). Above this range, at 25.4°C, the percentage of hatch deformities increases significantly (Fig. 5), while the hatch rate, and survival and size of larvae at yolk absorption, decrease significantly (Figs. 2-4). These results are similar to those found by Camus and Koutsikopoulos (1984) and Polo *et al.* (1991) for another sparid, the gilthead seabream *Sparus auratus*, which had a thermal optimum of 14.5-19°C where hatch rate, yolk utilisation efficiency, and survival to, and size at, yolk absorption were maximal, and hatch deformities minimal.

Below the optimum range, at 19.4°C, the percent hatch decreased and the percentage of hatch deformities increased, each significantly ($p < 0.05$) (Figs. 2 and 5). From these data, it is apparent that 19.4°C and 25.4°C are approaching the lower and upper temperature tolerance limits for this population of yellowfin bream.

As has been found in several other studies (e.g. Houde, 1974; Freddi *et al.*, 1981; Camus and Koutsikopoulos, 1984) determined optimum temperatures are comparable with mean ocean temperatures during the spawning season, which for yellowfin bream ranges from approximately 22-23.5°C (Fig. 1, Chapter 2).

The existence of an optimum temperature for yolk utilisation has been discussed by Ryland and Nichols (1967) and Johns and Howell (1980), and reviewed by Blaxter (1969). Ryland and Nichols (1967) found a distinct optimum for plaice larvae, *Pleuronectes platessa*, as did Marr (1966) studying two salmonid species and Polo *et al.* (1991) studying the gilthead seabream *S. auratus*. Ryland and Nichols (1967) state that temperature alone may cause a length variation at the commencement of feeding of 10% in plaice, with corresponding variations in swimming speed. Although yolk utilisation

efficiency was not measured quantitatively in the present study, the mean length variation at 95% yolk absorption of 5.8 % observed between larvae reared at 22.6°C and those at 25.4°C (Fig. 4) is a direct reflection of it.

Blaxter's (1969) review confirms that yolk utilisation efficiency is usually maximal at intermediate temperatures within the range of tolerance. This has been found also by Ryland and Nichols (1967) studying the plaice, *P. platessa*, Walsh *et al.* (1991) studying the mullet, *Mugil cephalus*, and Santerre and May (1977) studying the threadfin *Polydactylus sexfilis*.

In accordance with several other studies on the influence of temperature on size of larvae at hatch (Blaxter, 1957; Johns *et al.*, 1981; Laurence and Howell, 1981; Buckley, 1982), the present study did not find any significant difference in length at hatch of bream larvae reared at any of the experimental temperatures (Fig. 6; Table 1a). Other studies have either reported maximal hatch size at lower temperatures (Sweet and Kinne, 1964; Linden *et al.*, 1980; Bergston *et al.*, 1987), or at higher temperatures (Alderdice and Forrester, 1968, 1974; Laurence and Rogers, 1976; Rana, 1990). As stated by Blaxter and Hempel (1963), and Buckley (1982), there is apparently a variable relationship between maximal hatch size and incubation temperature between species.

(ii) salinity

At 29 and 23 ppt salinity, eggs were negatively buoyant, although at 29 ppt only just so, whereas at 35 ppt eggs were positively buoyant. The light aeration used, however, was sufficient to maintain eggs in suspension at each salinity. Negative buoyancy was considered an indication of the unsuitability of lower salinities for egg incubation. This was confirmed when hatch, survival and deformity data were analysed.

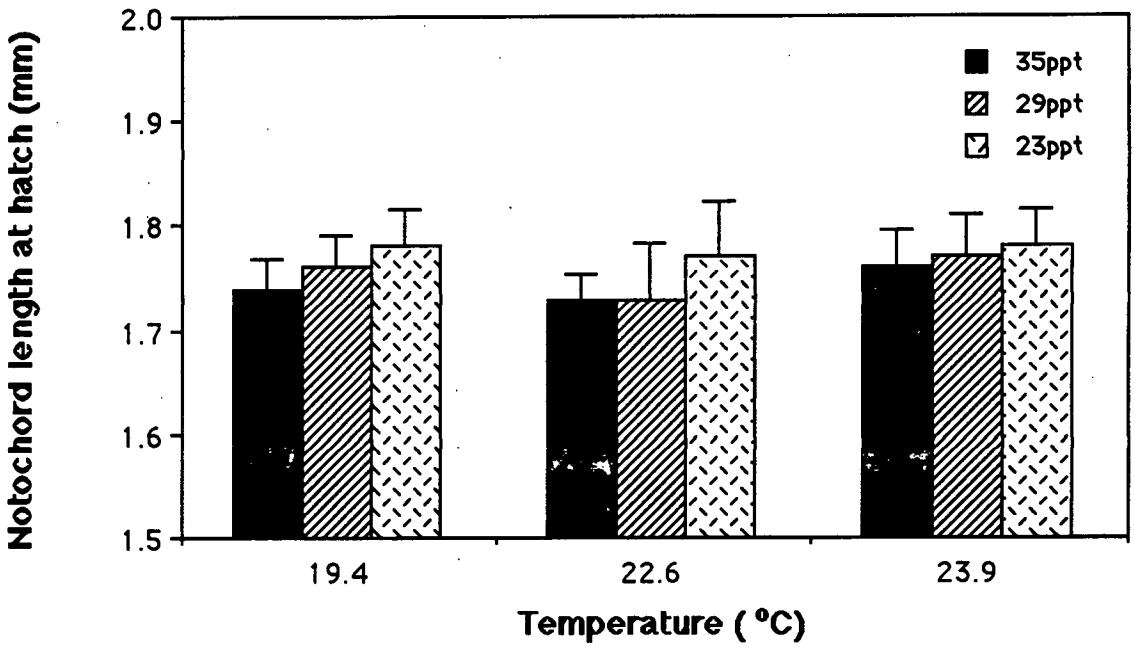


FIG. 6 - Influence of temperature and salinity on notochord length at hatch. Poor survival and high levels of deformity precluded data collection from higher temperatures. Data bars represent means (± s.d.) of three replicates of 5 larvae each. Two-way ANOVA showed temperature and salinity to have no significant influence on hatch size over this range ($p > 0.05$).

Salinities investigated below 35 ppt caused significant declines in hatch rate ($F=43.7$, d.f.=2, $p < 0.001$) and survival to yolk absorption ($F=21.1$, d.f.=2, $p < 0.001$) (Figs. 2 and 3), and significant increases in the percentage of hatch deformities (Chi-sq.=16.1, d.f.=8, $p < 0.05$) (Table 1b). At 29 ppt salinity, the percentage of hatch deformities exceeded 30% at a near-optimal temperature of 22.6°C (Table 1b), indicating that this salinity is close to the lower tolerance limit. This is despite presumably decreased osmotic demands (Holliday, 1965, 1969). A salinity of 35 ppt provided the widest thermal tolerance for eggs and yolksac larvae (Figs. 2 and 3). These results are consistent with observed ocean salinities during the yellowfin bream spawning season, which, in the 1992-1994 seasons, were in the range of 36 ppt to 40 ppt. Further experimentation (in the range 30-40ppt) is required to more narrowly define the optimum salinity range for eggs and larvae.

As found by other researchers investigating the influence of salinity on egg incubation and yolk absorption of marine fish larvae (Alderdice and Velsen, 1971; May, 1974; Van der Wal, 1985; Walsh *et al.*, 1991), salinity did not significantly affect hatch size (Fig. 6; Table 1b), and had a negligible effect on egg incubation and yolk absorption times in the present study.

Temperature and salinity interacted in such a way that the negative effects of sub-optimal levels of each compounded. For example no larvae survived to yolk absorption at temperatures above 23.9°C when held at 23 ppt salinity (Fig. 3). At higher temperatures, the range of salinity tolerance was lower (Figs. 2 and 3). This was also reported by Freddi *et al.* (1981) when studying the gilthead seabream *S. auratus*. Similarly, within the optimum temperature range, salinity had an insignificant effect on size at hatch or at yolk absorption (Fig. 6; Table 1b).

In summary, the recommended temperature range and salinity for

(a) 35 ppt

temperature (°C)	N.L. hatch (mm)
19.4	1.74 ± 0.03[a]
22.6	1.73 ± 0.02[a]
23.9	1.76 ± 0.04[a]
25.4	1.68 ± 0.08[a]
27.7	1.74 ± 0.06[a]

(b) 22.6°C

salinity (ppt)	N.L. hatch (mm)	N.L. 95% Y.A. (mm)	% hatch deformity
35	1.72 ± 0.02[b]	3.07 ± 0.01[c]	6.7 ± 3.3
29	1.73 ± 0.05[b]	3.08 ± 0.03[c]	33.3
23	1.77 ± 0.05[b]	3.08 ± 0.07[c]	45.5

TABLE 1 - (a) Effect of increasing temperature on notochord length (N.L.) at hatch, near the optimal salinity. (b) Effect of reducing salinity on notochord length at hatch and at 95% yolk absorption, and on percentage hatch deformity, near the optimal temperature. Values represent means (± s.d.) of three replicates of 10 larvae each, except deformity data at low salinity where replicates were pooled due to low survival. Superscripts represent statistical differences ($p<0.05$). Y.A. represents yolk absorption.

27.7°C

salinity (ppt)	% hatch	% hatch deformity	% survival to Y.A.
35	31.6 ± 3.0[a]	33.3 ± 3.3	10.5 ± 5.1[c]
29	15.8 ± 3.9[b]	91.7	2.5 ± 0.63[c]
23	4.5 ± 2.0[b]	71.4	0

TABLE 2 - Effect of salinity on three parameters, at the upper temperature tolerance limit for yellowfin bream. Percentages represent means of 3 replicates (± s.e.), except deformity data at low salinity where replicates were pooled due to low survival. Superscripts represent statistical differences ($p<0.05$). Y.A. represents yolk absorption.

rearing yellowfin bream eggs and larvae is 22.6-23.9°C and 35 ppt. Under these conditions survival and growth are maximised, and the occurrence of deformities minimised. Hence, this species can be considered both stenohaline and stenothermal. This is basic but important hatchery information if large-scale rearing is to be attempted. Upper temperature tolerance limits were not expected to extend far above spawning temperatures as Townsville is near the northern distribution limit for yellowfin bream, suggesting that temperatures in this region are becoming marginal. It should be borne in mind, however, that optima can vary with the particular population or race of the species being examined, as discovered for herring, *Clupea harengus*, by Blaxter (1956).

Effect of light intensity on yolk absorption efficiency

Figs. 7a and 7b depict the disappearance of yolk and simultaneous growth in length of larvae held under the three lighting regimes. No significant differences were found between the patterns of yolk disappearance or larval growth under any of the regimes. The depletion of yolk in darkness was initially slightly slower (Fig. 7a), although complete yolk absorption was reached simultaneously amongst treatments.

Similarly, and most importantly, the final sizes of larvae at yolk absorption were not significantly different amongst treatments, all approaching an asymptotic total length of approximately 3.1 mm. Hence, larvae from each treatment are presumably equally capable of capturing their first prey items.

Blaxter (1969) suggests that light has its most significant impact amongst species whose eggs or larvae naturally develop in darkness or semidarkness, as, for example, salmonid eggs do amongst gravel in the spawning redd. Yellowfin bream yolksac larvae, being pelagic, would naturally experience

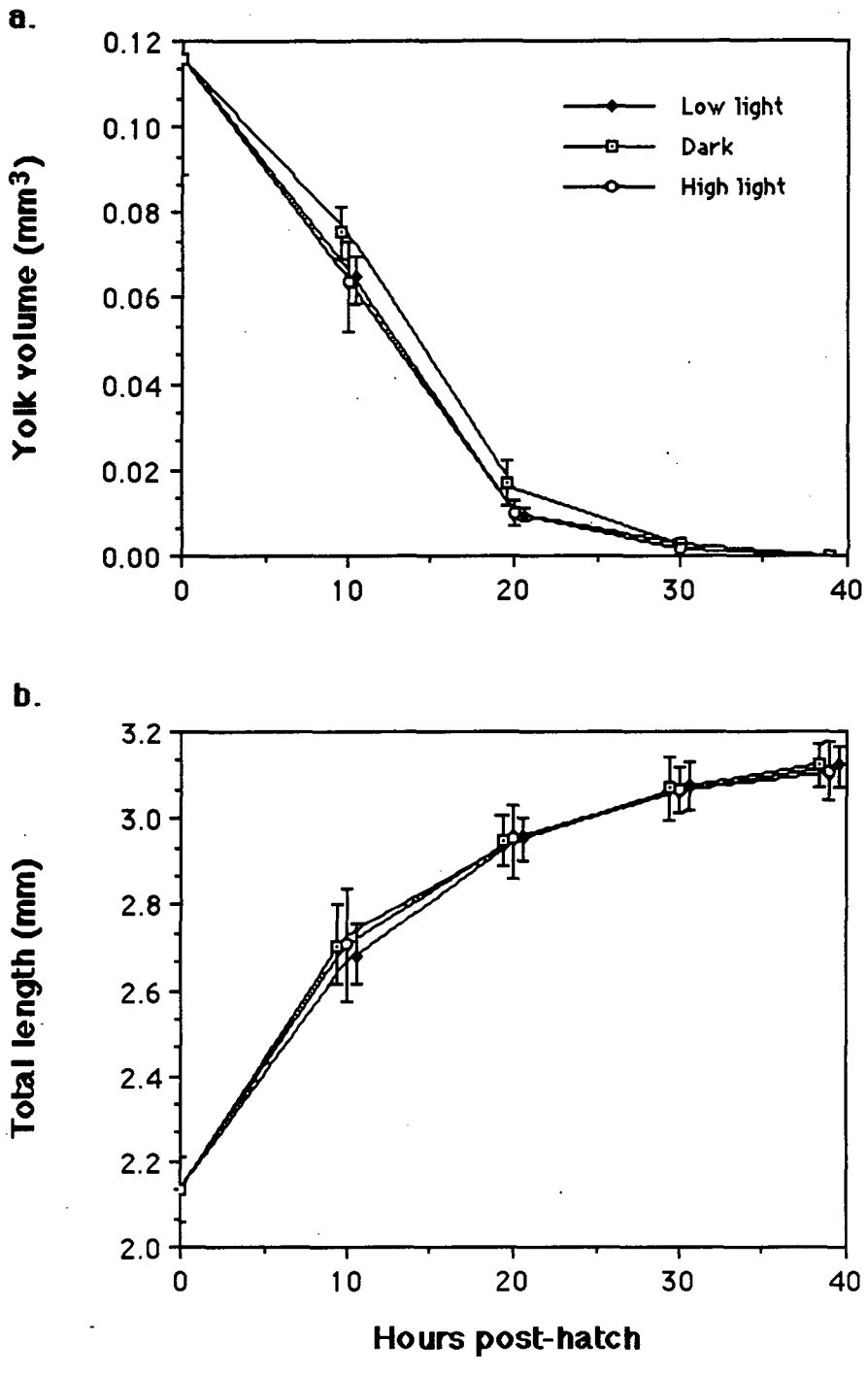


FIG. 7 - Yolk depletion (a) and simultaneous growth in length (b) from hatch, under three lighting regimes. Each data point represents the mean (± s.d.) of measurements from 45 larvae, 15 from each of three replicates.

high light levels and are apparently adapted to tolerate them. The significance of light intensity may become apparent from first-feeding onward, in terms of prey visibility, as shown for three species of marine fish larvae by Kiyono *et al.* (1989).

In summary, this experiment does not support the notion that maintaining fish larvae in darkness through yolksac absorption confers an advantage arising from more efficient yolk utilisation, and resulting in larger first-feeding larvae.

CHAPTER 4

Larval Rearing

Introduction

The larval rearing stage constitutes a bottleneck in the production of many marine food fish. Marine fish larvae are typically very small at hatch, and carry limited endogenous food reserves to sustain them through the transition to exogenous nutrition. Consequently, a very brief period of initial feeding opportunity exists, which, if missed, results in sudden large losses. The challenge for marine fish hatcheries has been to supply appropriate food (size, nutritional quality, digestibility and density), while maintaining the correct culture environment. To meet these requirements for food, hatcheries have had to rely on the culture of live foods, principally the rotifer, *Brachionus plicatilis*, and the brine shrimp, *Artemia* spp., as the use of inert, artificial diets has to date met with limited success.

The major rearing problems encountered, common to hatcheries world-wide, have been related to first-feeding success, nutritional quality of food (in particular essential fatty acid content), and swim bladder inflation failure. First-feeding success is being improved through use of smaller live foods such as 'super small' (SS strain) rotifers. Nutritional quality of live food is being improved either through the culture of nutritionally superior foods such as copepods, or through enhancement of traditional foods by feeding with commercial products high in essential nutrients such as the highly unsaturated fatty acids (HUFAs). Swim bladder inflation problems have been linked to the presence of oil on the water surface, which denies larvae access

to the air necessary for proper inflation, and have been overcome through use of water surface cleaning devices.

Culture techniques adopted can be broadly classified into 'clearwater' and 'greenwater'. The former relies on water exchange to maintain water quality, whereas the latter largely utilises the water conditioning properties of unicellular algae. More recently, an extension of the greenwater technique has been used, in which an algal bloom and subsequent zooplankton bloom are induced in an earthen pond by fertilising before stocking with fish larvae (Geiger 1983; Colura *et al.*, 1991; Rutledge and Rimmer 1991). Again little or no water exchange is carried out until harvest.

The aims of this component of the thesis were to rear the larvae of yellowfin bream, *A. australis*, using clearwater and greenwater techniques, and to investigate the timing of developmental events such as swim bladder inflation and the influence of light level on inflation success.

Nutritional studies

Of particular importance to marine larval fish diets is their protein and lipid quality (Tucker, 1992a). There are ten amino acids and at least two fatty acids considered essential to marine fish larvae and unable to be synthesised. If requirements for these are not met, growth and survival will be compromised. It has been suggested, and subsequently substantiated, that the requirements of a fish for essential amino acids will closely reflect the composition of tissues being formed, as the major use of essential amino acids is in the synthesis of body protein (Cowey and Tacon, 1983; Webb and Chu, 1983; Halver, 1985; Wilson and Cowey, 1985; Wilson and Poe, 1985; Gatlin, 1987; Mohanty and Kaushik, 1991). Consequently, in species for which quantitative amino acid requirements have not been determined through traditional digestibility and retention studies, an indication of requirements

can be gained through analysis of whole-body protein composition, first suggested by Phillips and Brockway (1956). The results do not indicate the quantitative requirements, just the most desirable essential amino acid profile of dietary protein. The technique is certainly useful in evaluating the limiting amino acids in a potential protein source (Wilson, 1989).

By similar reasoning, Tocher and Sargent (1984), Falk-Petersen *et al.* (1986) and others have suggested that the fatty acid profiles of fish eggs or whole-body tissues may allude to the dietary requirements of the species. This suggestion has proven useful in several species, such as the the Atlantic herring, *Clupea harengus* (Tocher *et al.*, 1985), the Dover sole, *Solea solea* (Dendrinos and Thorpe, 1987) and the dolphin fish, *Coryphaena hippurus* (Ostrowski and Divakaran, 1989).

In order to gain some understanding of both the requirements of yellowfin bream larvae for essential fatty and amino acids, and whether rotifers as an initial food item are likely to meet them, fatty acid analyses were performed on eggs and rotifers, and amino acid analyses on whole-body first-feeding larvae and rotifers. These profiles will provide useful guidelines for formulating diets, or selecting/enriching live foods, for cultured yellowfin bream.

Materials and Methods

Timing of swim bladder inflation and influence of light level

To investigate the timing of swim bladder inflation, and the influence of light intensity on inflation success, newly-hatched bream larvae were maintained at three light levels, under otherwise equivalent conditions, until the percentage of swim bladder inflation had stabilised. Three 10 l plastic

containers were used for each treatment, which included complete darkness, low light (250 lux), and high light (2000 lux). Fluorescent lighting ('cool white' tubes) was used to minimise heating effects, and a 24 h photoperiod was maintained. The three containers in darkness were completely covered with opaque black plastic.

Each container was filled with 8 l of 34 ppt salinity seawater, which was maintained at 23°C± 1°C. To this, 400 newly-hatched larvae were added by volumetric estimation (i.e. final density 50 larvae/l). No aeration was provided throughout this trial to avoid water circulation which has been found to influence swim bladder inflation in other species (e.g. Al-Abdul-Elah *et al.*, 1983; Foscarini, 1988).

At intervals of 12 h (i.e. twice daily) after the yolksac had been absorbed (first sample day 2, 62 h post-hatch), samples of 15 larvae were removed from each container, anaesthetised in 70 mg/l MS-222, and preserved in buffered 2.5% gluteraldehyde (Oozeki and Hirano, 1988). These were later examined for the presence of a swim bladder, allowing the mean number of larvae with a swim bladder to be determined for each of the 9 experimental containers at any one time. Results at each time sample were compared by one-way ANOVA ($p < 0.05$) and the Tukey pairwise comparison of means test. Homogeneity of variance was tested for sets of data by examining residuals.

Clearwater rearing trial

First attempts to rear yellowfin bream larvae employed the clearwater technique whereby 1 µm filtered seawater was used without the addition of microalgae. Only the first of three attempts with this method succeeded in producing metamorphosed larvae, and is the only trial described below. Although a similar rearing protocol was used in both subsequent trials, disease problems caused 100% mortality by day 6 in each. This was

apparently the result of using water common to the broodstock tank, which was not done in the first trial. The mortality was accompanied by behavioural abnormalities such as resting on the tank sides, spiralling movements, and apparent disorientation. These behaviours are symptomatic of microbial infection, probably bacterial or viral.

A 150 l black hemispherical tank, filled to 120 l, was used, with a central screened standpipe outlet. Water was circulated from a 1000 l sump, through a 1 µm cartridge filter, to the rearing tank. From here water flowed to a high-capacity biofilter before returning to the sump. Water was static for the first three days after which a nightime flowrate of 1.3 l/min. was begun to coincide with the commencement of feeding. From day 10 onwards, a permanent flow of 0.95 l/min. was maintained. A surface blower and floating trap (after Chatain and Ounais-Guschemann, 1990) were employed to remove any oily film that formed on the water surface during feeding. From day 10 the tank bottom was siphoned clean every second day. Very gentle aeration was provided through a finely-perforated tube. Lighting consisted of 75% filtered natural sunlight (12 h photoperiod) giving a peak noon light intensity of approximately 700 lux. Immersion heaters in the sump maintained water temperatures within the desired range. Water conditions throughout the trial were as follows:

Parameter	Range
temperature	20.4 - 23.4°C
salinity	34 - 38 ppt
pH	8.44 - 8.49
total ammonia	< 1.0 mg/l
nitrite	< 0.2 mg/l
nitrate	< 2.5 mg/l

Yolksac larvae, all derived from a single hormone-induced spawning, were stocked at the rate of 20 larvae/l (i.e. 2,400 larvae in 120 l). Food was first offered on the morning of day 3, when larvae had fully pigmented eyes and an open mouth and anus (Fig. 1a). Initial food consisted of sieved (125 µm screen) L-strain rotifers (*Brachionus plicatilis*), raised exclusively on the microalga *Nannochloropsis oculata*, and enriched with Frippak "Booster" (Frippak Feeds, U.K.) for 4 h prior to being introduced to the tank at 10-15 individuals/ml. Unscreened rotifers (up to 300 µm lorica length) were used from day 8, and previously decapsulated instar I brine shrimp (Great Salt Lake brand) were first introduced on day 17 at 2 individuals/ml (Fig. 1a). Larvae were fed brine shrimp exclusively from day 20 at 5 individuals/ml, and enriched brine shrimp metanauplii were provided from day 24 until weaning was completed. From day 28 the weaning diet "Sevbar" (Sanofi Aquaculture, France) was gradually substituted. After 5 further days juveniles were weaned and fed to satiation five times daily on a mixture of two dry commercial barramundi crumble diets.

Samples of 10 larvae were preserved every 1-2 days (in buffered 2.5% gluteraldehyde) to day 9 and then weekly to metamorphosis. Measurements of notochord length (N.L. ± 10 µm), and derived means, were recorded for each sample before preservation. Samples taken at first feeding were also used to measure mouth size. Mouth size was measured according to the method of Shirota (1970), where mouth diameter is estimated from top jaw length (AB), according to the formula:

$$\text{mouth diameter} = \sqrt{2} \times AB$$

Survival was estimated through observed larval densities and mortalities on the tank bottom. The age of fish in all records has been expressed as days after hatch (hatch = day 0).

Greenwater rearing trial

A rearing trial was conducted using the greenwater technique, in which microalgae are maintained in the rearing water, and minimal water exchange provided. Procedures were based on those described by Palmer *et al.* (1992) for rearing barramundi larvae, with some modifications. A 375 l circular flat-bottomed tank was filled with 15 µm filtered raw seawater, to which the soluble inorganic fertiliser "Aquasol" was added at the rate of 30 mg/l. A dense culture of *N. oculata* was added at the rate of 10% of the tank volume. Moderate aeration was then maintained for three days before stocking with yolksac larvae, after which very gentle aeration was provided through a finely-perforated tube. An immersion heater maintained water temperatures in the desired range, and a shadecloth cover was used to filter the midday natural sunlight to less than 500 lux (12 h photoperiod). No water exchange or tank cleaning was provided until day 22, when metamorphosis and weaning began. A surface blower and floating trap (after Chatain and Ounais-Guschemann, 1990) were employed intermittently to remove any oily film that formed on the water surface. Temperature and salinity were measured twice daily, other parameters were measured daily. Water conditions up to metamorphosis were as follows:

Parameter	Range
temperature	18.5 - 25.0°C
salinity	32 - 40 ppt
pH	7.9 - 8.5
total ammonia	< 1.0 mg/l
nitrite	< 0.3 mg/l
nitrate	< 5 mg/l
algal density	2×10^3 - 1×10^6 cells/ml

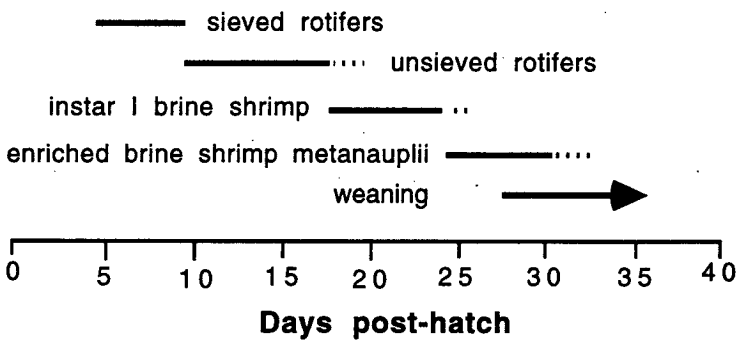
After three days, yolksac larvae from a single hormone-induced spawning (spawning No. 4 of the serial spawning trial, Chap. 2) were stocked at the rate of 20 larvae/l. The following day (day 1), when larvae were approaching first-feeding, unscreened L-strain rotifers (raised exclusively on *N. oculata*) were added at the rate of 5 individuals/ml (Fig. 1b). Rotifers were not enriched in this trial in an effort to reduce bacterial loads. Rotifer and algal densities were measured twice daily. Quantities of dense algal culture were added when necessary to maintain algal densities above 10^3 cells/ml. On day 15, feeding with previously decapsulated instar I *Artemia* nauplii (Great Salt Lake brand) was commenced, at approximately 2 individuals/ml, and from day 18 this was supplemented with *Artemia* metanauplii, up to 5/ml, while rotifers were no longer given (Fig. 1b). Weaning with "Sevbar" (Sanofi Aquaculture, France) commenced on day 21, and was complete by day 30. Larvae were viewed microscopically daily, and samples of 10 larvae preserved in 2.5% buffered gluteraldehyde every 3-4 days, for notochord length determinations, and recording of major developmental events such as swim bladder inflation, tail flexion, and metamorphosis. Survival was estimated through observed larval densities and, when visible, dead larvae on the tank bottom.

Nutritional studies

(i) amino acid analysis of larvae and rotifers

The bound and free amino acid profiles of yellowfin bream whole-body larval protein at first-feeding, and of rotifers being offered as food, were determined following acid hydrolysis of samples using reverse phase high performance liquid chromatography (HPLC) with pre-column derivitisation with orthophthaldialdehyde (OPA). Separation was achieved using a methanol-sodium acetate gradient buffer technique (Uhe *et al.*, 1991). Prior to

a. Clearwater trial



b. Greenwater trial

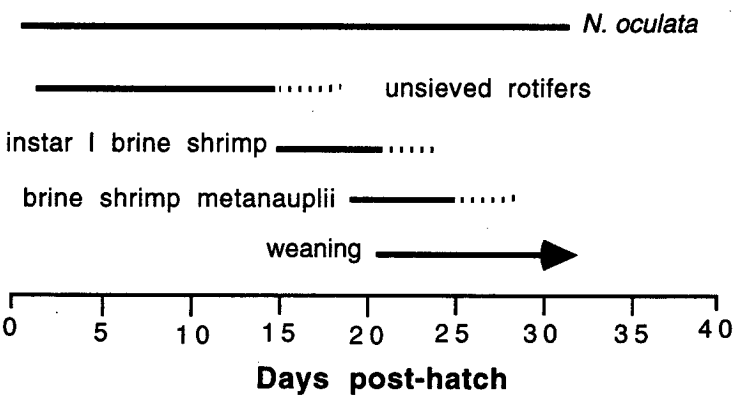


FIG. 1 - Feeding schedules used in the clearwater (a) and greenwater (b) larval rearing trials. All rotifers are L-strain. Sieved rotifers were passed through a 125µm sieve.

use buffers were filtered and degassed using a vacuum flask through a 0.45 μm membrane filter. All reagents used were of HPLC grade.

Samples were initially washed in 3.2% ammonium formate before being frozen to -80°C awaiting analysis. Free and protein amino acids were separated by duplicate washing with perchloric acid ($HClO_4$). Precipitated protein was solubilised in 2 ml of 1 N NaOH for 2 h at 70°C. This was then vortexed and a 50 μl sample hydrolysed with 5 ml of degassed 6 N HCL in an air oven at 110°C for 24 h. Samples were then frozen in liquid nitrogen, and freeze-dried. The lyophilised sample was resuspended in 500 μl of HPLC buffer A (95% methanol, 5% 0.1 M sodium acetate, pH 6.8). This was vortexed and 20 μl added to 80 μl of acetonitrile/mercaptoethanol/ homoserine (1:500 acetonitrile:mercaptoethanol; final homoserine concentration 125 μmol/ml). Similarly, free amino acid samples previously extracted with $HClO_4$ were vortexed and again 20 μl added to 80 μl of acetonitrile/mercaptoethanol/homoserine.

Using an automated sample injector (Waters 712 WISP, Millipore, Milford, Massachusetts, USA), 5 μl of this solution was injected into the column after addition of 10 μl of OPA. A C18 reverse phase column (Microsorb, C18 Cat. No. 80-215-C5, Rainin Instrument Co. Inc., Woburn, Massachusetts, USA) was used and maintained at 35°C. The amino acid derivatives were detected by fluorescence detection (CMA/280 Fluorescence detector, CMA Microanalysis, Sweden) at 425 nm, using gradient pumps (Waters 510 HPLC pump, Millipore, Milford, Massachusetts, USA), producing a chromatogram. Data collection and analysis were achieved using a programmable system controller (Maxima 820 Chromatography workstation, Waters, Millipore, Milford, Massachusetts, USA). Using peak area, the concentration of each amino acid was calculated relative to an internal standard (homoserine) and external standards containing 125

µmol/ml of each amino acid. Standards were run every six samples to ensure quality control. Free and bound amino acids, as mmol/g homogenate, were added to give total amino acid content.

Chemical score indices were calculated for the total (free + bound) of each essential amino acid in the rotifers as follows (Phillips and Brockway, 1956):

$$\text{Index 'I'} = \frac{\text{\% amino acid in rotifer x 100}}{\text{\% same amino acid in larval proteins}}$$

An 'I' value of 100 or more, therefore, indicates that the food meets the animal's dietary requirements for that amino acid. Values of less than 100 indicate limiting amino acids, the lowest 'I' value being termed the first limiting amino acid.

(ii) fatty acid analysis of eggs and rotifers

The fatty acid composition of eggs in early cleavage was determined for a single spawning, and of rotifers being offered as food. Rotifers analysed were unenriched, as provided in the successful greenwater trial, to investigate minimum HUFA requirements. Live samples were rinsed with 3.2% ammonium formate to remove salts before being frozen at -80°C awaiting analysis. Samples were prepared and analysed for fatty acids by the method of Southgate and Lou (1995). Briefly, a Hewlett Packard 5890 gas chromatograph, equipped with a fused silica capillary column was used to separate fatty acid methyl esters (FAMEs). The BPX70-coated (SGE, Melbourne, Australia) capillary column was 12 m long with 0.22 mm inside diameter. Helium was used as the carrier gas at 50 ml/min, and oven temperature was set to 150°C. FAME reference standards (Sigma) were used to identify individual fatty acids, with cod liver oil FAMEs used as secondary reference standards (Ackman and Burgher, 1965).

Results and Discussion

Timing of swim bladder inflation and influence of light level

Figure 2 shows that at 62 h post-hatch (day 2), no swim bladder inflation (SBI) was seen amongst any of the treatments. The first successful inflation was observed in samples from all treatments at 74 h post-hatch (day 3), but clearly no significant differences existed at this stage. From here percentage SBI increased rapidly amongst larvae in darkness, reaching 90% by 98 h post-hatch (day 4), and stabilising at this level. In the presence of low light, percentage SBI increased slowly up to 86 h, then rapidly thereafter to 71 % at 98 h. This again appeared to be the maximum level, with only a small increase in the following 12 h. Larvae held at high light intensity showed a similar pattern, although poor survival precluded a 110 h sample from being included. This light intensity is apparently detrimental for other reasons. The high initial inflation rates observed in all treatments indicate that feeding is not a requirement for inflation.

This pattern of initial swim bladder inflation is broadly similar to that recorded for many other marine fish larvae, although it occurs slightly earlier and more rapidly, despite similar water temperatures (e.g. Chatain, 1987, 1989; Foscarini, 1988; Battaglene and Talbot, 1990, 1992; Sweetman, 1992). In general, swim bladder inflation appears to coincide with the commencement of exogenous feeding.

The percentage SBI was significantly higher in darkness than at either light level at the 86 h ($F = 6.4$; d.f. = 2; $p < 0.05$) and 98 h ($F = 6.3$; d.f. = 2; $p < 0.05$) samples, but the improvement was not significant at the final, 110 h sample ($F = 0.6$; d.f. = 1; $p = 0.48$) (Fig. 2). The major impact of rearing in darkness, then, appears to be a 5-10 h advancement in the pattern of swim bladder inflation, with only a minor impact on final percentage inflation. This

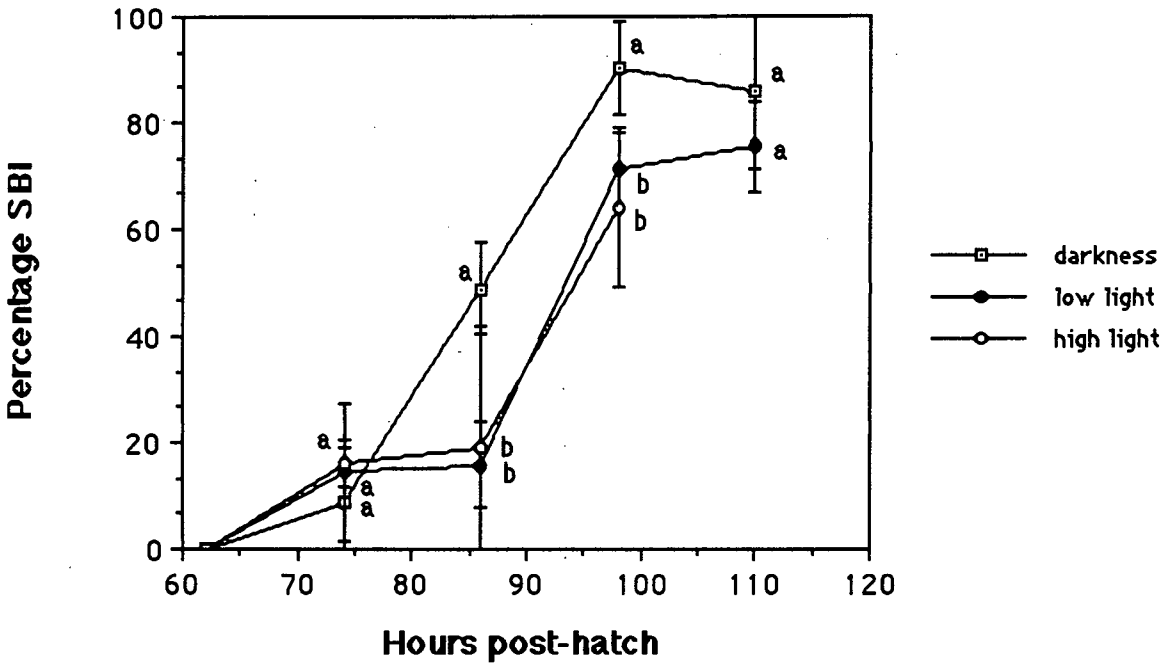


FIG. 2 - Patterns of swim bladder inflation (SBI) over time under three lighting situations. Data points represent mean percentages (± s.d.) of three replicates of 15 larvae each. Data points within a time sample sharing dissimilar superscripts represent statistically significant differences (one-way ANOVA, $p < 0.05$).

implies that yellowfin bream larvae are, to some extent, photonegative, although not to the extent of Australian bass where constant light of only 100-200 lux reduced the inflation rate from approximately 70% achieved in darkness to less than 1% (Battaglene and Talbot, 1990). Poor swim bladder inflation has been recorded for gilthead seabream, *S. auratus*, at light intensities exceeding 2000 lux (Barnabe, 1990). In practice, maintaining larvae in darkness during this stage is impractical as it coincides with first-feeding, and assuming yellowfin bream larvae are visual feeders, as proven for the two sparids *A. schlegeli* and *Pagrus auratus* (Kiyono *et al.*, 1989), some light will be necessary for larvae to locate food items.

From the above, it can be recommended to maintain larvae during SBI at the lowest light level compatible with successful first feeding. Although this has not been determined, personal observations of larvae feeding at light intensities of 50-100 lux would suggest that this may be a good compromise. A lower feeding threshold of approximately 10 lux is common (Tucker, 1992a), although a level of 100-150 lux was identified by Ounais-Guschemann (1989) for gilthead seabream larvae.

Failure to inflate the swim bladder has several deleterious consequences. It has been linked to larval deformities, poor growth performance, poor stress tolerance, and lordosis in older fish (Takashimi *et al.*, 1980; Kitajima *et al.*, 1981; Chatain, 1987; Battaglene and Talbot, 1990; Fukusho, 1991). Research into the mechanisms of swim bladder inflation has been given high priority in the Mediterranean, and has been instrumental in the development of the finfish mariculture industry there (Sweetman, 1992). Like the gilthead seabream *S. auratus*, and red seabream *P. auratus*, yellowfin bream almost certainly have a pneumatic duct and require access to the water surface in order to inflate the swim bladder. Yellowfin bream larvae could be seen breaking the water surface during SBI (personal observation). By analogy

with the red seabream, it is likely that the pneumatic duct degenerates beyond approximately 5 days post-hatch.

Several reports have emphasised the importance of maintaining a clean, oil-free water surface during the time of swim bladder inflation for larvae that require access to the water/air interface to achieve this (Foscarini 1988; Chatain and Ounais-Guschemann, 1990; Sweetman 1992). Similarly, excessive water disturbance (e.g. through aeration) has been found to interfere with access of other sparid larvae to the water surface (Al-Abdul-Elah *et al.*, 1983; Foscarini, 1988). The high inflation rates observed in the present study were probably a result of the clean water surfaces and low levels of water disturbance (as no feeding or aeration were supplied), allowing easy access of larvae to the water surface.

Clearwater rearing trial

Larvae were apparently capable of feeding (eyes pigmented, mouth and anus open) soon after the yolksac was absorbed. Although food was first offered on day 3, rotifers were not seen in the gut of larvae until day 4. An examination of larvae on day 6 showed many to have still not commenced feeding. This failure to commence feeding is most likely responsible for the large mortality peak on days 6/7 (Fig. 3), in which an estimated 40% of larvae died. An examination of dead larvae revealed most of their stomachs to be empty. The timing of the observed mortality peak coincides with that at which unfed larvae are observed to die. Such a mortality peak related to first feeding is common to many larval marine fish, and losses of up to 50% have been common in rearing trials with species such as *Sparus auratus* (Person Le Ruyet and Verillaud, 1980), *Pagrus auratus* (Kuronuma and Fukusho, 1984; Foscarini, 1988; Pankhurst *et al.*, 1991), *Diplodus sargus* (Mazzola *et al.*, 1983),

D. vulgaris (Jug-Dujakovic and Glamuzina, 1988), and *Acanthopagrus berda* (Mok, 1985).

It is well known that the maximum size of potential prey is set by larval mouth size (Hunter, 1981). However, the optimal prey size is only approximately 25% of maximum mouth width at first feeding (De Ciechomsky, 1967; Hunter, 1984; Fernandez-Diaz *et al.*, 1994). In a study of food size selectivity by gilthead seabream larvae, *S. auratus*, Polo *et al.* (1992) showed that up to day 8 from hatch, larvae selected small-strain rotifers (92-176 µm), and after day 13 selected large-strain rotifers (140-276 µm). Growth and survival were improved when both rotifer strains were available to the larvae, clearly demonstrating the importance of appropriate food size. Fernandez-Diaz *et al.* (1994) showed the same species to select food particles of 25-50 µm diameter at first-feeding. Mouth width increased linearly with larval size. Similar selectivity or functional mouth sizes have been demonstrated for other larval sparids such as *Archosargus rhomboidalis* (Stepien, 1976), *Puntazzo puntazzo* (Franicevic, 1989), and *D. vulgaris* (Jug-Dujakovic and Glamuzina, 1988). Yellowfin bream were found to have an initial mouth width of approximately 390 µm, representing an initial optimal prey width of approximately 90-100 µm. Sieved rotifers in the present study ranged in width from 85-180 µm immediately after sieving, but the average size would have increased in the following hours as neonates continued to grow in the rearing water (Lubzens *et al.*, 1989). It therefore appears that despite having passed through a 125 µm mesh, many of the rotifers offered in the present study were still too large to be optimal-sized prey for yellowfin bream larvae.

Traditionally, these problems have been remedied by the use of smaller rotifer strains (e.g. Hussain and Higuchi, 1980; Lim, 1993), by sieving larger-strain rotifers more finely (e.g. 90 µm sieves), or by using smaller food

organisms such as oyster trochophores (approximately 60 µm width). A small-strain rotifer from Japan will soon be available in Australia (S. Battaglene, pers. comm., 1994).

Another mortality peak, in which a further 40% of larvae are estimated to have died, followed on days 11/12 (Fig. 3). The cause of this is uncertain, although it may be linked to the poor rate of initial swim bladder inflation. Only approximately 30% of larvae inflated the swim bladder correctly (detailed below). Battaglene *et al.* (1989) observed amongst Australian bass, *Macquaria novemaculeata*, and Chatain (1989) amongst European sea bass, *Dicentrarchus labrax*, and gilthead seabream, *S. auratus*, that larvae with non-functional swim bladders were much more prone to stress-related mortality. An examination of dead larvae on days 11/12 revealed 74% to have non-functional swim bladders, although all had food present in the gut. Water quality was at its worst on these days, with total ammonia and nitrite nearing 1 mg/l and 0.2 mg/l respectively, which may have stressed the larvae.

The food schedule followed was similar to that used successfully on most other sparids, which also involves rotifers followed by brine shrimp (Person-Le Ruyet and Verillaud, 1980; Hussain *et al.*, 1981; Mok, 1985; Foscarini, 1988; Garratt *et al.*, 1989; Battaglene and Talbot, 1992). In Taiwan, however, the commercial culture of sparids is often accomplished through use of oyster trochophores from days 3-10 (Chen, 1990). Although once common practice in Japan for red seabream, the use of oyster larvae has given way to the use of small-strain rotifers because of the difficulty in preparing large quantities of oyster larvae, and their relatively poor nutritional value (Kuronuma and Fukusho, 1984; Fukuhara, 1987).

Tail flexion occurred from day 19, followed by metamorphosis between days 24-30 when larvae averaged 6.51 ± 0.80 mm (s.d., n = 30). This size at

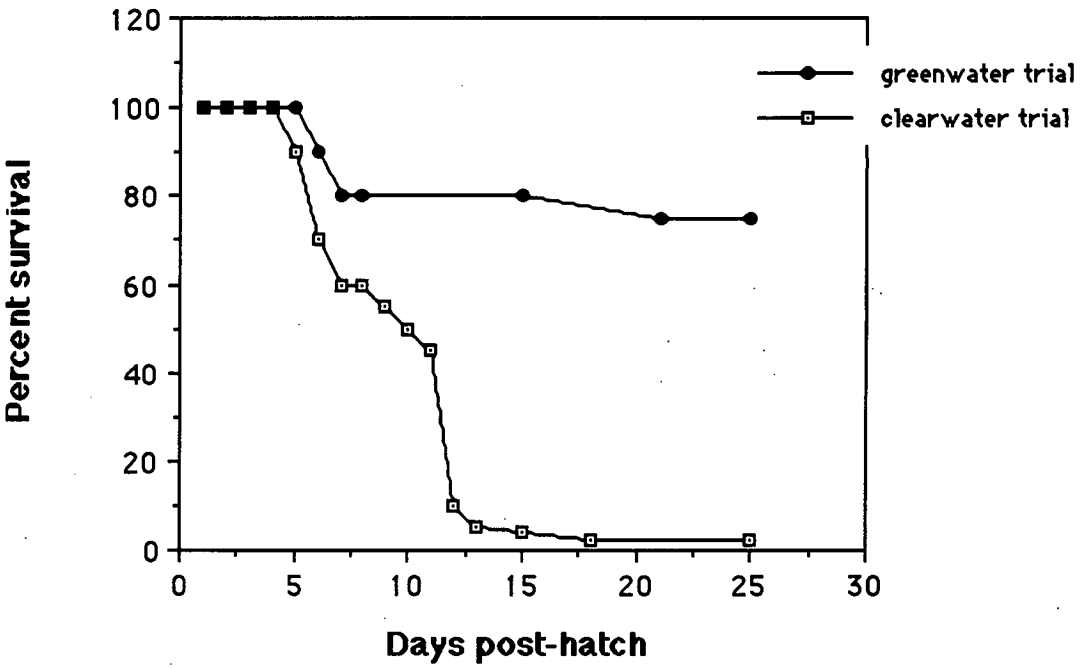


FIG. 3 - Survival curves to metamorphosis during larval rearing trials employing clearwater and greenwater techniques. Data points represent estimates of percent survival based on larval densities and mortalities seen on the tank bottom.

metamorphosis is small compared with other sparids (Foscarini, 1988). At this stage only 2.5 % of larvae remained (Fig. 3). Juveniles readily accepted artificial food by day 35, and survival from metamorphosis was high with little evidence of cannibalism. This developmental timing is almost identical to that observed for *P. auratus* from Australian waters (Battaglene and Talbot, 1992), but fast relative to other sparids. For example metamorphosis is not reached until day 60 for gilthead seabream, and weaning is not started until day 40-45 (Person-Le Ruyet and Verillaud, 1980; Tandler and Helps, 1985; Barnabe, 1990).

Growth rates to metamorphosis (Fig. 4) were similar to those observed for other sparids such as *P. auratus* (Foscarini, 1988; Pankhurst *et al.*, 1991; Battaglene and Talbot, 1992), *S. auratus* (Person-Le Ruyet and Verillaud, 1980), *A. cuvieri* (Hussain *et al.*, 1981), *A. schlegeli* (Kuronuma and Fukusho, 1984; Chen, 1990), *A. berda* (Mok, 1985), and *Archosargus rhomboidalis* (Stepien, 1976). Also, all of these studies showed a common "lag" period during and shortly after the transition from endogenous to exogenous nutrition, before positive growth was again recorded (Fig. 4).

The first swim bladders were seen on day 3 and no increase in the percentage of larvae with swim bladders was seen after day 5. Only approximately 30% of larvae inflated the swim bladder correctly. This is similar to rates achieved at commercial gilthead seabream hatcheries before the advent of surface oil skimmers. The percentage successful inflation was well below the level achieved in the swim bladder inflation experiment, where no feeding was carried out. The single blower and floating trap in operation in this trial may not have been adequate to remove all of the surface oil and debris that arose from rotifer feeding and enrichment.

The swim bladder of healthy larvae was oval-shaped, whereas approximately 20% of larvae had larger swim bladders that were spherical and did not enlarge with increasing larval size. These larvae may have inflated the swim bladder with water rather than air, an occurrence noted amongst red seabream larvae (Takashima *et al.*, 1980) and Australian bass larvae, *M. novemaculeata* (Battaglene and Talbot, 1990). Although the cause for this is unknown, it may also be related to difficulties in gaining access to the water surface.

Greenwater rearing trial

The timing of larval development was to some extent advanced in this trial, relative to the clearwater trial. Growth proceeded at a faster rate (Fig. 4), and tail flexion and metamorphosis occurred 1 day and 3 days earlier, respectively. While a greater size-for-age may be partly attributable to a larger mean hatch size (1.75 versus 1.63 mm N.L.), improvements in growth rate were evident (Fig. 4). A possible explanation related to swim bladder inflation success is presented below. Contrarily, weaning in this trial appeared to be more difficult to complete, taking an extra 4 days.

Numerous small protozoa (~5-10 μm) were observed in the rearing water throughout the trial. It is not known whether larvae were consuming these protozoa, although it is unlikely based on their extremely rapid and erratic movement. If so, however, this may have contributed to the high rate of initial feeding success (Fig. 3). Alternatively, the microalgae may have provided a better background against which rotifers could be seen, which is considered to be one of its major functions (Sweetman, 1992).

Successful swim bladder inflation, at 77%, was far in excess of that achieved in the clearwater trial, and similar to that achieved for barramundi larvae using the same greenwater technique (Palmer *et al.*, 1992). This was

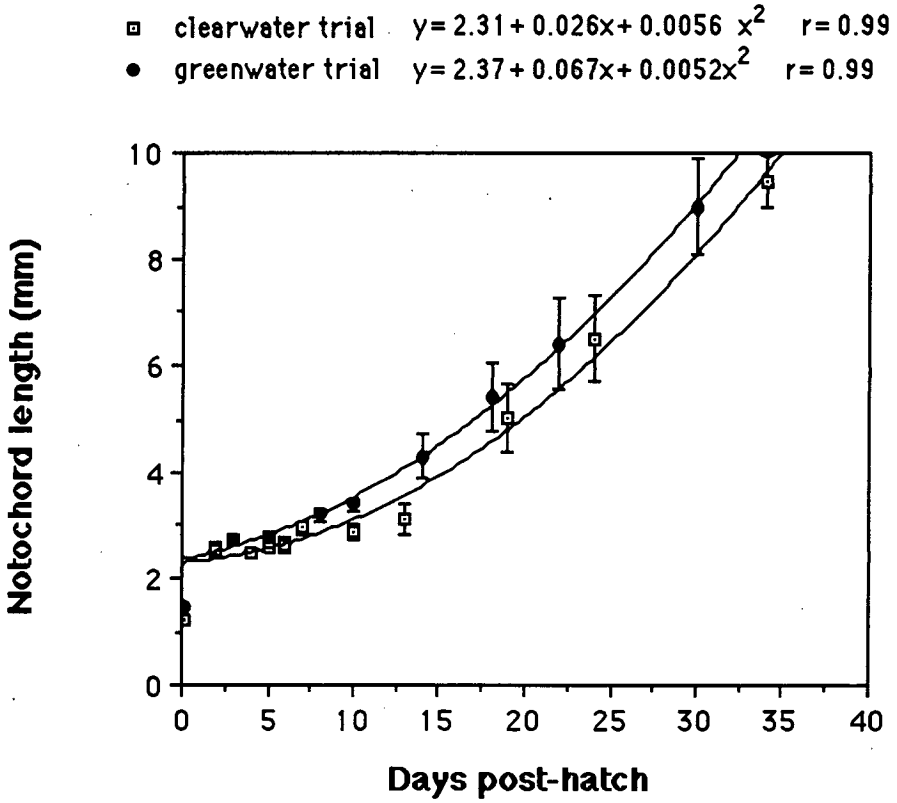


FIG. 4 - Growth of bream larvae in clearwater and greenwater trials to approximately 10 days beyond metamorphosis. Each data point represents the mean notochord length (± s.d.) of approximately 10 larvae. Quadratic equations have been fitted to each data set from day 2 onward, and the equations and 'r' values are given.

somewhat surprising as the surface skimmer was only used intermittently throughout this trial. The fact that live foods were not enriched with commercial nutritional enhancers is likely to be responsible for this improvement. However, an oily surface film during larval rearing of *P. auratus* has been attributed to the addition of algae to the rearing water (Chatain, 1982).

Chatain (1989) found, for gilthead seabream, that larvae not carrying functional swim bladders by metamorphosis were, on average, 20-30% smaller than those with swim bladders. Similarly, Battaglene and Talbot (1992) found, for *P. auratus*, that growth was significantly slower amongst larvae without functional swim bladders. This effect on growth can be attributed to the greater energetic demands involved in maintaining position in the water column, and less efficient predatory ability. Thus, the higher swim bladder inflation rate in the greenwater trial may explain the greater mean growth rate observed (Fig. 4).

Fig. 3 compares the survival curves observed in the two trials, and shows the greenwater, in this instance, to be far superior. Again, a major mortality peak is observed on days 7-8, and once again this can be attributed to a failure of approximately 20% of larvae to successfully overcome the transition to exogenous nutrition. From this time the survival curve for larvae in greenwater is relatively stable, showing approximately 75% survival to metamorphosis. The second mortality peak observed in the clearwater trial is absent. No noticeable increase in mortality occurred during weaning, which has been found to be a common occurrence in other species, such as the red seabream (Kuronuma and Fukusho, 1984).

The survival of yellowfin bream achieved in this experiment is considerably better than rates achieved at commercial gilthead seabream

hatcheries in Europe and Israel (approximately 10% and 20-40% respectively, Sweetman, 1992; Tandler, 1993) and red seabream hatcheries in Japan (40-50%, Fukusho, 1991). Sparids in general do not have a reputation for achieving high survival rates, and some species are quite problematic, such as, for example, the santer seabream, *Cheimerius nufar*, which failed to achieve greater than 0.5% survival in 19 initial rearing trials (Garratt *et al.*, 1989). The present results are indicative of the suitability of yellowfin bream for hatchery production.

The superiority of the greenwater over the clearwater rearing trial, while only a tentative finding as the greenwater trial was unreplicated, appears to be linked to the use of unicellular alge in the rearing water. This is common practice in fish hatcheries world-wide, for example in culturing groupers (Tookwinas, 1989; Lim, 1993), barramundi, *Lates calcarifer* (Dhert *et al.*, 1992), gilthead seabream, *S. auratus* (Person-Le Ruyet and Verillaud, 1980; Sweetman, 1992; Tandler, 1993), and red seabream, *P. auratus* (Fukusho, 1991). Benefits include the continued nutritional enhancement of live foods, water 'conditioning', and provision of a background against which larvae are better able to see food organisms (Fukusho, 1991; Sweetman, 1992). No direct nutritional value from the microalgae is thought to occur.

The nutritional state of broodfish up to spawning has been shown to dramatically influence egg composition and quality (e.g. Craik and Harvey, 1984b; Watanabe *et al.*, 1984a-d; Watanabe, 1985, 1989; Devauchelle *et al.*, 1988; Leu and Wu, 1990; Mourente and Odriozola, 1990b; Harel *et al.*, 1992). For example, concentrations of protein, vitamins A and E, and in particular fatty acid content and composition have all been shown to depend on levels in the broodstock diet of red seabream, *P. auratus* (Watanabe *et al.*, 1984a-d; Watanabe, 1985). As broodfish in the present study were wild-caught, the likelihood of dietary deficiency is remote. Indeed, from the viewpoints of

hatch rates and egg appearance, egg quality in both of the above trials is considered to have been good (Kjorsvik *et al.*, 1990).

However, larvae were obtained for rearing trials through hormone-induced spawning (Chapter 2). Egg quality is widely regarded as being inferior from hormone-induced spawnings than from natural spawnings, presumably due to the advancement of natural processes and stress involved in anaesthetisation and administration of hormone. Furthermore, Ako *et al.* (1994) found significantly lesser amounts of most fatty acids and essential amino acids in hormonally-induced than in naturally-spawned eggs from the milkfish, *Chanos chanos*. Superior egg quality has also been observed from completely spontaneous spawnings than from hormone-induced spawnings of red seabream, *P. auratus* (Foscarini, 1988). Billard *et al.* (1981) have reviewed the effects of stress on teleost reproduction, and cite numerous examples of stress having adverse effects on egg quality. The effects of stress on broodfish in the present project were minimised, as fish were only anaesthetised once to administer the single hormone injection. No second hormone dose was given, and fish were not hand-stripped. As such, egg quality in the present study is considered from most perspectives to have been high.

Nutritional studies

The significant mortality observed in the present study over days 7-8 coincides with that observed amongst unfed larvae, and is suggestive of starvation as the cause (energetic requirements not met). The high survival and growth of larvae beyond the transition to external foods is indicative of nutritional requirements being met through the foods then available to them. However, it is still of value to identify potentially limiting nutrients so that in future trials, growth can be maximised through live food enrichment. The

supply of amino and fatty acid requirements is considered to be particularly critical to marine fish larvae (Watanabe *et al.*, 1983; Tucker, 1992a).

(i) amino acid analysis of larvae and rotifers

Results of amino acid analyses indicate that rotifers are, from an essential amino acid profile point of view, a high value protein source for yellowfin bream larvae. The essential amino acid profiles of rotifers and yellowfin bream whole-body protein are similar (Table 1). Furthermore, these amino acids are likely to be largely available as rotifer protein is highly digestible, as shown for red seabream, *P. auratus*, larvae (Watanabe *et al.*, 1983). The chemical score indices (I) indicate that four essential amino acids (tryptophan, histidine, methionine and phenylalanine) occur in slightly lower proportions in rotifers than whole-body larval protein, with tryptophan being the first limiting amino acid ('I' value 87.9%, Table 1). Tryptophan and methionine are known to be adversely affected by acid hydrolysis (Gehrke and Takeda, 1973; Varadi and Pongor, 1979), so results are likely to underestimate their true content in rotifer and larval protein. Other 'I' values equalled or exceeded 100%, indicating that these amino acids occur in adequate amounts in rotifers.

Most other reported analyses of rotifers have similarly shown them to be adequate in essential amino acids (e.g. Watanabe *et al.*, 1983). *Artemia* nauplii and metanauplii are also considered to be a high quality protein source for marine fish larvae, although methionine may be limited to some extent (Watanabe *et al.*, 1983; Leger *et al.*, 1986, 1987).

(ii) fatty acid analysis of eggs and rotifers

The fatty acid profiles of eggs used in the greenwater rearing trial, and of rotifers, are given in Table 2. Oleic and palmitic acids (18:1n9 and 16:0

Amino Acid	FREE mmol/g homogenate		BOUND mmol/g homogenate		TOTAL % total (free + bound)		
	Larvae	Rotifers	Larvae	Rotifers	Larvae	Rotifers	'I' Value
ASP	1.71 ± 0.038	1.34 ± 0.069	19.62 ± 0.98	13.83 ± 0.97	4.76	4.94	
GLU	3.82 ± 0.047	2.44 ± 0.078	20.75 ± 1.00	14.07 ± 0.62	5.48	5.37	
ASN	0.16 ± 0.002	1.44 ± 0.030	U	U	0.04	0.47	
SER	3.41 ± 0.012	3.86 ± 0.032	23.01 ± 0.14	14.91 ± 0.17	5.90	6.11	
GLN	0.56 ± 0.005	1.89 ± 0.039	U	U	0.13	0.62	
GLY	6.24 ± 0.031	5.83 ± 0.290	27.06 ± 1.78	16.28 ± 1.11	7.44	7.20	
ALA	11.08 ± 0.106	1.50 ± 0.031	U	U	2.47	0.49	
TYR	4.39 ± 0.075	4.96 ± 0.075	21.39 ± 2.64	14.79 ± 0.31	5.76	6.42	
HIS*	4.98 ± 0.028	2.21 ± 0.010	24.08 ± 0.52	15.43 ± 0.10	6.49	5.74	88.5 %
THR*	2.61 ± 0.002	3.64 ± 0.023	22.30 ± 0.02	14.75 ± 0.13	5.56	5.98	107.6 %
ARG*	3.47 ± 0.072	6.91 ± 0.121	24.99 ± 0.12	15.78 ± 0.38	6.35	7.38	116.2 %
TRP*	7.79 ± 0.015	3.03 ± 0.001	22.67 ± 1.32	15.32 ± 0.01	6.80	5.97	87.9 %
MET*	8.62 ± 0.084	6.68 ± 0.009	59.40 ± 1.35	38.10 ± 0.07	15.18	14.57	96.0 %
VAL*	2.40 ± 0.024	2.55 ± 0.060	23.62 ± 0.38	15.47 ± 0.52	5.80	5.86	101.0 %
PHE*	4.29 ± 0.006	3.28 ± 0.059	23.39 ± 0.51	15.02 ± 0.38	6.18	5.96	96.4 %
ILE*	2.25 ± 0.040	2.45 ± 0.024	23.39 ± 0.10	15.44 ± 0.21	5.72	5.82	101.8 %
LEU*	3.04 ± 0.032	3.24 ± 0.043	24.10 ± 0.39	15.37 ± 0.28	6.06	6.06	99.9 %
LYS*	4.61 ± 0.027	7.60 ± 0.231	12.79 ± 0.60	7.82 ± 0.33	3.99	5.02	129.2 %

TABLE 1 - Free, bound and total amino acid profiles of first-feeding yellowfin bream larvae and rotifers, *B. plicatilis*, reared on the alga *N. oculata*. The chemical score indices ('I', refer to text) for total essential amino acids have been included to provide some indication of the value of rotifers as a protein source. Values represent means (± s.d.) of two replicate samples. Asterisks represent essential amino acids. 'U' represents undetected.

respectively) were quantitatively the major fatty acids present in eggs (18.64 and 23.54% of total fatty acids, respectively), while docosahexaenoic acid (DHA; 22:6n3) was the third major constituent at 11.89% of total fatty acids (Table 2). These three fatty acids are similarly the greatest constituents of eggs or yolksac larvae of other marine and freshwater species which have been studied, such as the dolphin fish, *Coryphaena hippurus* (Ostrowski and Divakaran, 1989, 1991; Ako *et al.*, 1991), gilthead seabream, *S. auratus* (Mourente and Odriozola, 1990a,b; Rodriguez *et al.*, 1994), rainbow trout, *O. mykis* (Watanabe *et al.*, 1978), red seabream, *P. auratus* (Watanabe *et al.*,1984a,d), Atlantic cod, *Gadus morhua* (Ulvund and Grahl-Nielsen, 1988), and Dover sole, *Solea solea* (Dendrinos and Thorpe, 1987). However, DHA is generally a greater constituent than observed here for yellowfin bream (up to 40% for dolphin fish, Kraul *et al.*, 1992). Levels of eicosapentaenoic acid (EPA) were also very low (2.98% of total fatty acids), giving a total n-3 HUFA content of only 19.67% of total fatty acids (Table 2). It should be noted, however, that the fatty acid profiles of eggs can vary to a considerable extent, even between different spawnings from the same female (Chapter 2).

Falk-Petersen *et al.* (1986), Dendrinos and Thorpe (1987), and others have suggested that the fatty acid requirements of a larva are a reflection of egg composition. This makes sense, particularly for essential fatty acids, as we would expect the mother to pass on to eggs a significant amount of any fatty acids which are necessary for the pre-feeding larva, but unable to be synthesised by the larva and therefore likely to be limiting. This is in fact observed, for example in DHA levels, which often occur at very high levels in eggs of species showing a high DHA requirement (Ostrowski and Divakaran, 1989; Mourente and Odriozola, 1990a,b). If this is correct, n-3 HUFA requirements of yellowfin bream would appear to be small. In fact the fatty

Fatty acid	Fertilised eggs	Rotifers fed *N. oculata*
14:0	2.63 ± 0.14	-
16:0	23.54 ± 0.42	28.1
16:1n7	5.24 ± 0.26	29.3
18:0	7.78 ± 0.52	4.4
18:1n7	-	4.1
18:1n9	18.64 ± 1.51	17.1
18:1n6	2.29 ± 0.36	-
18:2n6	4.89 ± 0.73	2.1
20:1n9	-	2.6
20:4n3	-	4.1
20:4n6	3.60 ± 0.13	-
20:5n3 (EPA)	2.98 ± 1.54	3.0
22:5n3	4.80 ± 0.11	2.2
22:6n3 (DHA)	11.89 ± 0.10	-
Σn-3 HUFA	19.67	9.3

TABLE 2 - Fatty acid composition (as percentage of fatty acids) of yellowfin bream eggs and rotifers, *B. plicatilis*, reared on the alga *N. oculata*. Egg values represent the mean (± s.d.) of two replicates. An analytical error prevented rotifer replication. Dashes represent values less than 1%.

acid profile would suggest a minimal EPA requirement, which occurred in very low amounts in the egg (3.0%).

It is widely believed that n-3 HUFAs play a critical role in the phospholipid fraction of biomembranes, and are essential dietary components for marine fish which, unlike freshwater species, have limited ability to elongate and desaturate shorter-chain fatty acids (Owen *et al.*, 1975; Kanazawa *et al.*, 1979, 1982, 1985; Yamada *et al.*, 1980; Watanabe, 1982; Lemm and Lemarie, 1991). The nutritional value of a particular food to marine fish larvae has often been linked directly with essential fatty acid (EFA) content (e.g. Watanabe *et al.*, 1983; Lubzens *et al.*, 1989; Koven *et al.*, 1990; Kraul *et al.*, 1993). Recent studies have suggested that the roles of the two major n-3 HUFAs, DHA and EPA, are distinct (Kraul *et al.*, 1993; Mourente *et al.*, 1993; Watanabe, 1993), and that DHA plays the more critical role (Watanabe, 1991, 1993; Tucker, 1992a) and is more strongly conserved in starved marine fish larvae than EPA (Ako *et al.*, 1991). It now appears that the requirements for DHA and EPA are species-specific, some fish requiring both and others only one or the other, or specific ratios of the two (Lubzens *et al.*, 1989; Sargent, 1991; Mourente *et al.*, 1993; Watanabe, 1993).

Unenriched rotifers in the present study, used in the greenwater trial, were very deficient in EPA and DHA, which together constituted only 3.0% of total fatty acids (Table 2). DHA occurred only in trace amounts. This is expected as the fatty acid profile of rotifers is chiefly determined by diet (Watanabe *et al.*, 1983). Rotifers in the present study were raised on the alga *Nannocloropsis oculata* (Eustigmatophyceae), which is known to be deficient in DHA (Mourente *et al.*, 1990; Dunstan *et al.*, 1993). Other analyses of rotifers raised on this alga (e.g. Watanabe *et al.*, 1983; Dendrinos and Thorpe, 1987; Rimmer and Reed, 1990; Mourente *et al.*, 1993) have similarly shown low DHA levels. Furthermore, it is well known that without enrichment, *Artemia*

too are deficient in DHA, particularly Great Salt Lake brand (Watanabe *et al.*, 1978; Dendrinos and Thorpe, 1987; Dhert *et al*, 1990). It therefore appears that larvae in the greenwater trial had a negligible dietary n-3 HUFA intake, certainly well below the 2-4% of dry food weight recommended by Tucker (1992a). This is of great interest as the larvae did not exhibit any signs of nutritional stress, implying either a low requirement for n-3 HUFA, or a substantial ability to bioconvert n-3 fatty acid precursors to higher-chain fatty acids. Although being extremely unusual, a similar low HUFA requirement has also been shown for the plaice, *Pleuronectes platessa* (Dickey-Collas and Geffen, 1992). These authors found plaice larvae to grow and survive equally well to metamorphosis when fed either n-3 HUFA-enriched or n-3 HUFA-deficient live foods. At least several species of marine fish have a slight ability to convert linolenic acid to DHA (e.g. Yone and Fujii, 1975; Hardy *et al.*, 1987), but apparently not enough to meet their requirements when DHA is limiting in the diet (Schauer and Simpson, 1985). Larvae suffering n-3 HUFA deficiency are normally characterised by a fainting or shock response after being subjected to any form of stress, as reported for Australian bass (Battaglene *et al.*, 1989), barramundi (Rodgers and Barlow, 1987) and European sea bass larvae (Katavic, 1986).

In several species, enrichment of live foods with EFAs has been shown to be most important in the later stages of larval development, rather than in the first few weeks of feeding (e.g. Rimmer and Reed, 1990; Kraul *et al.*, 1992; Stottrup and Attramadal, 1992). This has been explained by maternally-derived reserves of EFAs sustaining the larvae over the first few weeks (Ostrowski and Divakaran, 1990). This again supports the notion that yellowfin bream have a very low n-3 HUFA requirement and/or the ability to meet their requirement, as not only were no signs of n-3 HUFA deficiency shown in the last weeks of larval life, but levels of n-3 HUFA were initially

very low in the egg. The possibility exists that yellowfin bream may possess substantial Δ4, Δ5, and Δ6 desaturase activities, allowing this species to synthesise DHA to meet its requirements. This idea could be assessed through use of tritiated linoleic and linolenic acids, in the same way as has been used to show that marine fish in general have a low ability to manufacture C20 and C22 fatty acids from these precursors (Owen *et al.*, 1975; Yamada *et al.*, 1980), and certainly demands further investigation.

CHAPTER 5

Growout

Introduction

Growout performance is of paramount importance when assessing the economic viability of commercial culture of a species. Factors such as growth rate, survival, food conversion, disease susceptibility, tolerance of crowding and netcage conditions, and feeding habits should all be considered. While no species will be ideal from all of these perspectives, a knowledge of the shortfalls of a species, and ways to minimise the effects of these, is important when assessing its aquaculture potential.

No previous studies have examined the growout performance of captive yellowfin bream. However, several studies have estimated the growth of wild fish, mainly for the purpose of assessing the economically important wild fishery. Munro (1944) and Dredge (1976) recorded growth rates of wild fish in south-east Queensland of approximately 7.6 cm and 12.2-12.5 cm in the first and second years. More recently Pollock (1982b) and Pollock *et al.* (1983), using tag-recapture and length-frequency analysis, revealed much faster growth rates from fish in the same area, reaching 10-14.5 cm and 20.5 cm in their first and second years. This result cast doubt on the accuracy of otolith and scale annuli ageing techniques used in the former two studies. While these growth rates are considered poor by aquaculture standards, growth rates under culture conditions often substantially exceed those in the wild, and demand separate consideration.

The aims of this component of the project were to record growth rates, survival, food utilisation and other aspects of growout performance under netcage conditions for juvenile yellowfin bream originally obtained through hormone-induced spawning. This information will be useful when assessing the economics of large-scale rearing of yellowfin bream.

Materials and Methods

Assessment of growout performance

Yellowfin bream reared to metamorphosis in the greenwater larval rearing trial (Chapter 4) were maintained in a clearwater recirculation system until they were nearly three months old. Then, on 30th September 1993, 220 were transferred to a netcage of approximately 4 m^3 at the 'Hart Fisheries' barramundi farm in Ross Creek, Townsville. Thirty of these were measured for length (± 0.5 mm) and weight (± 0.1 g) prior to introduction to the netcage as a baseline measurement. Net mesh size was initially 4 mm, and was increased as fish grew. After 9 months, fish were transferred to a 24 m^3 net, and then at 12 months, due to space and net limitations, fish were transferred to a similar net shared with barramundi, *Lates calcarifer*, of smaller size. Nets were cleaned of fouling at irregular intervals as considered necessary.

Unfortunately, exceptionally large tides in January 1994 caused the netcage to tear, allowing all but 22 bream to escape. Fish were approximately 80 mm (TL) at this time. As wild juveniles of similar size are common in nearby waters at this time, a further 11 fish were caught by small hook and line, and substituted for some of those lost, allowing the growth trial to proceed. By analogy with the gilthead seabream, for which wild-caught and artificially-reared fish show similar growth, survival and food conversion

under netcage conditions (Franceston *et al.*, 1988), this was considered valid. Another baseline length and weight measurement was taken at this time.

Fish were initially (up to 60 mm) fed to satiation five times daily with barramundi starter 'crumble no. 1' (Aqua-Feed Products, P.O. Box 187, Deception Bay, Queensland, Australia) by way of an automatic feeder. Pellet size was gradually increased as fish grew. Feeding frequency was reduced to twice/day when, at 12 months, bream were placed in the netcage with barramundi. At this time, Aqua-Feed barramundi grower was offered as food (protein level 45%).

Samples of 10-15 fish were caught and anaesthetised with MS-222 (70 mg/l) every month, and their mean weights (± 0.1 g) and lengths (± 0.5 mm) determined. After 6 months an 'inventory' sample was taken in which the number of surviving fish was determined for survival information. When bream were transferred to the netcage containing barramundi, subsequent difficulties in catching them precluded samples from being taken for a 12 month period, until the barramundi were harvested. General observations of feeding habits and behaviour (aggression), tolerance to netcage conditions and handling, and susceptibility to disease were made.

Food conversion ratio

During the 12-day period 24th February to 8th March 1994, a preliminary food conversion ratio was determined. Thirty fish were transferred to a 1000 l fibreglass tank receiving recirculated water for this trial to avoid possible inaccuracies due to any natural food occurring in netcages. Fish were approximately 100 mm at this time, and were acclimated to tank conditions for four days prior to commencement of the trial. The weight of food given to the juvenile bream over this period was determined (± 0.1 g). Fish were starved for one day prior to the commencement, and again at the termination

of the trial, to avoid inaccuracies due to gut contents. Fish were fed manually five times daily. Extra care was taken through the trial to ensure that most or all of the food was ingested at each feeding. All fish were measured for weight before and after this period (± 0.1 g).

The food conversion ratio was calculated on the basis of the ratio of dry food consumed to live weight gained by the fish. Gross growth efficiency was presented as the fraction of wet weight gain to dry food consumed. The protein efficiency ratio was calculated on the basis of the ratio of wet weight gain to dry weight of protein fed over the experimental period. Water temperature and salinity throughout the trial were 29.5 ± 0.8°C and 36 ± 1 ppt respectively.

Results and Discussion

Growth assessment

The growth of yellowfin bream in length and weight to 25 months of age is shown in Figs. 1 and 2, and resultant fish are illustrated in Plates 1a and 1b. After 12 months from hatch, fish had reached a mean size of approximately 15 cm and 85 g, and by 25 months had reached 22.2 ± 1.5 cm and 252.4 ± 55.2 g (± s.d.) (range 162-350 g). These represent the first growth data for this species in captivity. Pollock (1982b) and Pollock *et al.* (1983), using tag-recapture data and length-frequency analysis of wild yellowfin bream populations inhabiting Moreton Bay, found that the modal size of yellowfin bream after 12 months was between 10-14.5 cm, and after 2 years was approximately 20.5 cm. Munro (1944) and Dredge (1976), using otolith and scale annuli interpretation, independently recorded slower growth rates of 7.6 cm and 12.2-12.5 cm in the first and second years, which may reflect difficulties in interpreting annuli. The faster growth observed in the present

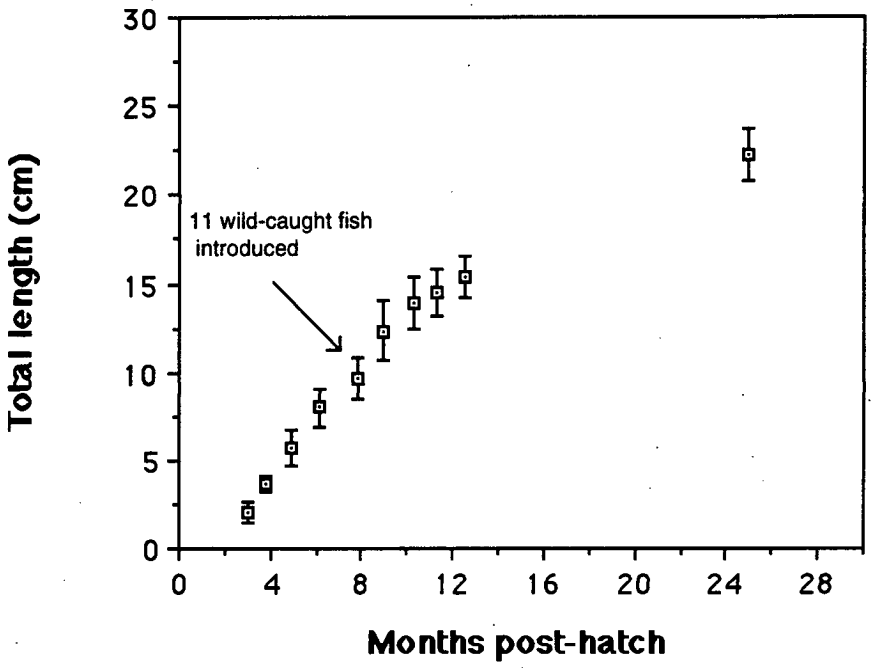


FIG. 1 - Growth in length of yellowfin bream under netcage conditions to 25 months of age. Fish were first introduced to the netcage at 3 months. Data points represent means (± s.d.) of samples of 10-30 fish.

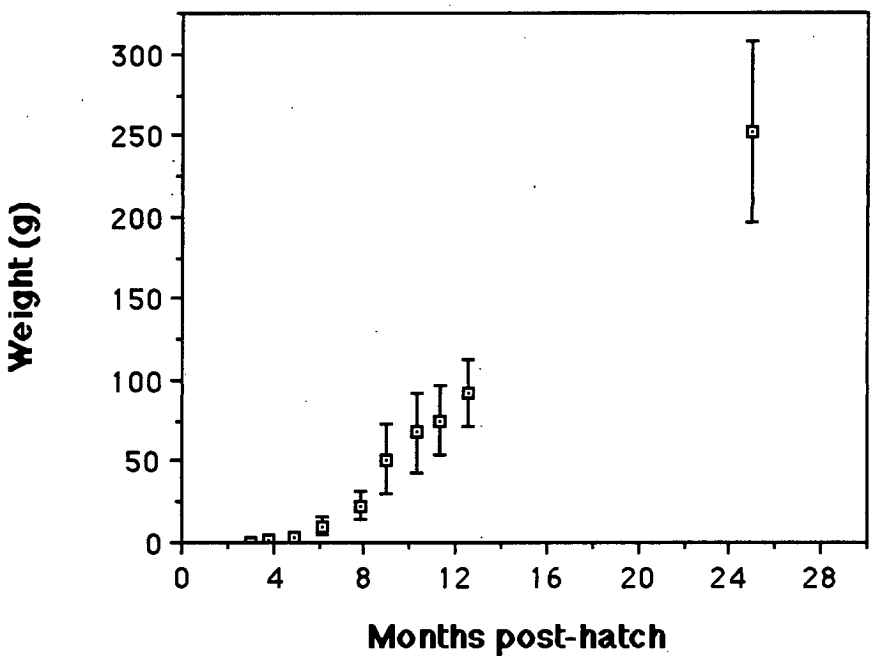


FIG. 2 - Growth in weight of yellowfin bream from introduction to a growout netcage at 3 months of age, to 25 months. Data points represent the means (± s.d.) of samples of 10-30 fish.

PLATES 1a (above) and 1b (right) - Yellowfin bream at the end of the two-year netcage growout trial. Mean fish weight was 252 ± 55.2 g (range 162-350 g).

study could be expected due to higher water temperatures (latitude of Moreton Bay and Townsville, 27°S and 19°S respectively). Furthermore, growth under culture conditions has often been found to exceed that observed in wild fish populations, mainly due to improved food availability (e.g. Chen, 1990; Bell *et al.*, 1991; Fukusho, 1991).

The regular sample of 10 fish taken for growth measurements in the August following transfer to the netcage showed all 10 fish to have matured into functional males at an age of 13 months. Slight abdominal pressure caused the release of milt from each fish. This observation confirmed earlier indications (Chapter 2) that yellowfin bream, at least in the Townsville region, mature in their first year. The diversion of food energy to gonadal rather than somatic growth is well known to reduce growth rate, and may well have done so in the present study. Interestingly, in the final sampling when fish were 25 months of age, six of the 25 fish (24%) had changed sex to female (mean length 22.7 ± 1.4 cm). This also confirmed earlier data indicating that yellowfin bream change sex to female from a size of approximately 21 cm (Chapter 2). As females generally invest more energy into reproduction, the impact of gonadal development on growth rate could be expected to increase with time as more fish change sex.

The above observations on growth indicate that yellowfin bream are not particularly fast growers, but still approach a marketable size in two years from hatch. This is very similar to growth rates attained in early growth trials with the now successfully cultured gilthead seabream, *S. auratus* (Pitt *et al.*, 1977). Today this species attains a size of 110 g in the first year, and 350-400 g in the second (Girin, 1982; Barnabe, 1990; Larrazabal, 1992). Red seabream, *P. auratus*, grow faster, reaching up to 600 g and 1.2 kg in 18 and 30 months respectively, under optimal conditions in Japan (Foscarini, 1988; Fukusho, 1991). However, in colder parts of Japan this growth rate is halved. The same

species, in a wild-caught juvenile growth trial in Australia, grew to a mean size of 24.9 cm and 403 g in 21 months (Bell *et al.*, 1991), while in Taiwan it can reach 1.5 kg in 18 months (Chen, 1990), again apparently a reflection of water temperature differences. Another sparid, the sheepshead *Archosargus probatocephalus*, can reach a large market size of 480 g in only 12 months from hatch at similar temperatures to those in the present study (Tucker, 1987).

Other species from the genus *Acanthopagrus* appear to grow faster than yellowfin bream. *A. cuvieri*, for example, was reported to grow to 32.8 mm and 0.53 g in 63 days in the Arabian Gulf (Hussain *et al.*, 1981), *A. schlegeli* reaches 22 cm (180 g) in 14 months and 29 cm (475 g) in 20 months in Taiwan (Chen, 1990), and *A. latus* reaches a market size of 600 g in 12 months in Taiwan (Chen, 1990). These growth rates are similar to those recorded for *A. berda* in Hong Kong (Mok, 1985), and are 50-100% faster than recorded here for yellowfin bream. In several instances, sparid growth rates under culture conditions have exceeded natural growth rates by up to 100% (Foscarini, 1988; Chen, 1990).

The relatively slow growth rate presented here for yellowfin bream, however, is not optimal, and could be improved in several ways. Food quality is very important as its effects are cumulative over long periods of time, creating the potential to have major influences on time to market size. Fish in the present trial were fed a diet formulated for another species, the barramundi *Lates calcarifer*. The compositional information is given in Table 1. While both species are likely to have similar protein requirements (in the region of 50%) as they are both carnivorous, their abilities to utilise fat and carbohydrate for energy are likely to differ as the capacity to utilise these nutrients varies between species (Steffens, 1989; Tucker, 1992b). Barramundi are largely piscivorous, while bream are naturally macrobenthos predators feeding principally on crustaceans, molluscs and polychaetes. However, if

similar to *A. schlegeli* , the gilthead seabream, and the sheepshead *Archosargus probatocephalus*, yellowfin bream may also be to some extent omnivorous.

Optimal protein levels for other sparids, such as the red and gilthead seabreams, have been in the range of 46-60% (Sabaut and Luquet, 1973; Alliot and Postoureaud, 1983; Foscarini, 1988; Takeuchi *et al.*, 1991), while fat levels of 10-15% have been used successfully on red seabream and the sheepshead (Tucker, 1987; Foscarini, 1988; Takeuchi *et al.*, 1991). Red seabream have been shown to have a poor ability to assimilate carbohydrates, and levels of 10-15% have been found optimal (Foscarini, 1988). Determination of alpha-amylase activity in the gut of yellowfin bream would be indicative of potential carbohydrate utilisation.

Improvements in muscle composition, food conversion and disease resistance have been observed in red seabream when various seaweeds, such as *Ulva pertusa*, have been included in the diet at 0.5-5.0% (Nakagawa *et al.*, 1984; Satoh *et al.*, 1987). Similar specific nutritional requirements need to be researched for yellowfin bream.

Growth rate could also likely be improved through increasing feeding frequency. Feeding frequency, and hence food intake, must be taken into account in bioenergetic models of fish growth potential (Neill *et al.*, 1983). Yellowfin bream have a relatively small mouth size and stomach, characteristics which would suggest them to be naturally adapted to having frequent small meals (Tucker, 1992b). Feeding frequency in the present trial was initially 5 times daily. This was reduced to two feeds/day when bream were placed in nets with barramundi (which having large mouths and stomachs are adapted to large infrequent meals). IFREMER recommend 7 feeds/day for gilthead seabream up to approximately 10 g, then 4 daily feeds up to 100 g (G. Lemarie, IFREMER, pers. comm., 1992). Red seabream

farmers in Japan initially use 10 feeds/day (Foscarini, 1988). Sparids can be trained to activate demand feeders (Tucker, 1992b), which may be the best way to guarantee adequate food and optimal feeding frequency.

Finally, the regular growth measurement checks, in which nets were raised and 10 fish caught, anaesthetised, and measured, may have caused some stress-related growth suppression. This would be particularly applicable after January 1994, when only 35 fish were being grown, as nearly one third of the group was being netted during each sampling. Sharing the netcage with barramundi is also likely to have had some deleterious influence on growth through crowding, competition for food and behavioural interactions.

A variety of techniques for improving fish growth are known, some of which could possibly be applied to yellowfin bream. If water temperatures could be maintained at high levels through the winter months, growth rates would likely be improved substantially. Pre-metamorphosis bream are unsuited to water temperatures over 25°C (Chapter 3), which apparently restricts the species to its present distribution. However, metamorphosed fish could take advantage of warm water effluent from power stations, or could be grown out in lower latitudes where mean water temperatures are higher.

Related fish species may be crossed to obtain hybrids showing characters intermediate between the parents. Inter-generic crosses of *Sparus auratus* with both *Diplodus puntazzo* and *D. vulgaris* in Europe, have been investigated as a means of improving culture qualities of the gilthead bream with some success (Jug-Dujakovic and Glamuzina, 1990). Similarly, the striped bass *Morone saxatilis* has been crossed with *M. chrysops* in the U.S.A. to produce the palmetto bass. The natural distribution of *A. australis* overlaps with those of two other sparids of the same genus, *A. butcheri* in the south,

and *A. berda* in the north (Munro, 1949). Both of these species are known to show faster growth than yellowfin bream (Mok, 1985; Munro, 1949). *A. butcheri*, in particular, is highly regarded as a table fish and could possibly be crossed with *A. australis* for benefit. Even in the wild this species reaches 21.6 cm in the second year (Butcher, 1945). This cross is known to occur naturally and successfully in certain coastal land-locked lakes in southern N.S.W. (Rowland, 1984). Furthermore, the hybrids are known to be fertile (Rowland, 1984).

Certain growth-promoting substances included in the feed have also been shown to effectively improve growth in fish. Weatherley and Gill (1987) suggested that some of these techniques could be applicable to species whose slow growth rate precludes economic aquaculture development. For example the compound dimethyl-B-propiothetin (DMPT) occurs naturally in various aquatic organisms, including fish, and is able to enhance food conversion and growth in several species tested, including red seabream (Nakajima *et al.*, 1990). Similarly, L-carnitine has been shown to improve growth in larval and juvenile European sea bass, *Dicentrarchus labrax*, apparently by increasing fat utilisation for energy (Santulli and D'Amelio, 1986; Santulli *et al.*, 1990). T3 (triiodothyronine) included in the diet of red seabream, increased growth and food conversion significantly by increasing appetite, digestion and absorption (Woo *et al.*, 1991). If these, and similar compounds, could be approved for use on fish designated for human consumption, the economics of yellowfin bream culture may become more attractive.

Finally, the negative effects of gonadal maturation on growth of yellowfin bream could be minimised in several ways. Gonadal recrudescence causes an arrestment of growth in gilthead seabream prior to and during the spawning season but manipulation of photoperiod during a growout trial with this species, to simulate that experienced out of the spawning season,

prevented gonadal recrudescence, allowing fish to reach market size six months earlier (Kadmon *et al.*, 1985). Growout of triploid animals could be expected to yield similar results, and is often used when the species of concern matures sexually before reaching market size.

Food conversion ratio

Results of the food conversion trial are given in Table 2. Relatively good food conversion and protein efficiency ratios were recorded (FCR=1.72:1 and PER=1.13:1), considering the fact that food used was not formulated for this species. This FCR is more efficient than the 2:1 recorded for snapper, *P. auratus*, in early growout trials in Australia (Bell *et al.*, 1991). Takeuchi *et al.* (1991), working with various high quality diets on this species in Japan, recorded FCRs in the range of 0.97-1.8:1, and PERs of 1.2-2.1:1, clearly demonstrating the sensitivity of these figures to small changes in diet composition. Jafri *et al.* (1981) reported a FCR, gross growth efficiency and PER of 2.6:1, 0.38, and 0.77:1 for the closely related species, *A. latus*, in Kuwait. Also from the genus *Acanthopagrus*, *A. schlegeli* was recorded to have a good food conversion of 1.4:1 (Chuang *et al.*, 1985). Marais and Kissil (1979), experimenting with energy and protein content in the diet of the gilthead seabream recorded FCRs between 2.1 and 2.5:1, and PERs of 1.01-1.10:1. Kissil *et al.* (1983) recorded food conversion and protein efficiency ratios in the range of 2.1-2.7:1 and 0.93-1.2:1, respectively, when studying this species.

Results presented here for yellowfin bream compare very favourably with these trials on other species, although food conversion can depend heavily on fish size and environmental factors such as temperature, salinity, and other water conditions, as well as diet composition (Brett, 1979), making comparisons difficult. Food utilisation information is important when assessing the economic feasibility of a species for aquaculture. The high PER

Crude protein %	51.5
Crude fat %	10.5
Crude fibre %	7.0
Moisture %	4.3

TABLE 1 - Proximate chemical composition of the test diet given to juvenile yellowfin bream during a 12-day feeding trial (as % dried pellets). Percent carbohydrate and ash data unavailable.

Initial number of fish	30
Percent survival	100
Initial mean total length (mm)	97.0 ± 11.7
Initial mean wet weight (g)	22.3 ± 8.8
Final mean total length (mm)	101.2 ± 11.4
Final mean wet weight (g)	25.9 ± 9.6
Mean increase in weight (%)	16.1%
Mean weight food consumed/fish/day (g)	0.54
Gross food conversion ratio (F.C.R.)	1.72:1
Gross growth efficiency	0.58
Protein efficiency ratio (P.E.R.)	1.13:1

TABLE 2 - Results of a 12-day feeding trial on yellowfin bream using 'Aqua-Feed' barramundi starter as food. Means are given ± s.d.

value recorded in the present study indicates an ability of *A. australis* to utilise proteins efficiently for somatic growth.

Survival, behaviour and susceptibility to disease

Survival between the time juveniles were introduced to netcage conditions and 7 months of age when most fish escaped, was estimated at 85%. This was based largely on the 'inventory' sampling which took place 6 months into the growout trial. The mortality peak observed amongst red seabream when introduced to netcages (Foscarini, 1988) was not observed. Survival past 7 months to 25 months was 81%. These high survival rates are indicative of the hardiness of this species, its tolerance of, and adaptation to, netcage conditions, and its resistance to disease. Survival rates during netcage culture of approximately 60% are common for red seabream (Foscarini, 1988), while over 80% is common for gilthead seabream (G. Lemarie, IFREMER, pers. comm., 1992).

Some aggression was observed in the two months following metamorphosis. During this time juveniles have an array of sharp teeth, sometimes used for 'tail-nipping'. This appeared to be more aggressive than cannibalistic behaviour as, although it sometimes resulted in the death of a fish, the killed individual was rarely eaten to any extent. This behaviour, termed 'agonistic behaviour induced mortality' by Hecht and Pienaar (1993), largely accounted for the 8.5% mortality observed between metamorphosis and introduction to netcages. Similar behaviour for a short period following metamorphosis has been reported for many other cultured sparids, such as red seabream (Foscarini, 1988), gilthead seabream (Tandler *et al.*, 1989a), and the silvery black porgy, *A. cuvieri* (Hussain *et al.*, 1981). Effective measures taken to minimise this have included reducing size variation amongst fish by grading, or adjusting densities and increasing food availability (Tandler *et al.*,

1989a; Hecht and Pienaar, 1993). The problem is particularly severe amongst gilthead seabream, due mainly to the exceptionally asynchronous growth of postlarvae. It has been suggested for this species that the ratio of smallest to largest fish should not exceed 1:4 to minimise aggression (G. Lemarie, IFREMER, pers. comm., 1992). Cannibalistic tendencies could be expected to be weaker for non-piscivorous species like yellowfin bream, than for example barramundi *L. calcarifer*, a true piscivore (Hecht and Pienaar, 1993).

Yellowfin bream appeared to be remarkably resistant to disease. *Flexibacter columnaris* and *Streptococcus* spp., may cause a significant mortality amongst cultured barramundi. Bream were apparently unaffected by these diseases, even when sharing a netcage with barramundi amongst which these diseases were prevalent. Red seabream in Japan have also gained a reputation for high disease resistance (Fukusho, 1991).

Most sparid culture world-wide is conducted in netcages (Girin, 1982; Foscarini, 1988; Chen, 1990), although seawater ponds have been used successfully for several species in the Mediterranean and Middle East in particular (Pitt *et al.*, 1977; Eisawy and Wassef, 1984; Kadmon *et al.*, 1985; Larrazabal, 1992). As well as having considerable potential for culture in netcages, yellowfin bream would be likely to adapt well to pond conditions also, and being largely benthic feeders could take full advantage of pond productivity.

SECTION B

INDUCED SPAWNING AND CULTURE OF MANGROVE JACK, *Lutjanus argentimaculatus*

CHAPTER 6

Induced Spawning

Introduction

Controlled spawning is fundamental to the development of new aquaculture species. Hormonal or environmental manipulations are now widely used to accurately time broodstock spawnings, allowing smooth hatchery running. Unfortunately, responses of fish to hormones are species-specific (Peter, 1986) and optimum application protocols need to be determined for each individual species. Of the lutjanids, six species, all of the genus *Lutjanus*, have been reported to spawn in captivity. Three of these responded to hormone treatment - *L. campechanus* (Minton *et al.*, 1983), *L. johni* (Lim *et al.*, 1985), and *L. argentimaculatus* (Doi and Singhagraiwan, 1993; Emata, 1994; Emata *et al.*, 1994). Spawnings of the other species occurred spontaneously when broodfish were held under conditions simulating the natural environment.

Research into the induced spawning of mangrove jack has been underway in Thailand since the mid 1980's to reduce the dependance of farmers on wild-caught juveniles (Doi and Singhagraiwan, 1993). Now, projects are also underway in the Philippines, Singapore, Malaysia, Taiwan and Australia (Anon., 1994; Emata *et al.*, 1994).

Despite the importance of mangrove jack as a food fish throughout its range, the natural reproductive biology and the early life stages have been poorly studied. It is thought that as fish approach sexual maturity, they migrate from inshore estuary habitats to offshore reef areas where they subsequently live and spawn (Marshall, 1982; Allen, 1985; R. Garrett, pers. comm., 1993; M. Sheaves, pers. comm., 1994). However, knowledge on exact

spawning sites has remained elusive. In mid to late summer, juveniles (~20 mm) appear on the coast where they often are seen in freshwater streams (personal observation).

Work on the induced spawning of mangrove jack commenced in Australia in 1992, both by staff at the Northern Fisheries Research Centre, Cairns, and as part of the present study. The aims of this component of the project were to apply recent hormonal spawning-induction technology (hormones and methods of administration) to develop reliable techniques for the induced spawning of local mangrove jack, thus enabling studies of egg incubation, yolk absorption and larval rearing to proceed.

Materials and Methods

Broodstock collection and examination

Broodstock originated as juveniles line-caught from the Hinchinbrook channel. They were subsequently maintained in netcages (5 m^3) at the 'Sea Harvest' (now 'Bluewater Barramundi') farm, within the Hinchinbrook channel (18°25' S, 146°10' E), until mature. Up to 25 fish were kept per netcage. All fish were weighed and tagged, and at the commencement of this project ranged in size from 2.5-5 kg. The diet consisted of local fresh baitfish and frozen pilchards, and occasionally barramundi pellet food. Most of the spawning trials described in the present study were performed on fish held in these netcages, although four induction trials used broodfish that had been moved to the nearby hatchery site at Mourilyan. These latter fish were maintained with barramundi broodfish in 30 m^3 tanks on a recirculation system, and were fed frozen pilchards injected with a vitamin supplement.

During the spawning season, from late October, broodfish were caught and anaesthetised with MS-222 at 70 mg/l. Males were examined for free milt, and females were cannulated with a fine plastic tube (1 mm I.D.) to draw oocytes from the ovaries. Oocytes were subsequently examined microscopically and measured by eyepiece micrometer to determine the mean diameter of the largest size-class. Hatchery fish were examined fortnightly in a similar way until found mature. Females considered sufficiently mature were then given one of three hormone treatments, between 8 a.m. and 10:30 a.m. Males were treated when milt production was considered sub-maximal, as detailed below.

Hormonal induction

(i) pelletised LHRHa

First spawning induction attempts used luteinising hormone-releasing hormone analogue, LHRHa (des-gly^{10}, D-ala^{6}, pro^{9}-ethylamide) (Peptech, Sydney, Australia) administered in the form of pellet implants. Pellets were made using a method modified from that of Sherwood *et al.* (1988). Two pellet types were trialed, each in triplicate. The first consisted of a 95% cholesterol/5% cocoa butter formulation, administering 25-29 µg LHRHa/kg, and the second an 80%/20% formulation, administering 30-49 µg LHRHa/kg. The former pellet type was prepared by dissolving 4 mg of LHRHa in 0.4 ml 80% ethanol, and mixing this thoroughly with 380 mg of cholesterol. When dry this was mixed with 20 mg of cocoa butter and pressed into a pelletiser to produce pellets approximately 5 mm x 2 mm. The second pellet type was prepared by mixing 640 mg of cholesterol with 160 mg of cellulose. One milligram of LHRHa was then dissolved in 0.4 ml of 80% ethanol, which was added to the matrix powder and thoroughly mixed and allowed to dry. This was then similarly pelletised. These latter pellets were given in conjunction

with an injection of the dopamine antagonist pimozide (2 mg/kg), which had been dissolved in 0.9% saline. Pellets were administered intramuscularly with a 12 gauge needle into the epaxial region below the dorsal fin. All males in this trial series received an aqueous LHRHa injection at 12-30 μg/kg.

(ii) aqueous LHRHa

In light of problems experienced with using pellets and obtaining spontaneous spawnings, trials using aqueous LHRHa solutions, followed by hand-stripping, were begun. Trials at the Northern Fisheries Research Centre using single injections of LHRHa at up to 100 μg/kg had met with limited success (R. Garrett, pers. comm., 1993), so a series of trials began using priming and resolving doses (Marte, 1989) given 24 h apart. A 70 μg/ml aqueous solution of LHRHa was prepared by dissolving 1 mg of LHRHa in 14 ml of 0.9% saline. Fish were injected intramuscularly, in the epaxial region below the dorsal fin. Males, when treated, received dosages of 20-50 μg LHRHa/kg, and a stronger solution of hormone (100 μg/ml) was used. Females were injected twice at 24 h interval with total dosages ranging from 36-60 μg LHRHa/kg. A 2 ml syringe and 1.5 inch, 22 gauge needle were used. Trials were carried out at least in triplicate using the following regimes to determine minimum dosage requirements for successful spawning. Note that in one trial series pimozide was given concurrently at a dose of 2 mg/kg. The volume of solution injected was maintained at 0.5 ml/kg or less to minimise back-flow losses.

(i) 15 + 25 μg LHRHa/kg, 24 h interval.

(ii) 30-35 + 25 μg/kg, 24 h interval.

(iii) 25 (plus 2 mg/kg pimozide) + 25 μg/kg, 24 h interval.

(iv) 25 + 25 μg/kg, 24 h interval.

(iii) Ovaprim

The commercial hormonal preparation Ovaprim was tested in triplicate for its effectiveness on mangrove jack. This was administered as an aqueous injection at a dose of 1.0 ml/kg female body weight, given as two 0.5 ml/kg injections 24 h apart. The concentrations of active agents in 'Ovaprim' (gonadotropin-releasing hormone and domperidone) could not be ascertained due to proprietary confidentiality.

Spawning conditions

Mean maximum oocyte diameters for all females prior to injection are indicated in Tables 1-3. After injection spawners were placed in vacant 5 m^3 netcages, usually one or two females with two males. At the hatchery spawners were placed in covered 5000 l circular spawning tanks, one pair per tank. Water conditions in netcages ranged in various trials from 27.0-31.5°C and 20.5-38.0 ppt salinity; and in tanks from 27.6-30.0°C and 30.4-34.0 ppt salinity. The pre-spawning behaviour of hatchery fish was observed on several occasions between 24 and 36 h after injection. Disturbance was kept to a minimum, except where a second injection was administered, until approximately 36 h had passed from the first injection. At this stage hatchery fish were left to spawn spontaneously. All fish maintained in netcages, however, were then anaesthetised and hand-stripped of eggs and milt. Artificial fertilisation was achieved using the 'dry' method.

Spawning assessment

At the hatchery, fine mesh nets were placed under the outlet flow from spawning tanks after 36 h had passed since the priming injection, and checked every 15 min for eggs. When a spawning was confirmed, time was noted and eggs were collected from the water by net, and transferred to a

known volume of seawater where numbers were estimated volumetrically. Numbers of eggs from strip-spawnings were similarly estimated. When larval rearing was to be attempted, eggs were immersed in a 100 mg/l iodine bath for 10 min, at 4 h post-fertilisation, to reduce bacterial and/or fungal contamination.

Fertilisation rates were determined by examining 50 eggs at 1 h post-fertilisation. Mean egg and oil globule diameters of thirty eggs were recorded for each spawning by use of microscope and eyepiece micrometer. From one spawning, a sample of eggs rinsed with 3.2% ammonium formate to remove salts was separated into three groups of 10 eggs, placed briefly on absorbent paper to remove excess water, and then each group placed on a pre-dried, pre-weighed filter paper and weighed on a CAHN 21 automatic microbalance. Each paper and lot of eggs was then placed in a drying oven at 60°C for 24h before being weighed again for dry weight. Each weight was divided by 10 to obtain mean individual egg weight.

Latent period, oocyte maturation post-injection and the 'window of fertilisation'

A mature 3 kg female was given two injections of 25 μg aqueous LHRHa/kg, 24 h apart, and then examined regularly by intra-ovarian cannulation from the priming injection until oocytes reached a fully hydrated condition. The response of oocytes to the hormone application, as reflected in increasing diameter over time, was then plotted.

Preliminary results pointed to a very short 'window of fertilisation', or period of time in which fully hydrated eggs are able to be fertilised, occurring shortly after 36 h from the priming injection. The bounds of this window were determined accurately by similarly injecting two further females (3 and 3.3 kg), and stripping and artificially fertilising eggs from each every 5 min from

36 h 10 min to 36 h 45 min post-injection. Approximately 10,000 eggs were stripped per determination, to which one drop of milt was added, and fertilisation achieved using the 'dry' method. Triplicate samples of 30 eggs were observed microscopically from each determination after 1h to ascertain fertilisation rates.

Egg fertility during water hardening

To assess how long stripped eggs may be left in seawater and remain fertile, the eggs from one 3.4 kg female were stripped into seawater, and duplicate samples of approximately 10,000 eggs taken at 6 min intervals from 0-24 min, and fertilisation achieved with two drops of milt ('wet' fertilisation). The mean percent fertilisation was then plotted against time.

Male hormone induction trials

Three males were selected which were unable to be hand-stripped, when anaesthetised, due to low milt volume, but which showed active sperm in their cannula samples. Each received a single aqueous LHRHa injection of 50 µg/kg, and was placed in a separate netcage. These fish were subsequently anaesthetised and examined for free milt at 12, 24, and 36 h after injection. Milt volume was estimated at each examination by drawing the milt into a 1 ml syringe.

Results and Discussion

Spawning season and reproductive biology

The spawning season, as assessed from gonad maturity of broodfish held captive in netcages in the Hinchinbrook Channel, extended for over 6 months, from mid October to early April. During this time individual

females matured at different times, so that at any one time only a percentage of females were mature. Individual males, however, were often mature throughout the entire season. Peak season appeared to span from December to February. The relationships between the observed spawning season and average (6 yr) water temperature, salinity and photoperiod conditions are illustrated in Fig. 1, and show the spawning season to correlate with water temperatures over approximately 27°C, and photoperiods greater than 12 h. The spawning season also correlates reasonably well with decreasing salinities associated with monsoonal rains. It is unclear how these parameters control reproductive activity, although it seems likely that photoperiod and temperature would have a controlling influence over the timing of the spawning season, while monsoonal rainfall may act as a final cue. Reshetnikov and Claro (1976) suggested that spawning of *L. synagris* may be timed with periods of high rainfall as runoff introduces nutrients into coastal waters and supports greater food production for early life stages.

In the hatchery, where temperature and photoperiod conditions were adjusted to advance the spawning season, little maturation of oocytes occurred until the water temperature and photoperiod reached 30°C and 14 h daylight respectively (Fig. 2). Although this maturation pattern is also consistent with many fish experiencing natural conditions, it would be dangerous to draw conclusions from these broodfish as they were in their first year at the hatchery and may still have carried endogenous circannual rhythms with them, as has been reported for other fish confined to a hatchery (e.g. Whitehead *et al.*, 1978).

Mangrove jack show no sexual dimorphism, and are dioecious. These traits are consistent with other members of the family (Allen, 1985; Grimes, 1987). Allen (1985) recognises two reproductive patterns amongst the lutjanids - either a protracted summer season, or continuous reproduction

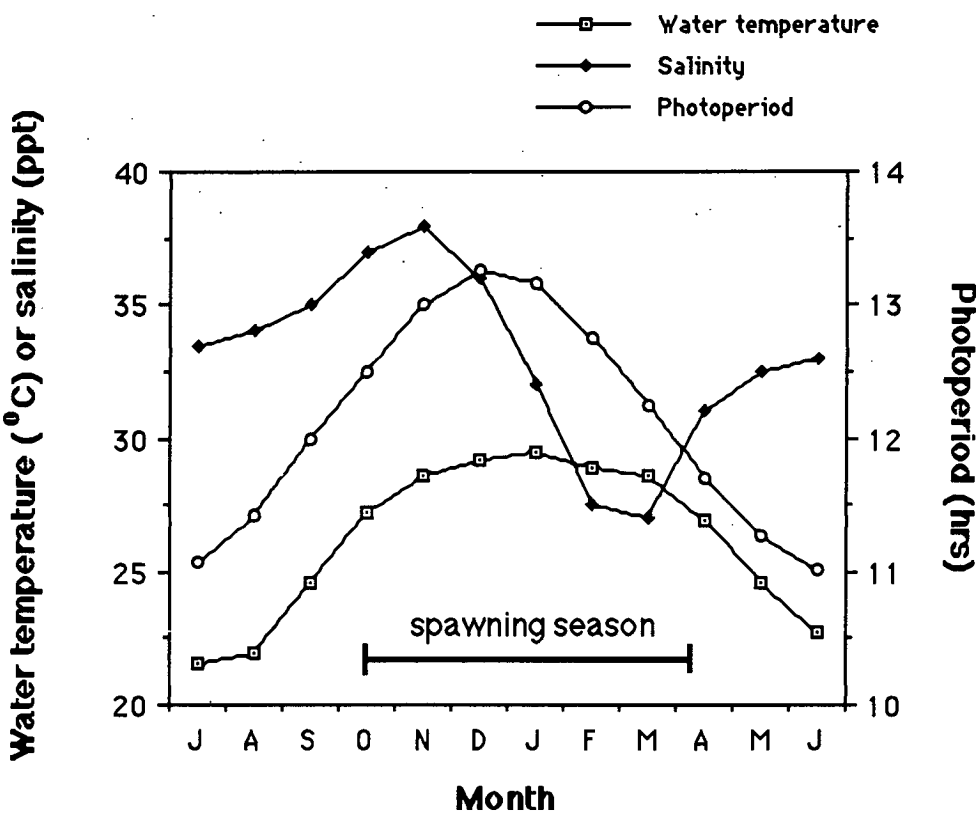


FIG 1 - Mean monthly water temperatures, salinities and photoperiods from 1990 to 1995 in the Hinchinbrook channel, where broodfish were maintained in netcages. The spawning season for mangrove jack, as observed in the present study, is seen to correspond with maximum water temperatures and photoperiods, and decreasing salinities associated with monsoonal rains. Temperature and salinity data supplied by 'Bluewater Barramundi'.

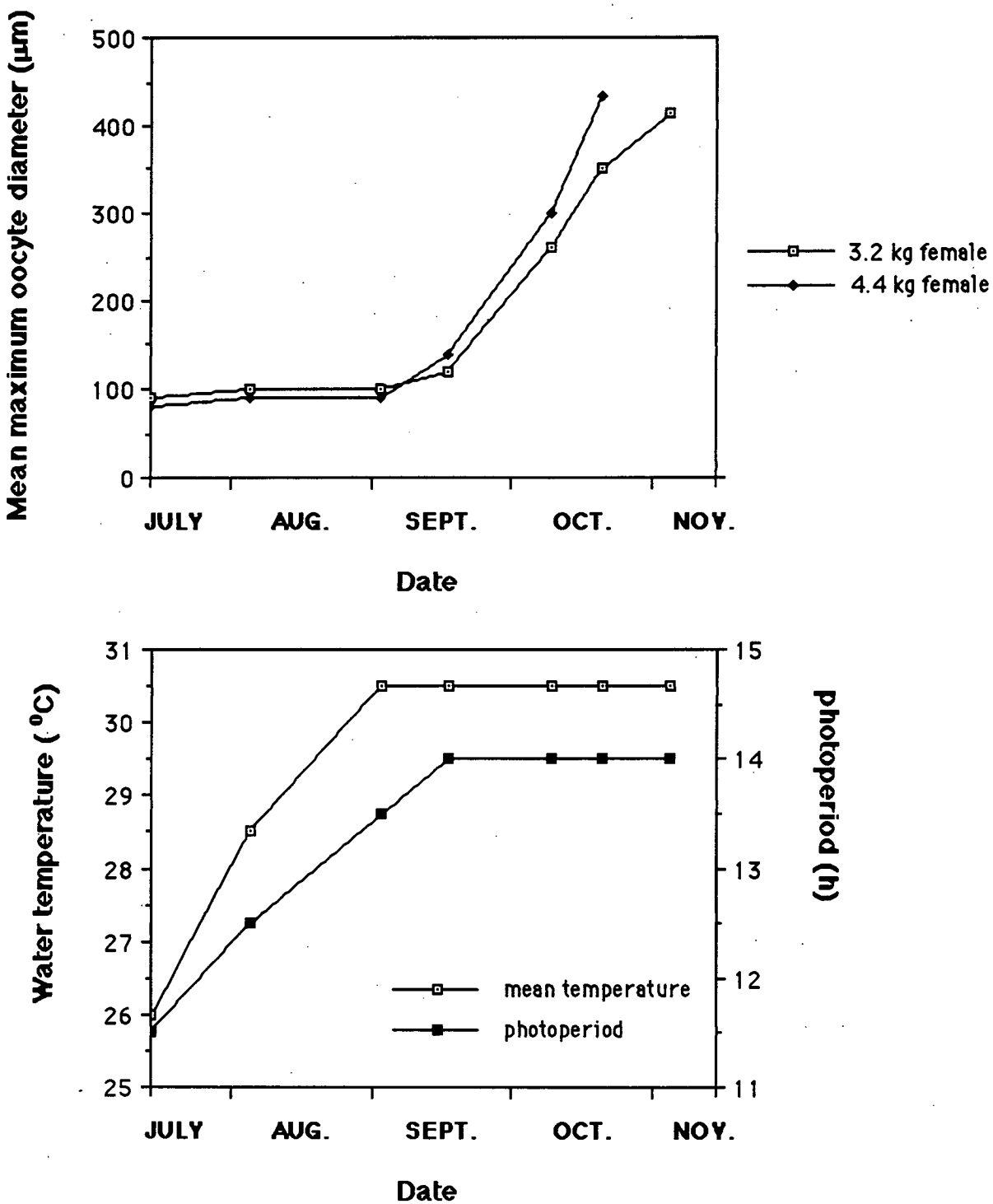


FIG 2 - Maturation of two female broodfish (above) held captive in a hatchery tank, and corresponding temperature and photoperiod conditions (below). Salinity was maintained at 30-34 ppt.

with peaks in spring and autumn. Mangrove jack clearly belong to the former. Mature males were not seen below a size of 1.6 kg (44 cm S.L.), and females below 2.4 kg (51 cm S.L.).

Pre-injection cannular biopsy

According to the classification of Wallace and Selman (1981) the mangrove jack ovary, like that of the yellowfin bream, is 'asynchronous'. Fig. 3 (top) is a frequency histogram of oocyte diameters appearing in a cannula sample from a typical mature female broodfish prior to LHRHa injection. Two major peaks may be seen, the first at approximately 80 μm and the second at approximately 425 μm. The former represents the primary oocyte stock from which maturing eggs are recruited; the latter are yolk globule oocytes, at the end of the vitellogenic stage of oocyte development. Apparently, oocytes mature to this latter stage, after which they remain dormant and do not advance further until appropriate hormonal stimulation, whereupon they advance rapidly towards the final maturation (germinal vesicle break down, GVBD, and yolk granule coalescence), hydration and ovulation stages (West, 1989). This progression is seen in the lower two frequency histograms (Fig. 3), from a female sampled at 24 h and 36 h after the priming injection.

Fig. 3 also shows mangrove jack to be serial spawners. Only a portion of oocytes within the 425 μm peak respond to hormone treatment, maturing and hydrating to form a third peak at 860 μm. The remainder of the 425 μm peak remain available for the next spawning. The capability of spawning several times within the spawning season is illustrated in Table 4, where a single 3.4 kg broodfish underwent six spawning inductions within a season. Five of these were successful. Most broodfish were in fact induced to spawn several times within a season, and many could be induced to spawn at least

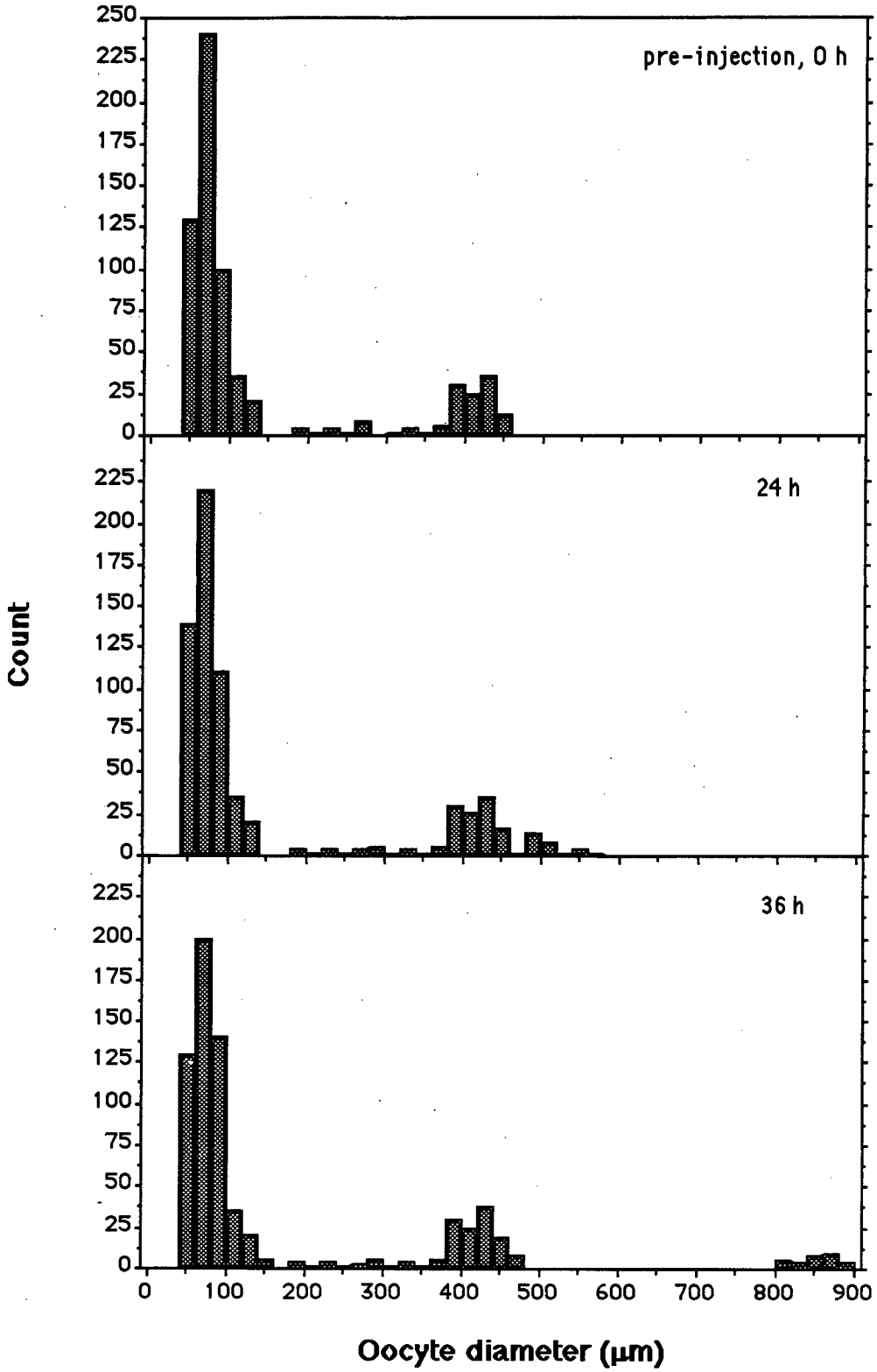


FIG 3 - Frequency histograms of oocyte diameters appearing in cannula samples taken from a typical broodfish prior to the priming injection, at 24 h (resolving injection) and at 36 h. A portion of the 425 µm peak responds to LHRHa treatment, producing fully hydrated, fertile eggs of approximately 860 µm.

fortnightly.

The threshold state of oocyte maturation required for successful hormonal induction was found to be near 400 µm. Two females with maximum oocyte diameters of 372 and 389 µm failed to spawn after appropriate hormonal stimulation; while females with maximum oocyte diameters of 403 and 408 µm did produce fully hydrated eggs, although in only small numbers, after similar treatment. Even within a single mature ovary, as shown in Fig. 3, only a fraction of oocytes, apparently over a threshold state of development, will respond to appropriate hormonal stimulation.

Hormonal induction

(ii) pelletised LHRHa

Low LHRHa dosages (< 30 µg/kg) in very slow release pellets (95% cholesterol, 5% cocoa butter; approx. 20% release by 24 h, Sherwood *et al.*, 1988) induced a spawning in only one of three females (Table 1). Although full oocyte hydration was achieved in the other two females, neither released eggs. Eggs from the first female microscopically appeared fertile (after Kjorsvik *et al.*, 1990), and the absence of fertilised eggs is thought to be due to a failure of the male to spawn.

Despite using slightly higher LHRHa dosages (30-32 µg/kg) in quicker-release pellets (80% cholesterol, 20% cellulose; approx. 94% release in 12 h, Carolsfeld *et al.*, 1988b), and with pimozide injected simultaneously, results were not improved (Table 1). Only the first female undergoing this treatment was allowed to spawn spontaneously, the others were hand-stripped. The first female did spawn, but again although appearing fertile, eggs were unfertilised, apparently because the male had again failed to spawn. Eggs were hand-stripped from the second female at hourly intervals between 36

LHRHa dose (µg/kg)	female size (kg)	pellet composition (%)	pre-injection oocyte diam. (µm)	hydration	strip-spawn or natural	spawning egg #/% fert'n	comments
24.6	2.6	95 chol./5 cocoa	425.0	full	natural	90,870/0%	male did not spawn
26.0	3.3	95 chol./5 cocoa	415.5	full	natural	-	no spawning
28.9	2.8	95 chol./5 cocoa	435.2	full	natural	-	no spawning
30.0 (P)	3.0	80 chol./20 cell.	473.1	full	natural	193,000/0%	male did not spawn
30.8 (P)	3.0	80 chol./20 cell.	436.4	full	stripped	550,000/0%	over-ripe
32.0 (P)	3.3	80 chol./20 cell.	428.8	partial	stripped	-	unstrippable

TABLE 1 - Results of spawning trials employing cholesterol/cocoa butter or cholesterol/cellulose matrix pellets at various LHRHa dosages. While full oocyte hydration was usually achieved, difficulties in synchronising male and female spawnings, and in estimating correct strip-spawning times, limited success. (P represents the additional administration of pimozide at the rate of 2 mg/kg).

and 44 h after injection, but the transient nature of the window of fertilisation for mangrove jack was not fully appreciated at this stage and no fertile eggs were obtained. The third female did not produce fully hydrated eggs, even by 46 h post-injection, so was unable to be stripped.

In summary, problems were encountered when using pellets, in both achieving female ovulation and spawning, and in synchronising male and female gamete release. This was despite often observing encouraging reproductive behaviour (see below) hours before an anticipated spawning. The former problem could possibly be solved through further experimentation with pellet composition and hormone dosage. In particular, higher levels of circulating gonadotropin (GtH) need to be achieved at the end of oocyte maturation, as this has been shown to be important for successful ovulation and spawning (Goetz, 1983). The problem of achieving synchronous male spawnings could be due to insufficient male hormonal stimulation (males received only 12-30 μg/kg LHRHa), or environmental factors such as tank size, etc.

Work in Thailand and the Philippines has demonstrated the potential to achieve successful spontaneous mangrove jack spawnings following hormone treatment, and occasionally without any intervention at all (Doi and Singhagraiwan, 1993; Emata *et al.*, 1994). The approaches employed in Thailand differed from the present study in three main ways - use of the hormonal preparation Human Chorionic Gonadatropin (HCG) instead of LHRHa, often injecting a group of mature fish instead of a pair (e.g. 15 females and 5 males together), and placing injected fish in larger tanks (18-190 m^3 instead of 5 m^3 tanks used in the present study) (Doi and Singhagraiwan, 1993; G. Schipp, pers. comm., 1994). Tank size has been found to have a significant effect on the ability of *L. johni* to spawn during trials conducted in Singapore (Lim *et al.*, 1985). In the Philippines, spawning trials have begun

using LHRHa as well as HCG, and successful spontaneous spawnings have been obtained when high dosages of 100 µg LHRHa/kg were given to male fish as well as females (Emata, 1994). Evidence suggests, then, that the use of larger tanks, greater numbers of spawners, and higher male hormone dosages may be necessary for successful natural spawnings. The use of several broodfish together seems reasonable as many lutjanids are naturally group spawners (Allen, 1985; Carter and Perrine, 1994).

The induced spawning of other lutjanid species has either involved the use of HCG at dosages varying from 250-1,100 IU/kg (Minton *et al.*, 1983; Lim *et al.*, 1985), or has occurred completely spontaneously, sometimes through photoperiod or temperature manipulation (Arnold *et al.*, 1978; Suzuki and Hioki, 1979; Rabalais *et al.*, 1980; Hamamoto *et al.*, 1992).

(ii) aqueous LHRHa

It was found that when two injections of at least 25 µg LHRHa/kg were given 24 h apart, final egg maturation, hydration, and ovulation could be reliably induced (Table 2). Lower dosages often resulted in only partial egg hydration. It was also found that the timing of processes involved in final egg maturation and hydration were very similar between individual females, allowing quite accurate estimates of ovulation time to be made, which facilitated hand-stripping.

The use of priming and resolving doses allows elevated blood GtH levels to be sustained over longer periods of time than single injections alone. Furthermore, it has been shown on several species of fish that a self-potentiation of the effects of LHRHa can occur when more than one injection is given (Peter, 1980; Carolsfeld *et al.* 1988a). For example, two injections of 25 µg/kg are often significantly more effective than one of 50 µg/kg. The

LHRHa dose (μg/kg)	female size (kg)	pre-injection oocyte diam. (μm)	oocyte hydration	hand strippable	egg number	% fert'n	comments
15 + 24	3.8	427.8	partial	no	-	-	insufficient hormone
15 + 25	3.2	456.1	partial	no	-	-	insufficient hormone
15 + 24	3.5	452.1	full	yes	211,000	4.4	over-ripe
34.4 + 25	3.2	449.5	full	yes	832,000	86.5	good
35 + 25	3.5	421.8	full	yes	1,566,000	16.1	low fertilisation
32.4 + 25	3.4	463.1	full	yes	561,000	57.8	good
30 + 25	4.6	448.1	full	yes	429,000	33.3	fair
35 + 25	3.8	448.4	full	yes	95,400	22.2	low fertilisation
30 + 25	3.3	456.3	full	yes	937,000	32.7	fair
25 (P) + 25	3.0	436.0	full	yes	1,238,000	3.8	insufficient milt
25 (P) + 25	2.7	462.7	full	yes	78,500	14.7	over-ripe
25 (P) + 25	3.0	403.4	full	yes	8,400	55.0	few eggs

TABLE 2 (continued over)

LHRHa dose (µg/kg)	female size (kg)	pre-injection oocyte diam. (µm)	oocyte hydration	hand strippable	egg number	% fert'n	comments
25 + 25	2.8	433.3	full	yes	553,000	72.0	good
25 + 25	3.0	456.9	full	yes	1,190,000	90.0	good
25 + 25	3.8	438.0	full	yes	154,000	31.8	fair
25 + 25	2.6	423.6	full	yes	150,000	60.8	good
25 + 25	3.4	471.0	full	yes	2,260,000	85.8	good
25 + 25	3.0	460.0	full	yes	23,380	17.4	low fertilisation
25 + 25	3.0	441.3	full	yes	289,000	92.8	good
25 + 25	4.6	420.1	full	yes	136,000	55.9	good
25 + 25	3.3	423.2	full	yes	93,300	70.3	good
25 + 25	3.0	427.2	full	yes	91,900	50.6	good
25 + 25	3.4	433.0	full	yes	394,000	70.8	good

TABLE 2 - Results of spawning-induction trials employing double aqueous LHRHa injections given at 24 h interval. A minimum of two 25 µg LHRH/kg injections is required for reliable spawning inductions.

additional handling and stress to the fish, however, detracts somewhat from the benefits.

The use of pimozide was found to be unnecessary, as suggested in the pellet trials above. Pimozide is a dopamine antagonist administered to block the negative effect of dopamine on GtH release from the pituitary (Donaldson and Hunter, 1983). The fact that administration of pimozide has no noticeable effect on final maturation processes indicates that dopamine does not play a significant role in the regulation of GtH release from the mangrove jack pituitary. In other species such as many freshwater cyprinids, the catfish *Clarius batrachus*, the loach *Paramisgurnus dabryanus*, and perch *Anabas testudineus*, this blocking system is strong, and injection of a dopamine antagonist is beneficial or essential (Lin *et al.*, 1985; Peter *et al.*, 1988; Manickam and Joy, 1989; Halder *et al.*, 1991).

Fertilisation rates from all hand-strip spawnings varied from 3.8-92.8%, with a mean of 51.0% (Table 2). These rates are marginally better than those reported by Doi and Singhagraiwan (1993) for mangrove jack induced to spawn spontaneously after hormone treatment (mean 46.0%; range 0 - 86.4%). Low fertilisation rates in the present study were almost always the result of incorrect estimation of stripping time, which was found to be critical (see below). Fertilisation rates therefore improved following this finding, and with experience.

(iii) Ovaprim

The 'Ovaprim' treatment, given as two 0.5 ml/kg injections at 24 h interval, failed to promote final egg maturation. None of the three females treated produced fully hydrated, fertile eggs (Table 3). This was despite a dosage double that of the manufacturer's recommendation, which was chosen

Ovaprim dose (ml/kg)	female size (kg)	pre-injection oocyte diam. (μm)	oocyte hydration	hand strippable	comments
0.5 + 0.5	4.7	431.3	partial	no	insufficient hormonal stimulation
0.5 + 0.5	3.4	433.4	partial	no	insufficient hormonal stimulation
0.6 +0.6	3.2	440.1	partial	no	insufficient hormonal stimulation

TABLE 3 - Results of spawning trials employing the hormonal preparation 'Ovaprim' at double (or more) the suggested dosage, as two injections 24 h apart. The inability to induce full oocyte hydration at these dosages suggests that Ovaprim has limited efficacy when used on mangrove jack.

on the basis of earlier results with Ovaprim on yellowfin bream, and on the relatively high dosages of LHRHa required by mangrove jack.

Ovaprim consists of the fish gonadotropin-releasing hormone (D-arg^6, pro^9-NEt)sGnRH together with the dopamine antagonist domperidone in a propylene glycol solvent. This hormone molecule is different from the LHRHa molecule used successfully above (des-gly^{10}, D-ala^6, pro^9-ethylamide). Peter (1986) and Carolsfeld *et al.* (1988a) stress the specificity of these peptide hormones and their varied efficacy on different fish species. The molecule used in Ovaprim is apparently less potent on mangrove jack than the particular LHRHa molecule used previously, or it occurs at a lower concentration in Ovaprim.

Furthermore, the presence of the dopamine antagonist domperidone will have little positive effect on final oocyte maturation processes in mangrove jack, as the GtH release-inhibitory system is apparently weak, as stated previously.

Pre-spawning behaviour

Pre-spawning behaviour comprised of four main activities. The first involved the pair simply staying in close proximity to one another. Usually the male would follow the female, often swimming close underneath, but occasionally the positions were reversed. The second activity involved 'yawning', in which the fish opened its mouth wide while simultaneously flaring the gills. This behaviour was exhibited by both sexes, although more often by the male. Another activity often observed, again more often in the male, was an erection of the dorsal fin as a display to the partner. This was commonly seen in combination with the fourth activity in which the male inclined its body at an angle of 45-60°, head down, towards the female.

Spawning assessment

(i) egg characteristics

Mangrove jack eggs are spherical, transparent, pelagic, non-adhesive and positively buoyant. The mean diameter of fertilised, water-hardened eggs from all spawnings was 823.9 μm (range 764.4-880.4 μm). That of the oil globule was 158.0 μm (range 152.1-165.6 μm). These represent mean egg and oil globule volumes of 0.293 mm^3 and 0.00207 mm^3 respectively. Mean egg wet and dry weights were 303.0 ± 15.4 μg and 45.0 ± 6.1 μg respectively, indicating a water content of 85.1%.

These eggs are similar to those from other lutjanids described in the literature, such as *L. kasmira* (Suzuki and Hioki, 1979), *L. campechanus* (Rabalais *et al.*, 1980), *L. johni* (Lim *et al.*, 1985) and *L. stellatus* (Hamamoto *et al.*, 1992). Similarly, the oil globule size recorded in the present study is typical of lutjanid eggs (Leis, 1987). Interestingly, the mean sizes of mangrove jack eggs reported from Thailand (795 μm; Doi and Singhagraiwan, 1993) and the Philippines (740 μm; Emata *et al.*, 1994) are slightly smaller. Larger egg size does not, however, necessarily confer a survival advantage to larvae (Kjorsvik *et al.*, 1990).

(ii) fecundity

Mangrove jack proved to be highly fecund. Individual spawnings varied to a large extent in size, from 8,400 eggs to 2.26 million eggs (mean 518,800 ± 110,660 (± s.e.)) in fish of 2.6-4.7 kg (Table 2). This is consistent with results reported by Doi and Singhagraiwan (1993) and Emata *et al.* (1994), who both found mangrove jack females capable of spawning over 1 million eggs in one night. High fecundity is also a characteristic common to most lutjanids (Grimes, 1987).

Figs. 4 and 5 indicate that individual spawning size was not related to female size, nor to the mean maximum oocyte diameter observed in the ovary. Rather, it appeared to depend on the number of eggs in the ovary over a critical minimum state of development, corresponding with a diameter of approximately 400-450 μm. It was these eggs which responded to hormone treatment. The reason that little correlation exists between spawning size and mean maximum oocyte size (Fig. 5) is that mean maximum oocyte size gives little indication of the spread of oocyte sizes present. For this reason mean maximum oocyte size is only useful as an indicator of whether a particular female is likely to spawn at all, and not as an indicator of spawning size. To predict spawning size, the size distribution of yolk globule oocytes would be a better indicator. Tamaru *et al.* (1988) have stressed the importance of a unimodal size distribution in successfully inducing milkfish, *Chanos chanos*, to spawn. Female size has no indicative value of spawning size as it has no bearing on the state of maturity of the ovary.

As mangrove jack are partial or serial spawners, individual spawning size has little relation to fecundity which refers to the total number of eggs available for spawning in a season. An indication of true fecundity was provided by female #14116, which underwent six spawning inductions in the 1993/4 season, five of which were successful (Table 4). From this female a total of 5.8 million eggs were spawned, representing a minimum fecundity of 1.7 million eggs/kg. This fecundity falls in the upper range of that reported for lutjanids by Grimes (1987) of 0.15 - 1.9 x 10^6 eggs/kg.

Latent period, oocyte maturation post-injection and the 'window of fertilisation'

The enlargement of oocytes after hormone treatment, as they undergo the processes of final maturation and hydration, is shown in Fig. 6. Little

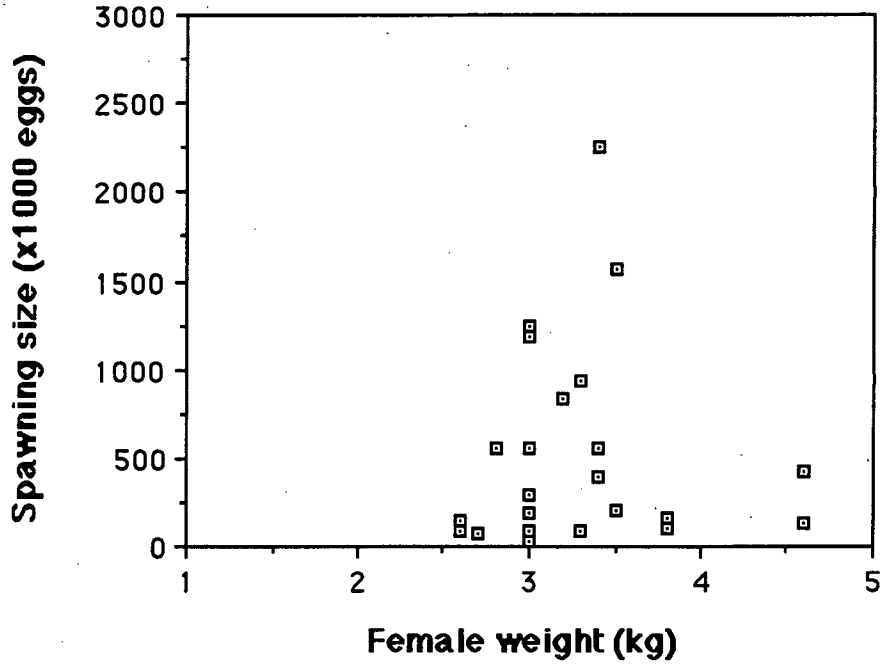


FIG 4 - Number of eggs shed from brood females relative to body weight. There is no trend for larger fish to release more eggs.

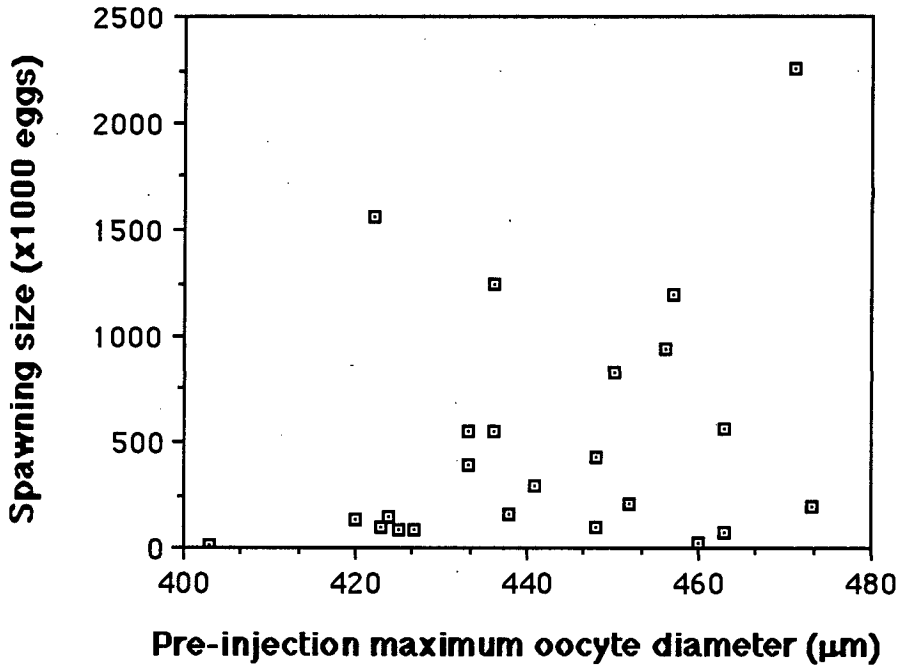


FIG 5 - Number of eggs shed from brood females relative to the mean diameter of the largest oocyte size-class prior to hormone treatment. There is little correlation between these parameters.

Female # 14116, 3.4 kg

Date	Max. oocyte diameter	Egg #/% fert'n
27/10/93	433.0 ± 19.0	0.394×10^6/70.8%
11/11/93	433.4 ± 21.2	partial hydration
14/11/93	517.1 ± 54.1	0.996×10^6/61.4%
11/1/94	471.0 ± 26.6	2.26×10^6/85.8%
28/2/94	463.1 ± 17.5	0.561×10^6/57.8%
15/3/94	421.8 ± 20.9	1.57×10^6/16.1%

TABLE 4 - Repeated spawning inductions from a single female broodfish in the 1993/94 spawning season. This clearly demonstrates that mangrove jack are serial spawners with high fecundity.

enlargement or yolk granule coalescence is observed until 24 h after the priming injection, after which the oocytes rapidly clear and hydrate to their terminal size, reaching it approximately 36 h from the priming injection. The female used to obtain Fig. 6 was hand-stripped at 36 h 20 min, resulting in 54.6% fertilisation. A very similar pattern of development, and hence latent period, was observed for all successful spawnings. A similar latent period has been observed for mangrove jack in Thailand (Doi and Singhagraiwan, 1993), and for *L. johni* in Singapore (Lim *et al.*, 1985) after injection with HCG. Emata *et al.* (1994) reported a shorter latent period of 27 h for mangrove jack after HCG treatment, while Minton *et al.* (1983) recorded a longer period of 42-56 h for *L. campechanus* after similar treatment.

After reaching their terminal size, oocytes were not fertile until entering their 'window of fertilisation', shortly after ovulation. Maximal fertility occurred for only a short period of time, as illustrated in Fig. 7. For the data presented in this figure two females were stripped, and egg fertility assessed, every 5 min from 36 h 10 min onward. Fig. 7 shows peak fertility to occur for a period of only approximately 10 min near 36 h and 20 min after the priming injection. This information has important implications for the timing of strip-spawnings. Apparently, small individual variations in the pattern of oocyte maturation and hydration post-injection prevented rates of fertilisation from being consistently high. Several studies on various fish species have shown an inverse relationship to exist between latent period and water temperature (e.g. Stacey *et al.* ,1979; Fortuny *et al.*, 1988). Small differences in latent period between mangrove jack females are also likely to be at least in part due to water temperature, which ranged from 27-31.5°C in various spawning trials.

The 'window of fertilisation' varies widely between fish species. At one extreme are species such as the rainbow trout, *Onchorynchus mykis*, whose

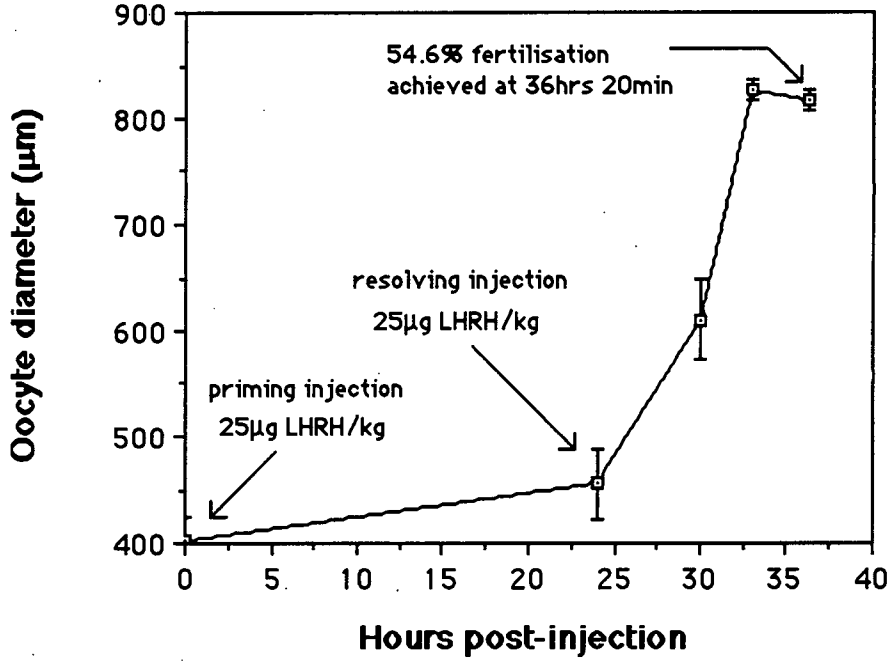


FIG 6 - Path of oocyte final maturation and hydration, as reflected in increasing oocyte diameter, after hormone treatment of a 3 kg female as indicated. The process culminates in the production of fertile eggs. Data points represent the means (± s.d.) of 30 oocyte diameters.

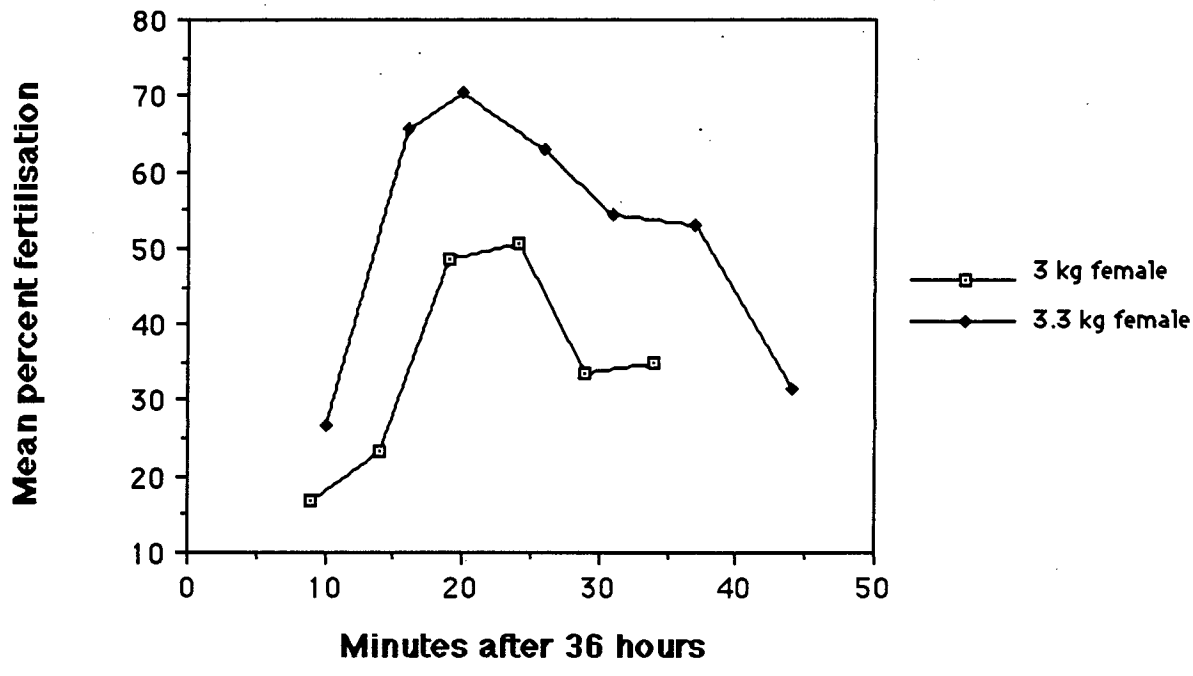


FIG 7 - The 'window of fertilisation' as measured for two female broodfish. Eggs retain maximum fertility for a space of only about 10 minutes near 36 h 20min, at 29-30°C.

eggs can remain fertile for weeks after ovulation has occurred (Nomura *et al.*, 1974). The mangrove jack lies at the other extreme, with species such as the barramundi, *Lates calcarifer*, which also has a very short window of fertilisation (R. Garrett, pers. comm., 1994). Between are species such as the sabalo, *Prochilodus platensis*, at 2 h (Fortuny *et al.*, 1988), the South American catfish, *Rhamdia sapo*, at 5-9 h (Espinich Ros *et al.*, 1984), and the ayu, *Plecoglossus altivelis*, at 48 h (Hirose *et al.*, 1977). A number of authors have presented data indicating the decline in egg quality, as reflected in hatch rates and hatch deformities, as eggs move out of their 'window of fertilisation' (e.g. Craik and Harvey, 1984a,c; Springate *et al.*, 1984; Fortuny *et al.*, 1988). Eventually over-ripe eggs begin to undergo biochemical changes which separate them from fertile eggs (Craik and Harvey, 1984a).

With some other species where hand-stripping is necessary, the timing of stripping is based on when ovulation occurs. Ovulation is often indicated by an enlargement of the anal papilla. In the present study where most fish were held in netcages, the anal papilla could not be observed, and the timing of stripping was based on time after hormone treatment alone. It is felt, for this reason, that fertilisation rates could be improved if fish were held under conditions allowing more ready and careful observation.

Many of the researchers working on lutjanids have commented on their ability to spawn repeatedly on consecutive nights. This is true of *L. campechanus* (Arnold *et al.*, 1978), *L. kasmira* (Suzuki and Hioki, 1979), *L. johni* (Lim *et al.*, 1985) and *L. stellatus* (Hamamoto *et al.*, 1992). It appears likely that mangrove jack also have this ability, although it was not observed in the present study, nor has it been reported from the Philippines (Emata *et al.*, 1994). In Thailand, where 20 or more injected broodfish are placed together in a single large spawning tank or pond, newly-spawned eggs can appear in

the tank for up to 11 days afterward (Doi and Singhagraiwan, 1993), suggesting that individual females are spawning repeatedly.

Egg fertilisability during water hardening

Stripped mangrove jack eggs retained maximal fertility for only approximately 6 minutes in seawater (Fig. 8). This has important implications when artificially fertilising these eggs using the 'wet' method, in which eggs are placed in water before milt is added for fertilisation. A rapid decline in egg fertility in the spawning medium is common to most fish species, and is related to processes of water hardening during which the micropyle closes accompanying separation of the chorion (Yamamoto, 1961). This limitation of the 'wet' method indicates that the 'dry' method of mixing milt with eggs may be preferred.

Male hormone induction trials

A single injection of LHRHa at 50 μg/kg, either in aqueous or pellet form, was capable of increasing milt production from zero to 0.9 ml within 24 h of injection (Fig. 9). Similar effects of the same LHRHa molecule on male salmon, *Salmo salar*, were reported by Weil and Crim (1983). These authors found that the volume of collectable milt correlated positively with blood GtH levels, and that the increase in milt volume was due to increased sperm production rather than sperm dilution.

In the present study, the male which received the treatment in pellet form (80% cholesterol/20% cellulose) showed a peak expressible milt volume at 24 h, whereas males receiving the hormone in aqueous form showed peak milt volume by 12 h. This is presumably a reflection of the rate of hormone release into the fish from the two methods, the pellet giving a relatively slower release. Weil and Crim (1983) also found the pattern of milt

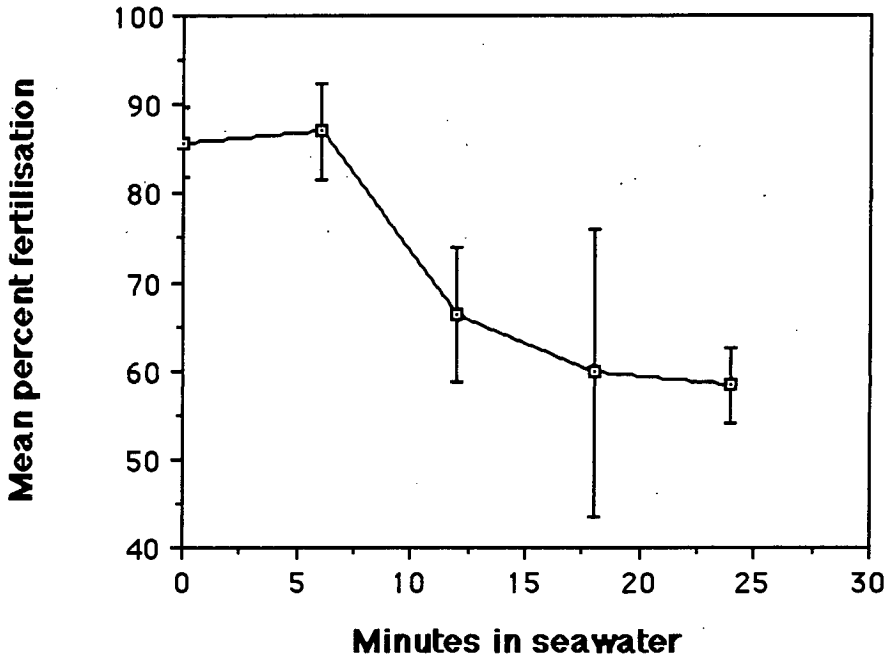


FIG 8 - The rapid decline in fertility of eggs kept in seawater after being stripped from a ripe broodfish. Data points represent the mean (± s.d.) of two replicate samples of 30 eggs.

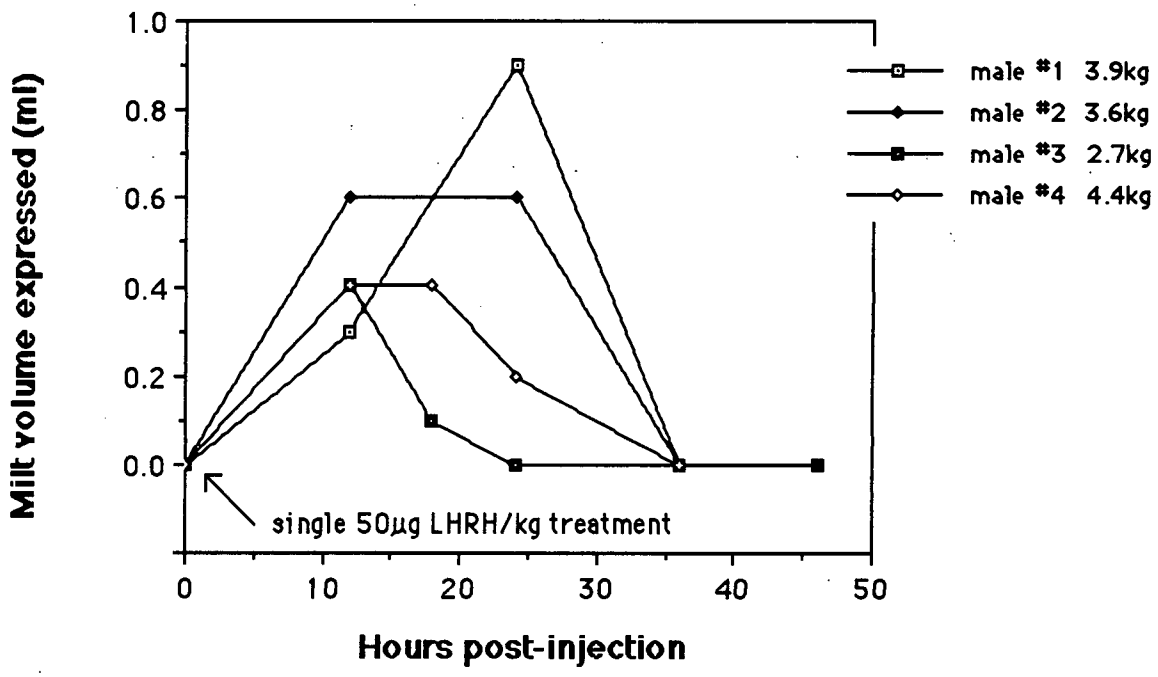


FIG 9 - The reaction of male broodfish to LHRHa treatment at 50 µg/kg, as reflected in expressible milt volume. Male #1 received the hormone treatment as an 80% cholesterol/20% cellulose pellet implant, while others received their treatment in aqueous form. The pellet has apparently delayed the peak effect from about 12-14 h to 24 h post-injection.

production to vary according to the method of hormone administration. In all males, however, expressible milt volume had again declined to zero by 36 h (Fig. 9). On contact with seawater, viable sperm remained active for only approximately 2-3 min. These results are of use when planning strip-spawnings, for formulating treatment protocols, and ensuring that sufficient milt will be available during the brief 'window of fertilisation' of the eggs.

CHAPTER 7

Egg Incubation and Yolk Absorption

Introduction

Data from laboratory studies suggest that the transition from endogenous to exogenous energy sources is possibly the most difficult event for fish larvae to survive (Ehrlich, 1974; May, 1974). This common mortality peak has generally been explained by a failure of larvae to capture first prey items, insufficient suitable prey items, and/or a low resistance to starvation (May, 1971, 1974). This is most apparent amongst marine fish, where larvae are particularly small, with minimal energy reserves to sustain them until suitable and adequate food is encountered. A 'window' of initial feeding opportunity of only a few days is common, compared with, for example, the Atlantic salmon *Salmo salar*, in which initial feeding can be delayed by 1-2 weeks without adversely affecting subsequent survival or growth (Koss and Bromage, 1990). This 'critical period' concept has been used by fisheries biologists to explain year-class strength in natural fish populations (Blaxter and Hempel, 1963; May, 1971, 1974; Ehrlich, 1974). Similarly, in fish hatcheries the survival rate to metamorphosis is often closely linked to the percentage of first-feeding success.

It is now widely accepted that larger larvae at first-feeding, produced by the most efficient use of endogenous energy, are more efficient predators, have a greater swimming and foraging ability, a larger mouth size, and show a greater resistance to starvation (Blaxter and Hempel, 1963; Ryland and Nichols, 1967; Blaxter, 1969; Johns and Howell, 1980), and, hence, have greater survival potential. Miller *et al.* (1988), in a review of 72 species of

marine and freshwater teleosts, established the highly size-dependent susceptibility of first-feeding larvae to starvation. The ability to capture larger prey items is advantageous by virtue of their rapidly increasing calorific values (Hunter, 1981).

It follows that by creating conditions which maximise the efficiency of endogenous energy use, and hence the size of first-feeding larvae, early mortality will be minimised. The physical environment, such as temperature and salinity conditions, have a direct impact on yolk utilisation efficiency, and there is often a combination of factors at which it is maximised (e.g. Marr, 1966; Ryland and Nichols, 1967; Polo *et al.*, 1991). Ryland and Nichols (1967), for example, found that temperature alone can cause a length variation at the commencement of feeding of 10% in plaice larvae. It has been suggested that temperature may be more critical in the culture of warmwater marine fish than for temperate species, where early life stages are often stenothermal (Brett, 1970; Saksena *et al.*, 1972; May, 1975; Santerre, 1976). Furthermore, survival rates and occurrence of deformities are also often closely tied to physical rearing conditions (Blaxter, 1969, 1988; Alderdice, 1988).

The aims of this component of the study were twofold - (i) to provide a brief description of embryological and early larval development for mangrove jack originating from the Hinchinbrook Channel, and to compare this with the descriptions for Thai fish (Doi and Singhagraiwan, 1993) and for fish from the Philippines (Emata *et al.*, 1994), and (ii) to identify optimal temperature and salinity conditions for maximisation of survival and first-feeding size at the completion of yolk absorption.

Materials and Methods

Egg and larval developmental sequences

Newly-spawned eggs, obtained through hormone-induction (Chapter 6), were placed in a 10 l opaque plastic tank receiving light aeration at a density of 20 eggs/l, and allowed to develop through hatch and beyond to complete oil globule absorption. Temperature and salinity conditions were maintained at 29.0 ± 0.8°C and 34 ppt, and shadecloth covers were used to reduce natural sunlight to approximately 1000 lux at midday. The timing of major developmental events was noted. Photos were taken from the early embryo stage of egg development, and not before, due to the requirement for completion of the sensitive development up to the gastrula stage prior to transportation, and due to transport time from the spawning site to the university.

Regular samples (at hatch, 12.5, 18, 24, and 36 h post-hatch) of 10 yolksac larvae were taken for total length (TL) and yolk length and width measurements, allowing graphs of yolk disappearance and simultaneous growth in larval length to be plotted. Yolk volume was estimated from the formula (Alderdice *et al.*, 1979):

$$\text{yolk volume} = \frac{4}{3}\pi\left(\frac{R_1}{2}\right)^2 \times \left(\frac{R_2}{2}\right)^2 \qquad \text{where } R_1 = \text{radius of yolksac length}, \; R_2 = \text{radius of yolksac width}$$

A second, similar experiment was carried out to enable monitoring of oil globule deletion. Water conditions and procedures were as above, except water temperature which was 27.4± 0.6°C throughout the 60h trial. Samples were taken at 15h intervals to 30h, then at approximately 5h intervals.

Optimum temperature/salinity conditions for yolksac larvae

A 5 x 3 factorial design (temperature x salinity) was used to determine optimum conditions for yolksac larvae. A similar experiment on eggs could not be carried out for the abovementioned logistical reasons. Specific

temperatures chosen were 25.5, 27.5, 30.5, 34.0, and 36.0°C, allowing assessment of temperatures either side of natural spawning temperatures. These did not vary more than ± 0.8°C from their settings. Salinities chosen were 22, 27, and 32 ppt, encompasing the salinity range encountered during the spawning season. These remained essentially unaltered throughout the trial. Three replicates of each of the 15 temperature/salinity combinations were used, amounting to 45 experimental containers.

Experimental tubs consisted of 1 litre flat-bottomed circular opaque plastic containers, with lids fitted to minimise evaporation. These were two-thirds submersed in temperature-controlled water baths, 9 tubs being placed randomly per bath. Each tub was equipped with an adjustable air supply via an airstone, and was completely covered with black plastic so that yolk absorption proceeded in darkness. Temperature was measured twice daily throughout the trial.

Newly-hatched larvae were allocated by volumetric estimation to each experimental tub (stocking density 103 larvae/l). All larvae were derived from a single hormone-induced spawning. Tubs to contain 32 ppt salinity were then filled to 1 litre, and allowed to equilibrate to bath temperature. Other tubs were adjusted to their lower salinities through slow addition of distilled water. Equilibration rates did not exceed 3°C or 10 ppt/h.

Three measurements were taken for each tub when 99% yolk absorption was reached - time to reach this stage, number of surviving larvae, and mean total length of larvae. Mean total length was determined by measuring 30 larvae from each tub with an ocular micrometer, except where low survival rates precluded 30 from being measured.

Differences in measured variables were detected using 2-way ANOVA ($p < 0.05$) and the Tukey pairwise comparison of means test. Homogeneity of variance was tested for sets of data by examining residuals.

Results and Discussion

Egg and larval developmental sequences

Egg development was very rapid, with first eggs hatching at 18 h 10 min post-fertilisation at 29.0°C. Mean total length at hatch was 2.12 mm (range 1.95-2.25 mm). Hatching larvae had a mean yolk volume of 0.161 ± 0.03 mm^3 (± s.d.), measured for larvae of mean total length 1.95 mm. An oil globule of mean volume 0.00207 mm^3 was situated in the anterior part of the yolk (Plate 4). These volumes are very similar to those reported by Doi and Singhagraiwan (1993) for mangrove jack larvae from Thailand.

The elliptical yolksac projecting anterior to the head, and anterior oil globule (Plate 4), are common characteristics of lutjanid larvae (Suzuki and Hioki, 1979; Rabalais *et al.*, 1980; Minton *et al.*, 1983; Lim *et al.*, 1985; Leis, 1987; Hamamoto *et al.*, 1992). A line of melanophores was present beneath the notochord, but otherwise were few in number (Plate 5). The mean total length of larvae at the completion of yolk absorption was 3.17 mm.

The patterns of yolk depletion post-hatch, and simultaneous growth in total length, are given in Fig. 1. It may be seen that by approximately 36 h post-hatch, larvae have completely used their yolk reserves and have almost reached their immediate asymptotic length of approximately 3.1 mm. The oil globule, however, remains until approximately 70 h, as shown in Fig. 2, measured at 27°C. From the rapid depletion of the oil globule, the 'window' of initial feeding opportunity can be predicted to be extremely brief.

Both of these absorption times are shorter than those reported by Doi and Singhagraiwan (1993) for Thai fish (approximately 70 h and 100 h for

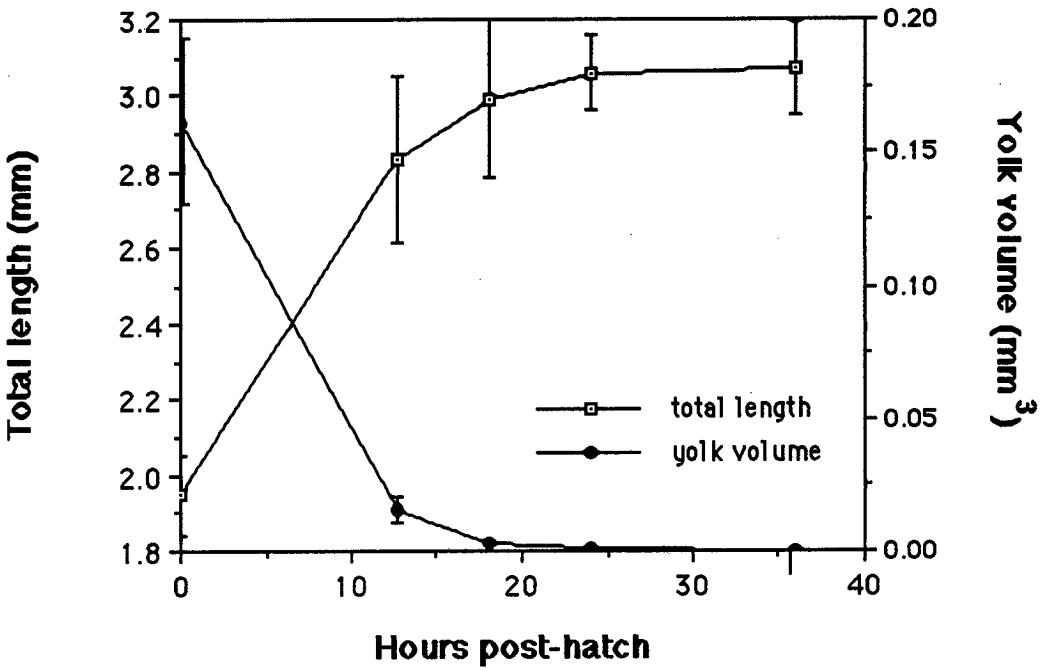


FIG. 1 - Patterns of yolk depletion and simultaneous growth in total length of mangrove jack larvae from hatch, at 29.0±0.8 C. Data points represent means (± s.d.) of samples of 10 larvae each.

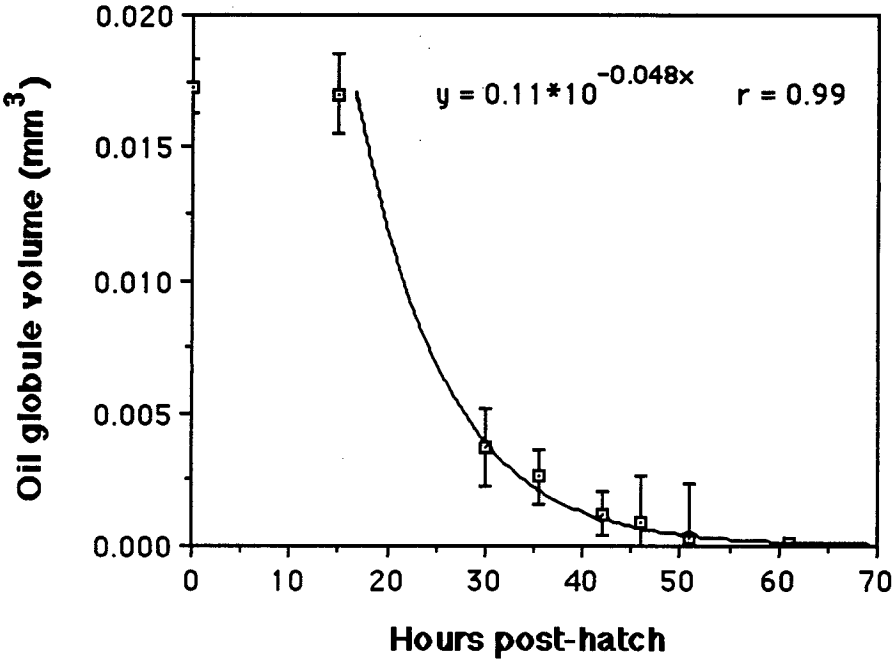


FIG. 2 - Pattern of oil globule usage from hatch, at 27.4±0.6 C. Data points represent means (± s.d.) of samples of 10 larvae each. An exponential curve has been fitted to the data from 15 h post-hatch, and equation and 'r' value included. Oil usage before this time is negligible.

yolksac and oil globule respectively), although this may be related to temperatures used. The development of mangrove jack eggs was similar to that of other lutjanids, and of pelagic eggs in general. The timing of major developmental events, at 29.0°C, was as follows:

fertilisation	0 min
first cell division	30 min
second cell division	43 min
third cell division	55 min
morula stage	1 h 45 min
blastula stage	2 h 50 min
late gastrula stage	5 h 30 min
neurula stage	8 h
early embryo	8 h 50 min
4 somite stage, otoliths, first melanophores	10 h 40 min
appearance of optic vesicles	12 h 30 min
heart starts to beat	14 h 30 min
HATCH	18 h 10 min - 19 h
yolk half absorbed	10 h post-hatch
yolk 95% absorbed	17 h post-hatch
yolk completely absorbed, eyes pigmented, mouth and anus open	36 h post-hatch
oil globule 95% absorbed	40 h post-hatch
oil globule completely absorbed	70 h post-hatch

Plates 1-7 depict major developmental events from the early embryo stage.

As stated in the introduction, Doi and Singhagraiwan (1993) have described the sequence of embryonic development of mangrove jack eggs spawned in Thailand. Their description is broadly consistent with the above, although they reported hatching commencing at 15 h post-fertilisation at 27.8-29.7°C, which represents a significantly shorter incubation time than observed at 29.0°C in the present study. Similarly, Emata *et al.* (1994) reported an incubation time of 16 h at 28°C for mangrove jack from the Philippines, which is also shorter than observed here. These differences may

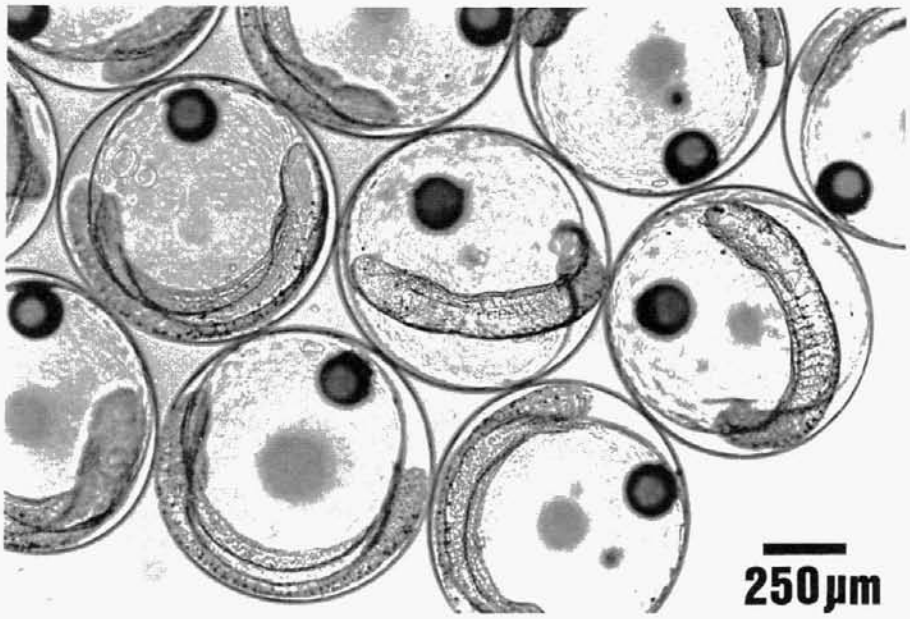


PLATE 1 - Early embryo showing somites and first melanophores, 13 h.

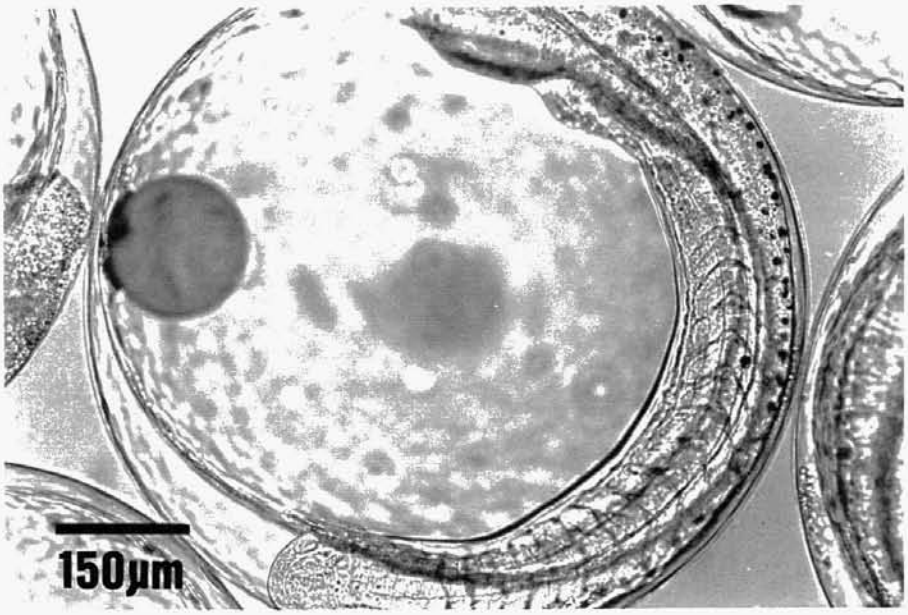


PLATE 2 - As Plate 1, at higher magnification.

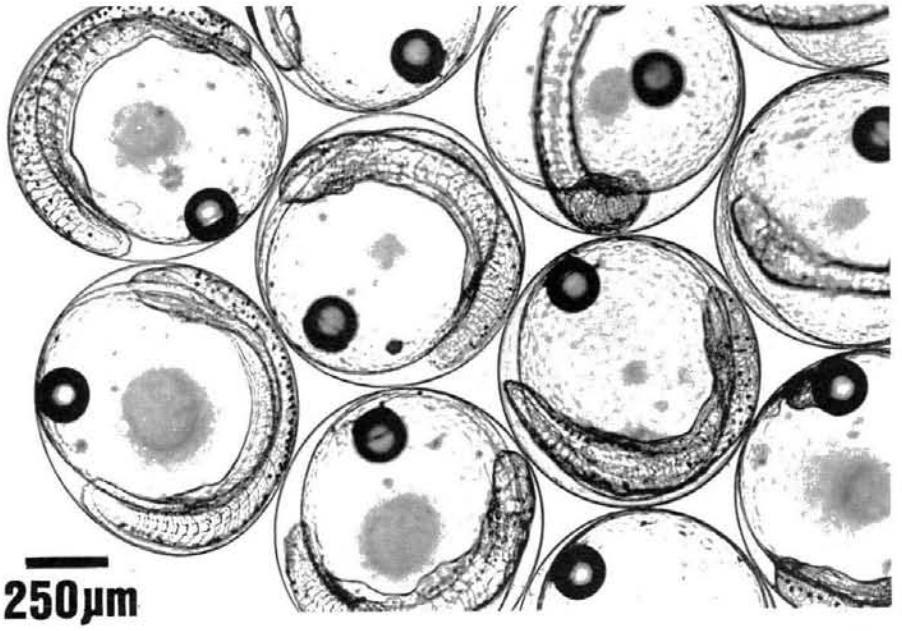


PLATE 3 - Pre-hatch, 15 h. Embryo has heartbeat and shows frequent movement.

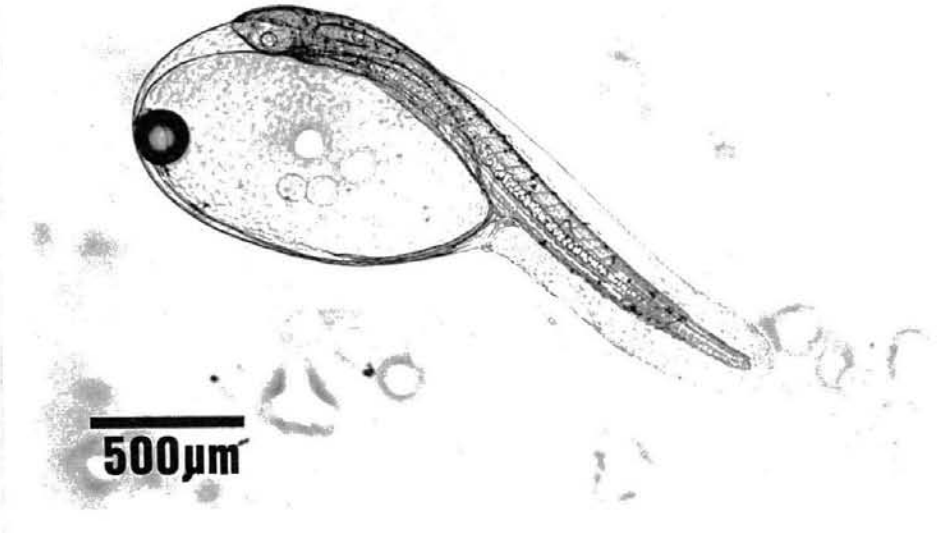


PLATE 4 - Hatch, 18 h 10 min.

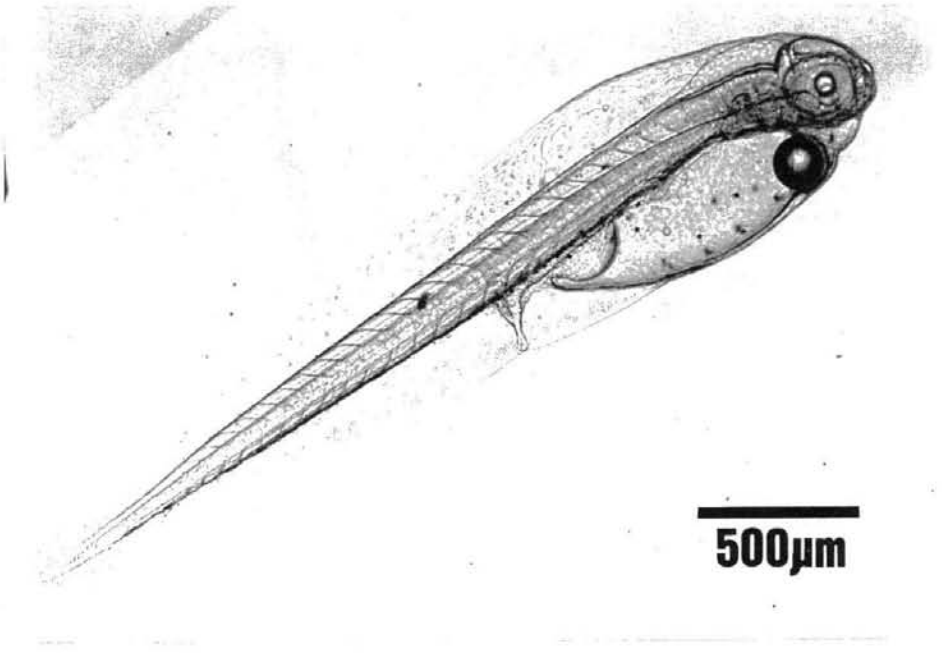


PLATE 5 - Half yolk absorption, 9 h post-hatch.

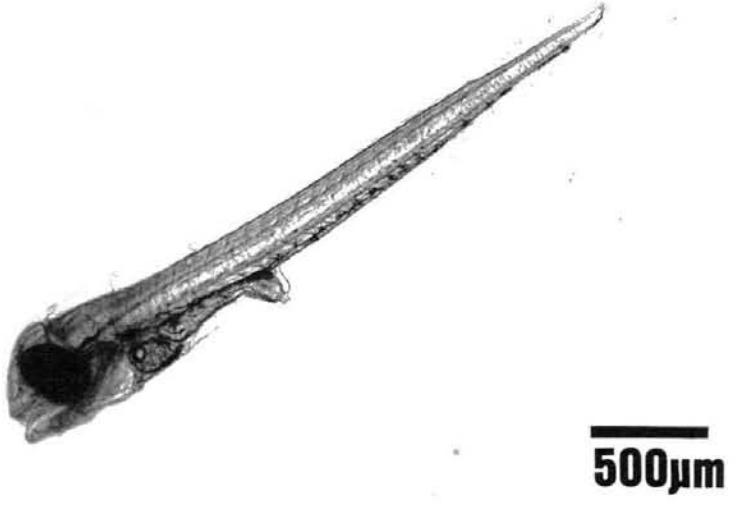


PLATE 6 - First-feeding larva, 42h post-hatch. Note fully pimented eyes, open mouth and anus, and partially absorbed oil globule.

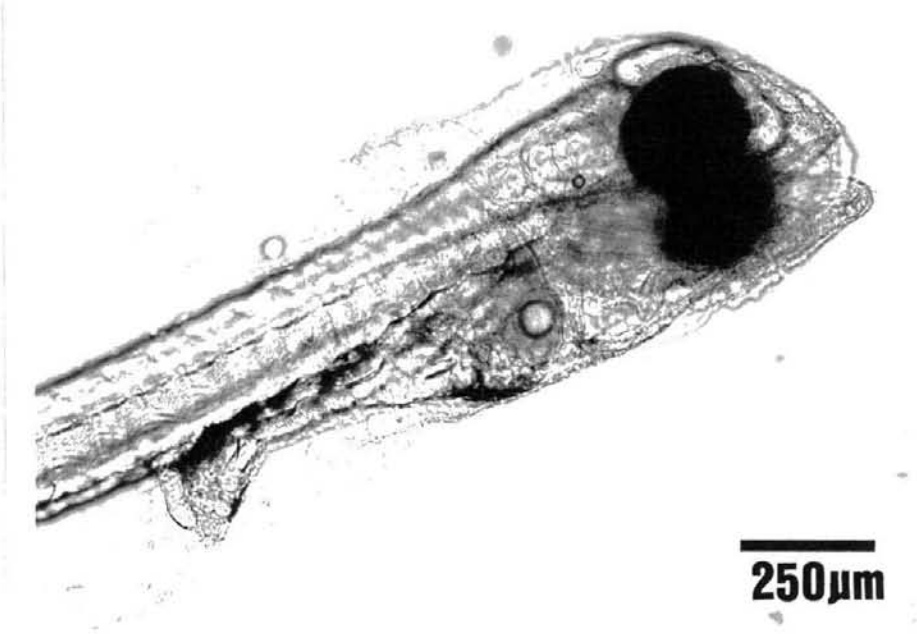


PLATE 7 - As Plate 6, at higher magnification.

reflect genetic differences between fish populations, or differences in other incubation conditions such as salinity or dissolved oxygen, both of which are known to affect incubation time (Blaxter, 1969).

Also, Doi and Singhagraiwan (1993) and Emata *et al.* (1994) report smaller larval sizes at hatch (1.56 - 1.87 mm, and 1.61 mm TL respectively), and at yolk absorption (both slightly less than 3.0 mm), than found in the present study. These differences may possibly be attributable to smaller reported egg size (means of 795 µm and 740 µm respectively compared with 824 µm in this study). Smaller hatch sizes have also been reported for the John's snapper, *L. johni* (1.65 mm) by Lim *et al.* (1985), the red snapper, *L. campechanus* (1.5 mm) by Minton *et al.* (1983), and the blue-banded snapper *L. kasmira* (1.83 mm) by Suzuki and Hioki (1979), but similar sizes have been reported for red snapper, *L. campechanus* (2.2 mm) by Rabalais *et al.* (1980) and the white spotted snapper, *L. stellatus* (2.5 mm) by Hamamoto *et al.* (1992). Size at yolk absorption is similar to that reported for *L. kasmira* (3.08-3.2 mm) by Suzuki and Hioki (1979), but larger than that reported for the John's snapper, *L. johni* (2.9 mm) by Lim *et al.* (1985).

Emata *et al.* (1994) state, for the mangrove jack population in the Philippines, that at hatch the oil globule is situated at the "midventral to posterior side of the yolksac" which differs from its extreme anterior position observed in the present study, and in Thailand (Doi and Singhagraiwan, 1993). Again this illustrates the subtle differences that exist between isolated populations of the same species.

Optimum temperature/salinity conditions for yolksac larvae

Two-way ANOVA showed only temperature to have a significant effect on larval length at yolk absorption ($F=30.2$, d.f.=4, $p< 0.001$), but both temperature and salinity to have significant effects on survival to yolk

absorption ($F=4.7$, d.f.=4, $p< 0.005$; and $F=10.6$, d.f.=2, $p< 0.001$ respectively), over the ranges of the experiment. Yolksac larvae were able to tolerate all temperature/salinity combinations investigated, although an optimum temperature was identified based on survival and first-feeding size data. The optimum salinity may have fallen below the range tested.

Temperature had a controlling influence on 99% yolk absorption time (Fig. 3), being 31 h at 25.5°C, and 16 h at 36°C. This is expected from recognised Q_{10} coefficients for metabolic processes (Blaxter, 1969). Salinity did not have any significant effect on yolk absorption time, as found for the bairdiella, *Bairdiella icistia*, by May (1974), for the gilthead seabream, *Sparus auratus*, by Freddi *et al.* (1981), and for the petrale sole, *Eopsetta jordani*, by Alderdice and Forrester (1971). A logarithmic curve best described the temperature data:

$$y = 10{,}301.\ x^{-1.807}$$

where y = yolk absorption time (h); x = temperature (°C).

Doi and Singhagraiwan (1993) have studied the relationship between temperature and incubation time for mangrove jack eggs, and expressed it as $T = 253.8/(t-13.41)$ where 'T' is incubation period in hours and 't' is water temperature in °C. However, as stated previously this appears to underestimate incubation time observed for the population of fish used in this study. These authors found a temperature of 37.5°C to be outside the thermal tolerance limits for eggs, and temperatures of 25.0 and 34.8°C to be marginal. Hatch rates were consistent between 26.0-31.5°C.

Fig. 4 illustrates the effects of temperature and salinity on size of larvae at yolk absorption. This is of importance due to the recognised association between first-feeding size and survival during the transition from

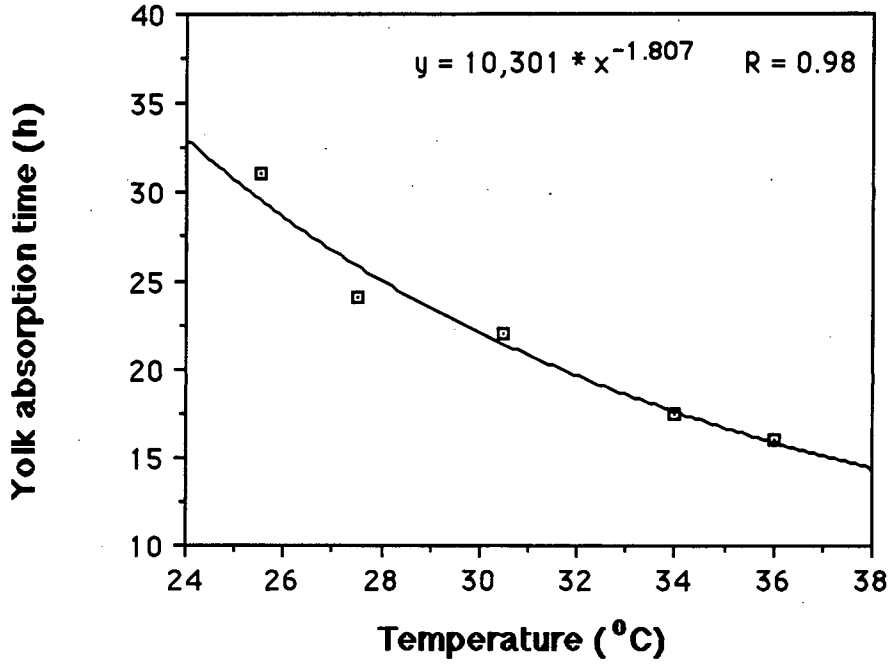


FIG. 3 - Influence of temperature on 99% yolk absorption time at 32 ppt salinity. A logarithmic curve has been fitted to the data, and 'r' value included.

endogenous to exogenous nutrition. This is a consequence of increased predation efficiency, swimming ability, mouth size (and hence potential prey size) and resistance to starvation (Blaxter and Hempel, 1963; Ryland and Nichols, 1967; Blaxter, 1969; May, 1974; Dabrowski, 1976; Hunter, 1981). First feeding length is a reflection of yolk utilisation efficiency, as it is a direct result of the conversion of yolk into tissue. Fig. 4 shows that, regardless of salinity, temperatures of 25.5 and 36°C are outside the optimum range for yolk utilisation efficiency. Greatest efficiency occurred in the range of 27.5-34.0°C. Although the statistical analysis showed there to be no significant interaction between temperature and salinity here ($F=2.0$, d.f.=8, $p=0.076$), it is common for the optimal temperature to vary to some extent with salinity (Blaxter, 1969; Freddi *et al.*, 1981).

The existence of a temperature range in which yolk is most efficiently converted to tissue (and hence larval length) is well established, and has been identified for numerous species (e.g. Marr, 1966; Ryland and Nichols, 1967; May, 1974; Santerre and May, 1977; Polo *et al*, 1991). At these temperatures size and hence survival potential can be expected to be at their maximum. Ryland and Nichols (1967) state that temperature alone may cause a length variation of up to 10% in plaice larvae at first feeding, with associated benefits to the larvae. In the present study temperature was responsible for a 3.9% variation in larval length at first feeding, between temperatures of 27.5 and 36.0°C. This is an important difference if large-scale rearing is to be attempted.

Fig. 5 illustrates the influences of temperature and salinity on survival through yolk absorption. The most striking result was the improved survival at reduced salinities, suggesting that larvae are under considerable osmotic stress at full-strength seawater. The poorest survival values occurred at the lowest two temperatures, 25.5 and 27.5°C. The thermal tolerance of yolksac

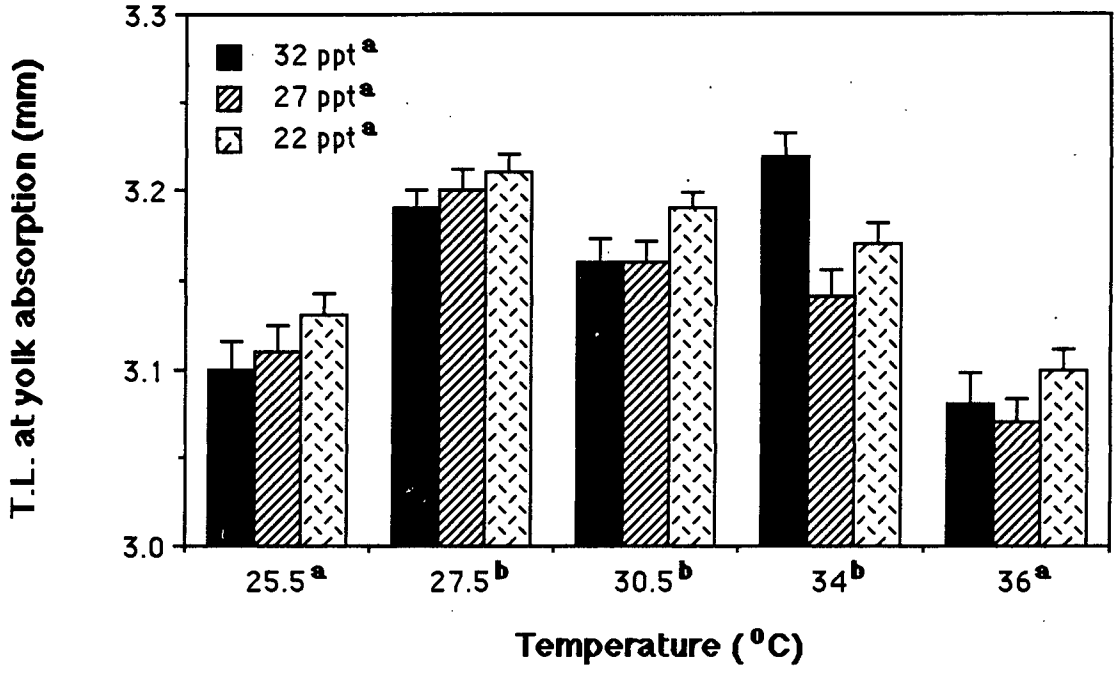


FIG. 4 - Effects of temperature and salinity on total length of larvae at completion of yolk absorption. Data bars represent means (± s.e.) of three replicates of 30 larvae each. Temperatures assigned dissimilar superscripts represent a statistically significant difference (2-way ANOVA, $p < 0.05$). No significant difference exists between salinity treatments.

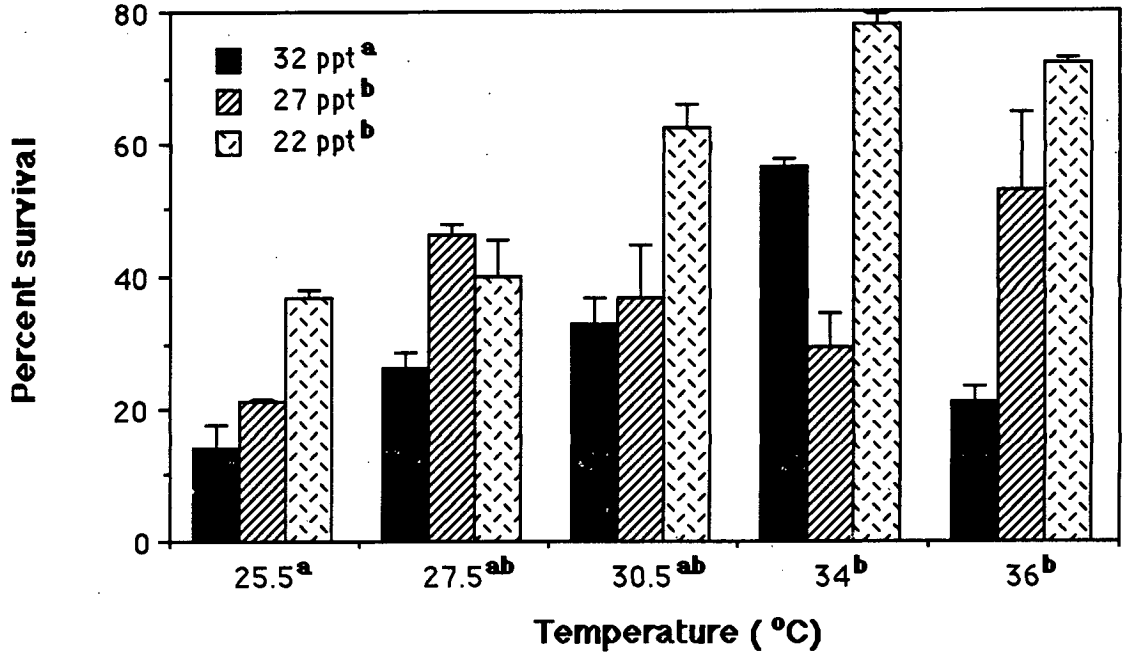


FIG. 5 - Effects of temperature and salinity on percent survival of hatched larvae to completion of yolk absorption. Data bars represent means (± s.e.) of three replicates of 103 larvae each. Temperatures or salinities assigned dissimilar superscripts represent statistically significant differences between them (2-way ANOVA, $p < 0.05$).

larvae was extended at lower salinities. Although again no significant temperature/salinity interaction was revealed ($F=2.0$, d.f.=8, $p=0.081$), increasing temperatures tended to be more beneficial at lower salinities. Mangrove jack yolksac larvae may therefore be considered quite euryhaline, with a tolerance range likely to exceed the range 22-32 ppt examined in this experiment.

A decrease in the optimal salinity level for early larval stages has also been found for grey mullet, *Mugil cephalus* (Nash and Shehadeh, 1980), and the gilthead seabream, *Sparus auratus* (Freddi *et al.*, 1981). Holliday (1965), working with unfed herring larvae, found that they could survive longest before starvation at lower salinities, and has explained his observations by the reduced osmotic demand placed on larvae at lower salinities, and adds that this in turn could allow a greater tolerance of the negative effects of other environmental factors such as suboptimal temperature, as observed in the present study. It is well known that the energetic cost of osmoregulation is lowest in adult fish at a salinity isotonic with the body fluids (e.g. Farmer and Beamish, 1969).

Laboratory-derived optima for marine fish larvae often correspond closely with conditions during the spawning season (e.g. Camus and Koutsikopoulos, 1984; Van der Wal, 1985). It is difficult to relate the identified optima in the present study to natural conditions during the spawning season, as the spawning location has not been positively identified. However, mean water temperatures are in the range of 27-30°C, and mean salinities in the range of 27-38 ppt, in the Hinchinbrook channel during the spawning season (Chapter 6, Fig. 1). Salinity would vary with proximity to the coast and river mouths, being relatively higher and more stable with distance from the coast. Salinities approaching freshwater are common to a depth of at least 1 m in the Hinchinbrook Channel after heavy rainfall. The

preference of yolksac larvae for water of lower salinity suggests that the spawning site may be closer inshore than has previously been suggested, where salinities are lowered from monsoonal rains. However, it does not necessarily follow that the optimum salinity for eggs and feeding larval stages is the same as for yolksac larvae. For example, Freddi *et al.* (1981) found, for the gilthead seabream, that the optimum salinity for yolksac larvae was considerably lower than for eggs, and was in fact at least 10 ppt lower than natural conditions during the spawning season, regardless of water temperature. A similar experiment needs to be done on eggs and feeding larvae of mangrove jack before any conclusions may be drawn.

In summary, optimum rearing conditions of 22 ppt salinity, and 30.5-34.0°C are suggested for the yolksac stage of mangrove jack. Whether or not these optima extend beyond to eggs and feeding larvae is yet to be determined.

CHAPTER 8

Larval Rearing

Introduction

Marine fish larvae present a challenge to those attempting to rear them by virtue of their very small size and limited supply of energy reserves to sustain them through the transition to exogenous nutrition. Furthermore, the simple gut, with limited digestive capability, relies to a large extent on enzymatic autolysis of live foods, making the use of conventional artificial or inert foods to date unviable. Traditionally, hatcheries have relied to a large extent on the rotifer, *Brachionus plicatilis*, and the brine shrimp, *Artemia* spp., to meet these food requirements, because of their ease of raising in large numbers, an important consideration for commercial hatcheries.

Commercial marine fish hatcheries world-wide have encountered common problems in the larval rearing of numerous species. These have included poor first-feeding success and associated mortality peak, nutritional inadequacies of live foods, and low percentages of correct swim bladder inflation, with associated problems. While swim bladder inflation has been dramatically improved through the use of surface oil skimmers (Chatain and Ounais-Guschemann, 1990), and nutritional inadequacies of rotifers and brine shrimp largely overcome through the use of commercial enrichment formulations (Sorgeloos and Leger, 1992; Sweetman, 1992; Tucker, 1992a), the low rate of first feeding success has persisted in many species. This has often been attributed to rotifers being too large an initial prey item. Unfortunately, no smaller substitute with similarly favourable intensive culture qualities has been found. On occasion the larval stages of oysters have been used, but

more recently small (type S) and super small (type SS)-strain rotifers have become available and are becoming widespread in their use.

Some species of marine fish larvae present additional problems to the aquaculturist. Species such as the groupers (*Epinephelus* spp.), the coral trouts (*Plectropomus* spp.), and many lutjanids (*Lutjanus* spp.) consistently show very poor survival rates. Possible reasons for this include particular sensitivity to poor egg and water quality, obscure nutritional or environmental requirements, particularly small mouth sizes and energy reserves, or even the unusual osteological development of the feeding apparatus (Kohno *et al.*, 1995). The mangrove jack, *L. argentimaculatus*, appears to fall into this category based on larval rearing trials in Southeast Asia in recent years. Survival rates have typically been variable and in the order of 1% or less.

The aims of this component of the project were to attempt rearing local mangrove jack larvae intensively to metamorphosis using 'clearwater' and 'greenwater' techniques, and to trace their development and growth, including the timing of first-feeding and swim bladder inflation.

Nutritional studies

Of particular importance to marine fish larval diets is their protein and lipid quality (Tucker, 1992a). A valuable dietary protein source will provide adequate amounts of the ten essential amino acids common to all fish (Wilson, 1989). The closer the essential amino acid composition in the diet resembles the larval requirements, the greater will be its nutritional value, assuming the protein is digestible and the amino acids are equally available. The larval requirements, in turn, are a close reflection of the composition of tissues being formed, as the major use of essential amino acids is in the synthesis of body protein (Webb and Chu, 1983; Gatlin, 1987). Consequently, in species for which quantitative amino acid requirements have not been

determined through traditional digestibility and retention studies, an indication of requirements can be gained through analysis of whole-body protein composition, first suggested by Phillips and Brockway (1956). The results do not indicate required quantities of essential amino acids, just the most desirable essential amino acid profile of dietary protein. Quantitative requirements can then be obtained through determination of one or two amino acid requirements, from which others can be predicted relatively. The close relationship between the essential amino acid pattern of whole body tissue and the known requirement pattern of many species is now well established (Cowey and Tacon, 1983; Halver, 1985; Wilson and Cowey, 1985; Wilson and Poe, 1985; Gatlin, 1987; Mohanty and Kaushik, 1991), and this technique is widely accepted as a suitable method for evaluating the limiting amino acids in a potential dietary protein source (Wilson, 1989).

By similar reasoning, Tocher and Sargent (1984), Falk-Petersen *et al.* (1986) and others have suggested that the fatty acid profiles of fish whole-body tissues may allude to the dietary requirements of the species. This concept has proven useful in several species including the Atlantic herring, *Clupea harengus* (Tocher *et al.*, 1985), the Dover sole, *Solea solea* (Dendrinos and Thorpe, 1987), and the dolphin fish, *Coryphaena hippurus* (Ostrowski and Divakaran, 1989).

As part of this component of the project, the free and bound amino acid composition and fatty acid composition of whole-body first-feeding mangrove jack larvae were determined and compared with that of rotifers, to gain some understanding of the value of rotifer lipid and protein to this species. The results presented here will provide useful guidelines for formulating diets and/or selecting and enriching live foods for cultured mangrove jack, and reveal any existing nutritional inadequacies which may be responsible for the poor larval survival rates characteristic of the species.

Materials and Methods

Rearing trials

A total of 6 mangrove jack larval rearing trials were carried out. Although all were carried out intensively (5-50 larvae/l), and in tanks of less than 1,500 l capacity, individual trials varied in terms of initial food organisms offered, and in the physical rearing environment provided. Trials could be categorised into 4 types based on these differences, and are listed in the order in which they were performed. In all cases the age of larvae has been expressed as days after hatch, where hatch is designated day 0.

(i) clearwater with rotifers as first food

The first attempt to rear mangrove jack larvae used clearwater with rotifers offered as first food. Fig. 1 schematically illustrates the culture system employed. Seawater was recirculated and continually filtered to 1 µm, and no unicellular algae were added to the rearing tank. A 200 l black hemispherical tank, filled to 150 l, was used as the larval rearing tank, with a central adjustable mesh standpipe outlet. Water was circulated from a 1000 l sump, through a 1 µm cartridge filter and UV steriliser, to the rearing tank. From here water flowed through live food traps to a high-capacity biofilter before returning to the sump.

Water was static for the first three days after which a nightime flowrate of 0.9 l/min. was begun to coincide with the commencement of feeding. From day 9 onwards, a permanent flow of 0.9 l/min. was maintained. A surface blower and floating trap (after Chatain and Ounais-Guschemann, 1990) was employed to remove any oily film that formed on the water surface during feeding. From day 6 the tank bottom was siphoned clean every second day. Very gentle aeration was provided through a finely-perforated

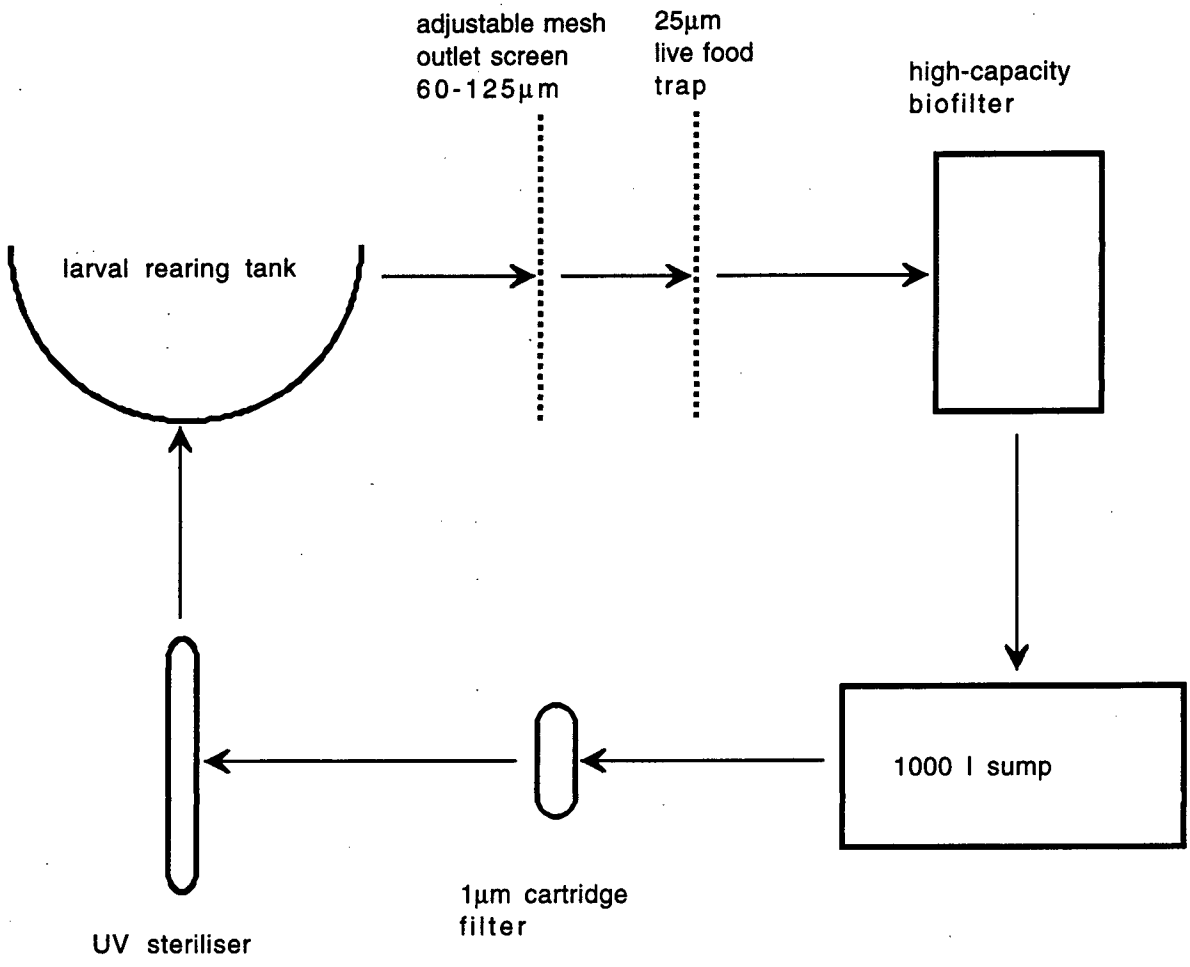


FIG. 1 - Schematic diagram of the culture system used in 'clearwater' rearing trials with mangrove jack larvae. The larval rearing tank ranged in size from 200 l to 1000 l in different trials, with corresponding changes in biofilter size.

tube. Lighting consisted of 75% filtered natural sunlight (12 h photoperiod) giving a peak noon light intensity of approximately 1000 lux. Immersion heaters in the sump maintained water temperatures within the desired range. Water conditions throughout the trial were as follows (measured daily):

Parameter	Range
temperature	27.5 - 30.8°C
salinity	32 - 34 ppt
pH	8.0 - 8.2
total ammonia	< 0.2 mg/l
nitrite	< 0.1 mg/l
nitrate	< 2.5 mg/l
light (inten./photop.)	< 1000 lux/12 h

Yolksac larvae, all derived from the one hormone-induced spawning, were stocked at the rate of 27 larvae/l (i.e. 4000 larvae in 150 l). Food was first offered on the morning of day 2, when larvae had fully pigmented eyes and an open mouth and anus. Initial food consisted of sieved (125 µm screen) L-strain rotifers, *B. plicatilis*. Rotifers were raised exclusively on *Nannochloropsis oculata*, and were enriched with Frippak "Booster" (Frippak Feeds, U.K.) for 4 h prior to being introduced to the tank at 10 individuals/ml. Unscreened rotifers (up to 300 µm lorica length) were used from day 7, and previously decapsulated instar I brine shrimp (Great Salt Lake brand) were first introduced on day 11 at 2 individuals/ml (Fig. 2).

Samples of 10 larvae were preserved every 1-2 days (in buffered 2.5% gluteraldehyde) to day 12 when no further larvae remained. Measurements of notochord length (N.L.), and derived means, were recorded for each

sample before preservation. These samples were also used to record the timing of major developmental events such as first exogenous feeding and swim bladder inflation, and for mouth size determinations. Mouth size was measured according to the method of Shirota (1970), where mouth height is estimated from top jaw length (AB), according to the formula:

$$\text{mouth height} = \sqrt{2} \times AB$$

Survival was estimated through observed larval densities and mortalities on the tank bottom.

(ii) greenwater with rotifers as first food

A rearing trial was carried out in static water containing a low density of the unicellular alga *N. oculata*. Procedures were based on those described by Palmer *et al.* (1992) for rearing barramundi larvae, with some modifications. A 1000 l, black-walled conical base tank was used. This was filled with 1 µm-filtered seawater to which a dense algal culture was added to achieve a density of 6×10^4 algal cells/ml. A surface blower and floating trap were employed to remove any oily film that formed on the water surface during feeding. Gentle aeration was provided in several areas of the tank through airstones. Fluorescent lighting ('cool white' tubes) provided 250 lux with a 12 h photoperiod. Water temperature was maintained within the desired range through room heating. Water conditions throughout the trial were as follows (measured daily):

Parameter	Range
algal density	$5 \times 10^3 - 1 \times 10^5$ cells/ml
temperature	27.5 - 29.3°C
salinity	33 - 35 ppt
pH	8.2 - 8.5
total ammonia	< 0.2 mg/l
nitrite	< 0.1 mg/l
nitrate	< 2.5 mg/l
light (intens./photop.)	250 lux/12 h

Eggs at an advanced stage of development, all derived from a single hormone-induced spawning, were stocked at the rate of 25 eggs/l (i.e. 25,000 eggs in 1,000 l). Egg shells were siphoned from the tank bottom soon after hatch. Food was first offered on the morning of day 2, when larvae had fully pigmented eyes and a functional mouth and anus. Initial food consisted of sieved (125 µm screen) L-strain rotifers, *B. plicatilis*. Rotifers were raised exclusively on *N. oculata*, and were enriched with Frippak "Booster" for 4 h prior to being introduced to the tank at 6 individuals/ml (Fig. 2). Unscreened rotifers (up to 300 µm lorica length) were used from day 6 until total mortality was observed on day 9. No larvae were preserved or measured from this trial. Survival was estimated daily through observed larval densities.

(iii) clearwater with oyster trochophores as first food

After suspecting inappropriate initial food size to be limiting success in the above trials, the rearing protocol was changed to incorporate trochophores of the local black-lip oyster, *Crassostrea echinata*. In these three trials, trochophores preceded rotifers in the feeding schedule (Fig. 2). Oyster trochophores of this species are 40-60 µm in diameter, and transform to the

shelled 'D-veliger' stage which is initially approximately 80 µm in width. Fertilised oyster eggs were obtained either through strip-spawning or induced spawning by temperature shock.

Once again Fig. 1 schematically illustrates the culture system employed. Seawater was recirculated and continually filtered to 1 µm, and no unicellular algae were added to the rearing tank. A 1,000 l black-walled, conical-base tank was used, with a central screened standpipe outlet. Water was circulated from a 1000 l sump, through a 1 µm cartridge filter and UV steriliser, to the rearing tank. From here water flowed through live food traps to a high-capacity biofilter before returning to the sump.

Water was static for the first three days after which a nightime flowrate of 2.0 l/min. was begun to coincide with the commencement of feeding. From day 8 onwards, a permanent flow of 2.0 l/min. was maintained. A surface blower and floating trap were employed to remove any oily film that formed on the water surface during feeding. From day 6 the tank bottom was siphoned clean every second day. Very gentle aeration was provided through a finely-perforated tube. Fluorescent lighting ('cool white' tubes) provided 250 lux with a 12 h photoperiod. Immersion heaters in the sump maintained water temperatures within the desired range. Water conditions throughout the three trials were as follows (measured daily):

Parameter	Range		
	trial 1	trial 2	trial 3
temperature	27.0 - 29.5°C	27.0 - 28.5°C	26.5 - 29.0°C
salinity	35 - 36 ppt	35 - 37 ppt	35 ppt
pH	7.7 - 8.0	8.0 - 8.2	8.0 - 8.3
total ammonia	< 0.2 mg/l	< 0.2 mg/l	< 0.2 mg/l
nitrite	< 0.1 mg/l	< 0.1 mg/l	< 0.1 mg/l
nitrate	< 2.5 mg/l	< 2.5 mg/l	< 2.5 mg/l
light (inten./photo.)	250 lux/12 h	250 lux/12 h	250 lux/12 h

Recently-hatched yolksac larvae, all derived from the one hormone-induced spawning, were stocked at the rate of 5, 25 and 50 larvae/l in the three trials. Food was first offered on the morning of day 2, when larvae had fully pigmented eyes and a functional mouth and anus. Initial food consisted of oyster trochophores (*C. echinata*) at 4-10/ml to day 4. On day 4 this was supplemented with sieved (125 µm screen) L-strain rotifers, *B. plicatilis*, at 3-6/ml (Fig. 2). Rotifers were raised exclusively on *N. oculata*, and were enriched with Frippak "Booster" for 4 h prior to being introduced to the tank. Trochophores were no longer added after day 5, although 'D-veligers' remained in the water column through day 6. Unscreened rotifers (up to 300 µm lorica length) were used from day 7 until no further larvae remained (Fig. 2).

Large losses were observed in the first trial from what appeared to be a microbial disease introduced with the oyster larvae. In the two subsequent trials, therefore, all food added to the tank was first disinfected in a 10 ppm furazolidone bath for 30 min.

Larvae from each trial were examined microscopically daily to assess feeding success, health and stage of development. Survival was estimated through observed larval densities and mortalities on the tank bottom.

(iv) greenwater with oyster trochophores, rotifers and wild-caught zooplankton as first food

Another 'greenwater' trial was conducted using a combination of initial food items, including oyster trochophores, sieved rotifers and sieved wild-caught zooplankton. Procedures were similar to those described in the previous greenwater trial, with a low density of microalgae being maintained in the rearing water, and minimal water exchange provided. A 1,500 l circular flat-bottomed tank was filled with 5 µm-filtered raw seawater, to which a quantity of dense *N. oculata* culture was added to provide a density of 2×10^5 cells/ml. Gentle aeration was provided in 4 areas in the tank via airstones. Two immersion heaters maintained water temperatures in the desired range. Fluorescent lighting ('cool white' tubes) provided 250 lux with a 12 h photoperiod. No water exchange or tank cleaning was provided. A surface blower and floating trap were employed intermittently to remove any oily film that formed on the water surface. Temperature was measured twice daily, other parameters were measured daily. Water conditions throughout the trial were as follows:

Parameter	Range
algal density	5×10^3 - 2×10^5 cells/ml
temperature	27.0 - 28.5°C
salinity	35 - 36 ppt
pH	8.1 - 8.5
total ammonia	< 0.5 mg/l
nitrite	< 0.1 mg/l
nitrate	< 2.5 mg/l
light (intens./photop.)	250 lux/12 h

Yolksac larvae from a single hormone-induced spawning were stocked immediately after algae were added to the tank, at a density of 15 larvae/l. Unscreened L-strain rotifers (raised exclusively on *N. oculata*) were added simultaneously at the rate of 1 individual/ml. On day 2, oyster trochophores were added at the rate of 7/ml, and screened wild-caught zooplankton added at 2/ml. The plankton net comprised an initial 100 µm screen to exclude larger zooplankton, followed by a 25 µm screen on which small zooplankton were retained. Wild zooplankton consisted of approximately 75% copepods (nauplii and copepodites), along with smaller numbers of crustacean and bivalve larvae, protozoa, and other unidentified organisms. Further additions of oyster trochophores and wild zooplankton were added daily to maintain live food densities at approximately 10 organisms/ml.

Live food densities were measured twice daily. Quantities of dense algal culture were added when necessary to maintain algal densities above 5×10^3 cells/ml. Larvae were viewed microscopically daily to assess feeding success, health and state of development. Survival was estimated through observed larval densities.

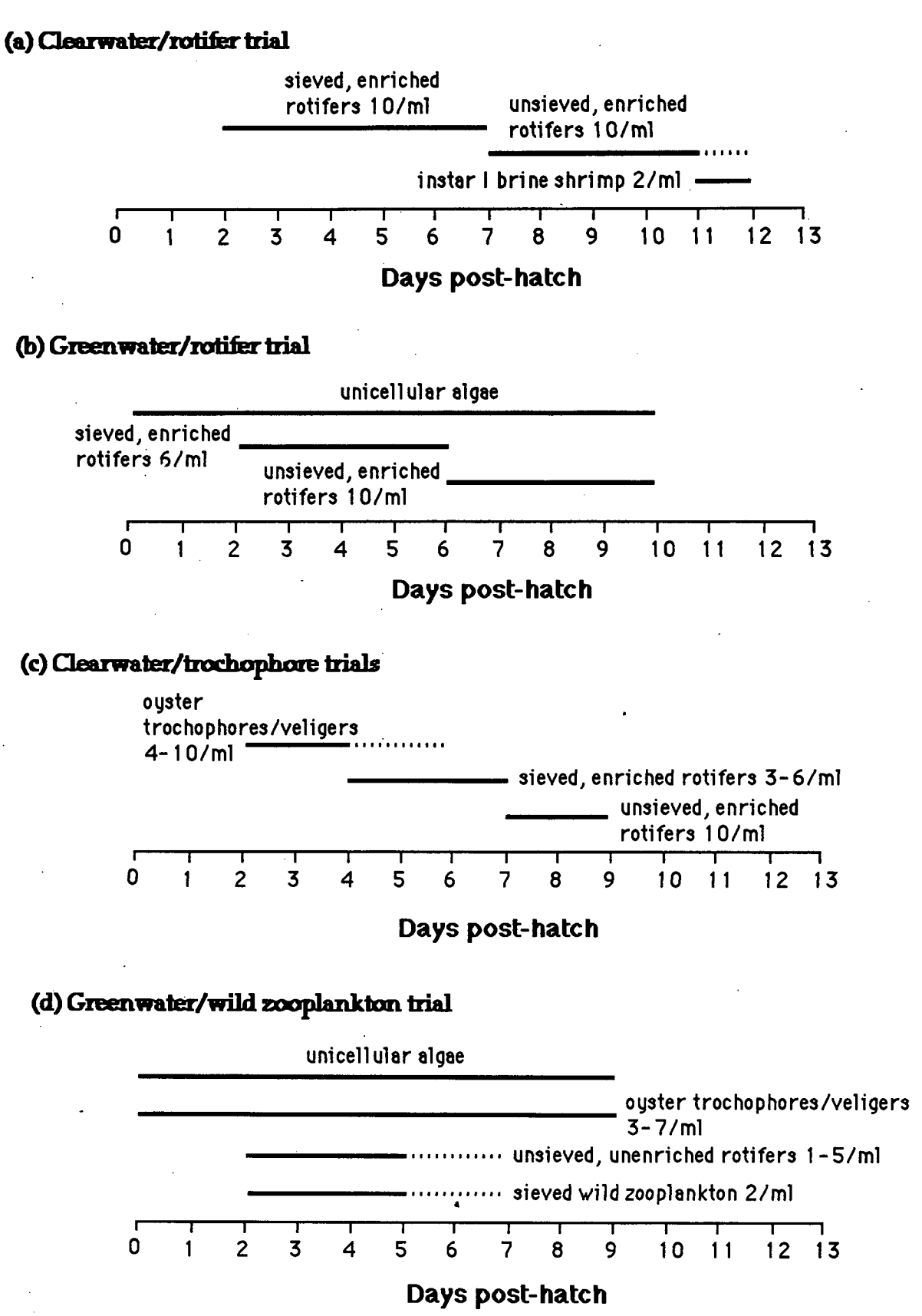


FIG. 2 - Feeding schedules used in mangrove jack larval rearing trials. All rotifers are L-strain; sieved rotifers have been passed through a 125 μm sieve.

Nutritional studies

In order to gain some understanding of the nutritional requirements of mangrove jack larvae, and to investigate possible causes for the large losses observed in larval rearing trials, two nutritional analyses were performed.

(i) fatty acid analysis of eggs and larvae

The fatty acid profiles of eggs just prior to hatch, yolksac larvae at half yolk absorption, and yolk-absorbed larvae at 65% oil globule absorption were determined for a single spawning, without any food being offered. These analyses provided information on changes in the fatty acid composition during the prefeeding yolksac stage, indicating fatty acid usage. Procedures followed those previously outlined on p. 107.

(ii) amino acid analysis of larvae

The protein bound and free amino acid profiles of mangrove jack larvae at first-feeding were determined to gain insight into the amino acid requirements of mangrove jack larvae. Comparison with previously established values for rotifers enabled an evaluation of rotifers in meeting these requirements. Procedures followed those outlined on pp. 104-107, and chemical score indices were calculated in like fashion.

Results and Discussion

Larval development

On day 2, mangrove jack larvae were apparently first capable of feeding, having fully pigmented eyes and an open mouth and anus. This was 42 h post-hatch at 29°C. In none of the trials, however, was food seen in the gut of larvae until day 3. The first inflated swim bladders were seen in the first trial (clearwater/rotifers) late on day 2, and increased in number rapidly through day 3, to stabilise at approximately 70% by the end of day 4. This rate is good but could possibly be improved further through more effective use of water surface skimmers. This is important if the consequences of swim bladder inflation failure, such as reduced growth, skeletal deformities and poor stress tolerance (Chatain, 1989; Chatain and Ounais-Guschemann, 1990) are to be minimised.

Growth (Fig. 3) over the 12 days of the most successful trial was similar to that reported by Doi and Singhagraiwan (1993) for this species in Thailand and by Emata *et al.* (1994) in the Philippines, over this period. A typical (of marine fish larvae) growth lag was observed over the first few days, but positive growth was recorded thereafter (Fig. 3). This appears to be associated with the transition from endogenous to exogenous nutrition, and not necessarily tied to the nutritional quality of rotifers as first food, as it is widely observed among early larvae regardless of the food type being offered (eg. Foscarini, 1988; Eda *et al.*, 1990; Battaglene and Talbot, 1992; Polo *et al.*, 1992; Doi and Singhagraiwan, 1993).

This preliminary growth data, and developmental data, parallels that reported for *L. kasmira*, *L. campechanus* and *L. johni* (Suzuki and Hioki, 1979; Rabalais *et al.*, 1980; Lim *et al.*, 1985). According to the method of Shirota (1970; see methods), the maximum mouth height of first-feeding mangrove

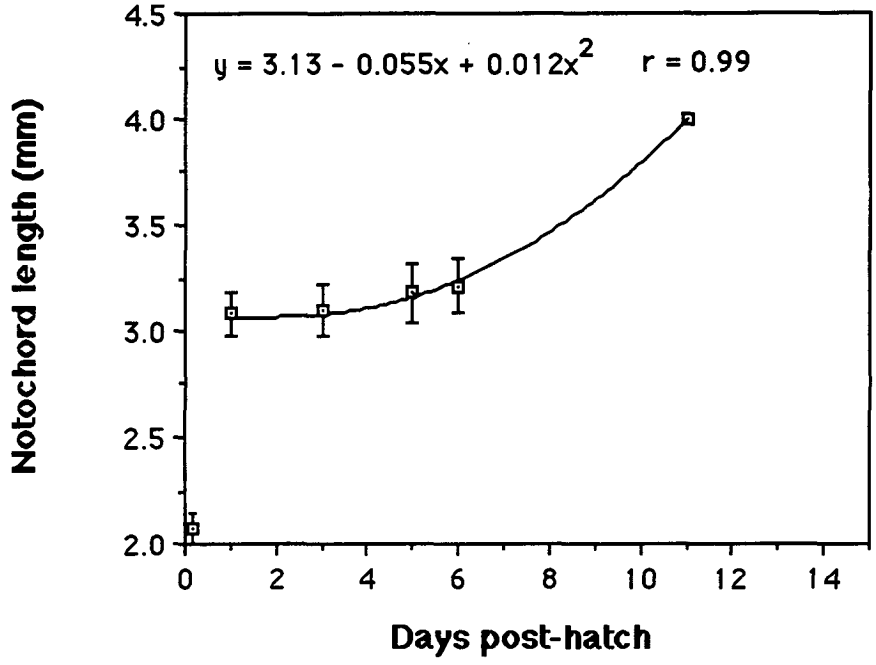


FIG. 3 - Growth curve for mangrove jack larvae from days 1 to 11 in the most successful rearing trial (clearwater/rotifers). A quadratic curve has been fitted, and its equation and 'r' value included. Each data point represents the mean (± s.d.) of a sample of 5-10 larvae, except the day 11 sample where only 1 larva, considered to be representative, was measured due to very low survival. The hatch size has also been included.

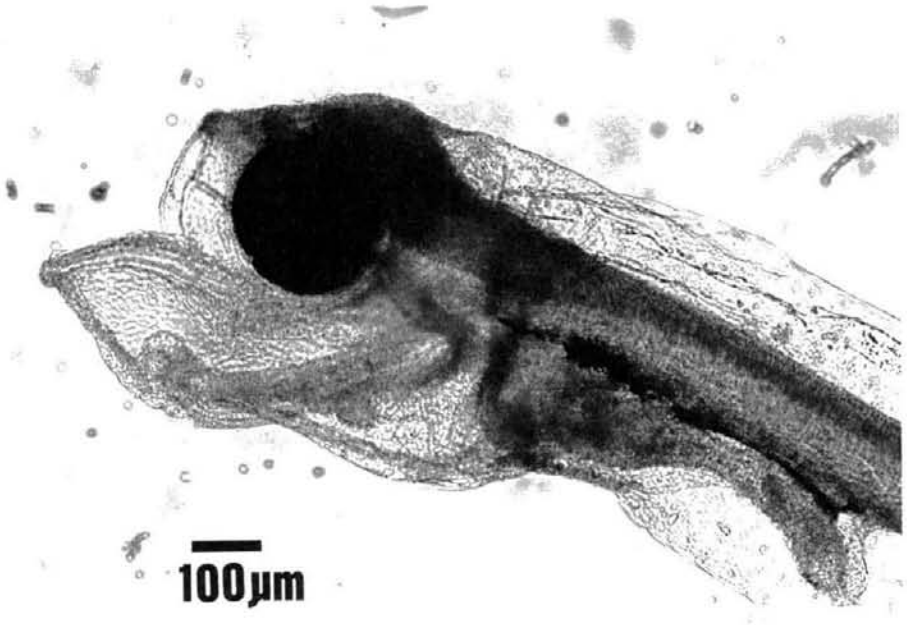


PLATE 1 - First-feeding mangrove jack larva showing mouth structure and apparent maximal mouth gape.

jack larvae is approximately 370 µm. However, the applicability of this method to mangrove jack larvae was uncertain due to the curved shape of the upper and lower jaws (Plate 1). Plate 1 depicts a larva preserved with the mouth at an apparent maximal gape. On this specimen mouth height is approximately 300 µm. The horizontal width of the mouth opening, when measured directly, was also approximately 300 µm. This mouth size is quite typical for a larva this size (Shirota, 1970). However, the optimal prey size is only approximately 25% of maximum mouth width at first feeding (De Ciechomsky, 1967; Hunter, 1984; Fernandez-Diaz *et al.*, 1994). Thus, for mangrove jack larvae, the optimal prey width at first feeding is in the order of 75 µm . This is consistent with observations of mangrove jack larvae with oyster 'D-veligers' of approximately 80 µm width in the gut at first feeding in the present study.

Rearing trials

In none of the rearing trials did larvae survive beyond 12 days post-hatch. Fig. 4 shows the consistent pattern of mortality in each trial. This pattern is characterised by a sudden and heavy mortality between days 3 and 6. Very low numbers (typically less than 1%) persist from day 6 until total mortality is observed. Variations between trials, while in part an artefact of different hatch times of day, probably also reflect egg quality differences between spawnings from different females and at different times within the spawning season. The mortality peak corresponds with that observed amongst starved larvae, and indicates that most larvae are not surviving the transition to exogenous energy sources. A first-food related problem is implicated as most dead larvae appeared to have empty guts. This may however be a secondary consequence of other problems, such as poor egg quality and suboptimal physical rearing conditions, as discussed below.

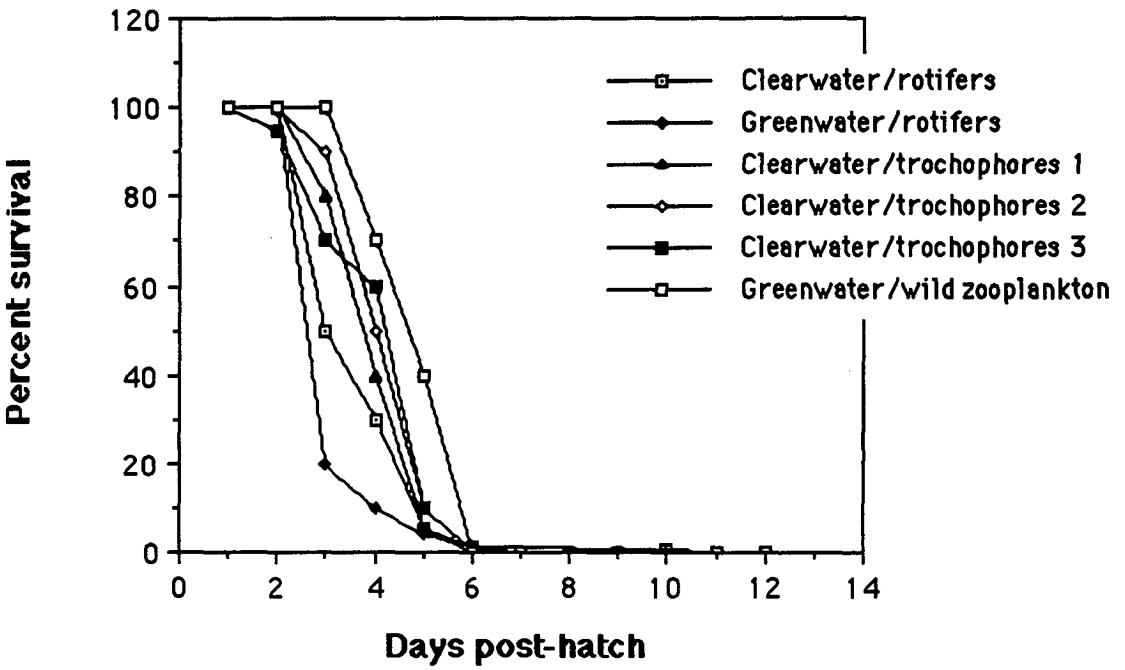


FIG. 4 - Mortality curves for the six mangrove jack larval rearing trials. Complete mortality was observed in all trials between days 6 and 12 post-hatch.

Significant mortality at first-feeding seems almost universal amongst marine fish larvae (Blaxter, 1969, 1988; Tucker, 1992a). However, that observed for mangrove jack larvae is particularly severe, and similar to that observed in grouper culture (Chen *et al.*, 1977; Hussain and Higuchi, 1980; Tookwinas 1989; Lim, 1993). Similar patterns of mortality with mangrove jack have been experienced by staff at the D.P.I. Northern Fisheries Research Centre, Cairns, Australia (R. Garrett, pers. comm., 1994), and by researchers in Thailand (Doi and Singhagraiwan, 1993) and in the Philippines (Emata *et al.*, 1994). However, using a 'semi-intensive' pond rearing method, Thai researchers have been able to quite consistently raise a small percentage of larvae to metamorphosis (typically < 1%). Whether or not this is due to specific rearing practices employed, or merely a reflection of the very large numbers of fertilised eggs used in each trial (tens of millions), is unknown. Survival rates, however, remain low and variable regardless, and clearly more information is required on the specific requirements of mangrove jack larvae before reliable juvenile production will be possible anywhere.

Difficulties in the rearing of mangrove jack larvae are not unique within the family. Rabalais *et al.* (1980) reported a total mortality of red snapper, *L. campechanus*, larvae by day 10 when offered rotifers as first food, and Suzuki and Hioki (1979) reported a similar result when attempting to rear larvae of *L. kasmira* with oyster trochophores. However, larvae of *L. griseus* were successfully reared to metamorphosis when given wild-caught zooplankton graded to 35-73 µm in size as first food, although survival information was not included (Richards and Saksena, 1980). Furthermore, poor survival (mean 1%, with frequent total mortality) has been observed in larval rearing trials of *L. johni* in Singapore (Lim *et al.*, 1985). Again, large losses are experienced between days 4-7 when larvae are fed mussel larvae and rotifers

as first food. Similar mortality patterns have been observed for this species in Australia by staff at the Northern Territory Department of Primary Industries and Fisheries, Darwin (G. Schipp, pers. comm., 1994).

Possible explanations for rearing difficulties

The following discussion explores possible reasons for the poor survival consistently observed for mangrove jack (and other lutjanid) larvae under the headings of physical and nutritional requirements of initial prey, egg quality and the physical rearing environment.

(i) initial prey organisms - physical aspects

If a food problem is involved, it could occur anywhere in the chain of events from perception and capture of food, to digestion and assimilation of nutrients. First, prey items must be visible, available, of correct size, and able to elicit a feeding response and be caught (Leger *et al.*, 1987; Sorgeloos and Leger, 1992; Tucker, 1992a). Lack of appropriate food organisms when the larva has consumed its yolk reserves will result in tissue autolysis and eventual death (Lasker *et al.*, 1970; Theilacker, 1981; Bagarinao, 1986). Chesney (1995) considers the low 'energy gathering potential' of small tropical marine fish larvae, coupled with the physiological disadvantage of development at high temperature, to greatly increase the influence of food limitation on such larvae.

Young (1994) has discussed the apparent obligate requirement of some marine fish larvae for copepod nauplii as first food. Young postulates that the slow, lazy circular swimming action of rotifers does not elicit a feeding response from these larvae, which strongly prefer the jerky motion characteristic of copepod nauplii. Mangrove jack may fall into this category. Indeed, in large rearing tanks in Thailand (190 m^3), approximately 90% of

larval gut contents up to day 15 comprised copepod nauplii despite rotifers being present (Singhagraiwan and Doi, 1993). Similar findings were reported by Lim (1993) for greasy grouper (*Epinephelus tauvina*) larvae, where larvae commonly had guts full of copepod nauplii despite them being at a concentration 5-10 times lower than that of rotifers. Young (1994) goes further to give examples of larval marine species which will not consume rotifers or copepod nauplii. These larvae are thought to require smaller organisms, such as motile ciliates and other protozoa, motile algae and larvae of coral reef fauna, as first food (Young, 1994). Similarly, this remains a possibility with mangrove jack larvae, for which low survival rates are still the norm even with use of copepod nauplii (Doi and Singhagraiwan, 1993).

Initial prey organisms used in the present study included sieved L-type ('large' type; Fu *et al.*, 1991) rotifers, oyster trochophores, and wild zooplankton of which approximately 75% were early planktonic copepod stages. Sieved rotifers ranged in width from 85-180 µm immediately after sieving, trochophores 40-60 µm, and copepod nauplii 90 µm and over. According to the optimal prey size given for first-feeding mangrove jack larvae above, approximately 75 µm, all but the smallest rotifers and copepods are too large to be ideal as first foods. Assuming only the smallest 10% of the rotifer or copepod (i.e. nauplii) population to be of suitable size, a density of 10 rotifers/ml or 1 copepod/ml, as used in these trials, would present an effective, or available, density of only 1 rotifer/ml or 1 copepod nauplius/10 ml. This density is likely to be inadequate for high first-feeding success. Furthermore, while sieving may effectively separate small neonates from adult rotifers, within a short period in the culture tank neonates will have grown to full adult size and become unavailable to the larvae (Lubzens *et al.*, 1989). The culture of genetically smaller strains of rotifers (S or SS-types)

therefore offers a better solution than sieving. An S-strain rotifer will soon be available in Australia (S. Battaglene, pers. comm., 1994).

Work on *L. johni* in Singapore has shown that larvae of this species will not consume rotifers (strain unspecified) until day 6 (Lim *et al.*, 1985). As these larvae are apparently very similar to mangrove jack, similar food selectivity is likely to exist. However, Emata *et al.* (1994) were able to rear low numbers of mangrove jack larvae to day 28 using rotifers (strain unspecified) followed by brine shrimp. Similarly, Pechmanee and Chungyampin (1988) observed first-feeding mangrove jack larvae to accept screened (100 µm mesh) S-type rotifers when provided alone, although only in small numbers. Singhagraiwan and Doi (1993) were unable to reproduce these results, and using S-type rotifers usually observed complete mortality by day 6, and explained this by excessive prey size. Similarly, Lim (1993) blamed excessive size of S-type rotifers for poor survival of greasy grouper (*E. tauvina*) larvae, and has recommended the use of SS-type ('super small' type) rotifers for this species. Best results were obtained from feeding SS-type rotifers from days 3-7 followed by S-type from days 8-24.

Rotifers have not been used to any great extent in Thailand as first food for mangrove jack larvae due to difficulties in obtaining large numbers of very small screened rotifers, and their subsequent enlargement in larval rearing tanks, as mentioned above. Instead, the first two naupliar stages of the copepod species *Acartia* sp. and *Oithona* sp. are used, and are now considered to be essential for the first two days of feeding, after which rotifers and other copepod species are given (Doi and Singhagraiwan, 1993; G. Schipp, pers. comm., 1994). A correlation was observed between larval survival and numbers of copepod nauplii in the rearing tank. Singhagraiwan and Doi (1993) mention densities of copepod nauplii and copepodites, together, of over 10 individuals/ml in rearing water.

Acartia (a calanoid) and *Oithona* (a cyclopoid) are planktonic copepods of approximately 100 μm at the naupliar stage. It is unclear whether the size, swimming movement or nutritional properties of these species are responsible for their value as a first food for mangrove jack larvae. According to larval mouth size, however, these copepods tend to be larger than optimal. Trials need to be carried out in Australia using a high density (e.g. 10 individuals/ml) of early copepod nauplii, or super-small (SS) strain rotifers when they become available.

Oyster trochophores and the following veliger stage, however, are certainly within the correct size-range. Feeding on oyster trochophores and the veliger stage was confirmed in this study, the veligers being easily discerned due to the refractive nature of the shell. As many as 4 veligers could be seen within the guts of larvae on day 4, and up to 7 on day 5. Despite the apparently suitable size of oyster trochophores and veligers, only approximately 50% of larvae accepted them, and the overall survival of larvae was not improved (Fig. 4). It would seem, assuming adequate larval vigour, that oyster larvae did not elicit a strong feeding response. Researchers in Thailand had similar results with oyster eggs and larvae, with all mangrove jack larvae dying by the fifth day post-hatch (Doi and Singhagraiwan, 1993). Work on *L. johni* in Singapore, however, has shown that larvae of this species will consume and survive on mussel trochophores as first food (Lim *et al.*, 1985).

In summary, the size of oyster larvae is suitable as first-food for mangrove jack larvae, although the movement may not elicit a strong feeding response, and digestibility is unknown. Copepod nauplii and enriched small-strain rotifers are becoming larger than optimal, although the movement of copepods, at least, may be more attractive to larvae. Further to this, research

into the natural diet of mangrove jack larvae is required to determine if any specific prey requirements exist for this species.

(ii) initial prey organisms - nutritional aspects

Nutritionally, live foods must be digestible and meet energetic and specific nutrient requirements of the culture organism (Sorgeloos and Leger, 1992). Most recent research into nutritional requirements of marine fish larvae has focused on n-3 HUFAs (highly unsaturated fatty acids), whereas other nutrients such as other lipid classes, particular peptides, free amino acids, pigments, vitamins and even hormone content may prove equally critical in some species (Fyhn, 1989; Sorgeloos and Leger, 1992; Lam, 1995). Indeed, the superior nutritional value of copepods over rotifers and *Artemia* cannot be wholly accounted for by essential amino acid or n-3 HUFA content (Kraul *et al.*, 1993). The mortality pattern observed in the present study, being dramatic over days 3-6, and the emaciated condition of larvae, is suggestive of starvation as the cause (energetic requirements not met). However, the continued slow mortality of larvae which had successfully made the transition to external foods is also of concern and may reflect nutritional inadequacies. Amino and fatty acid nutrition is considered to be particularly crucial for marine fish larvae (Watanabe *et al.*, 1983; Tucker, 1992a).

Amino acid analysis of larvae

Results of amino acid analyses indicate that rotifers appear to be a high value protein source for mangrove jack larvae (Table 1), assuming the larvae analysed are representative of the species. The essential amino acid profiles of rotifers and mangrove jack whole-body protein are similar (Table 1). Furthermore, these amino acids are likely to be largely available as it has been shown that rotifer protein is highly digestible, at least for red seabream *P. auratus* larvae (Watanabe *et al.*, 1983). The chemical score indices (I) indicate

that six essential amino acids occur in slightly lower proportions in rotifers than whole-body larval protein, but only three of these (tryptophan, histidine and methionine) have 'I' values less than 95%. Tryptophan is the first limiting amino acid, with a still high 'I' value 89.3% (Table 1). Tryptophan and methionine are known to be adversely affected by acid hydrolysis (Gehrke and Takeda, 1973; Varadi and Pongor, 1979), so results are likely to underestimate their true content (in larval proteins also), and they may, in fact, not be limiting. Actual 'I' values may differ slightly from those presented here as the rotifer analysis was derived from earlier work with yellowfin bream (Chap. 4, Table 1), and the biochemical composition of algae is known to vary under different culture conditions (eg. Renaud *et al.*, 1991). The comparison is however considered valid as algae and rotifers were raised under the same culture conditions (including temperature) in both trials, apart from natural photoperiod changes.

Most other reported analyses of rotifers have similarly shown them to be adequate in essential amino acids (e.g. Watanabe *et al.*, 1983). *Artemia* nauplii and metanauplii are also considered to be a high quality protein source for marine fish larvae, although methionine may be limiting to some extent (Watanabe *et al.*, 1983; Leger *et al.*, 1986, 1987).

Fatty acid analysis of eggs and larvae

DHA (22:6n3) occurs in very high levels in mangrove jack eggs and yolksac larvae, accounting for nearly one-third of total fatty acids (Table 2), again assuming the eggs and larvae analysed are representative of the species. DHA was quantitatively the major HUFA, while 16:0 was the major saturated fatty acid present in both eggs and early larvae. Oleic acid (18:1n9) was a third major constituent fatty acid. The dominance of these three fatty acids is common to the fatty acid profiles of other marine and freshwater

Amino Acid	FREE mmol/g homogenate		BOUND mmol/g homogenate		TOTAL % total (free + bound)		
	Larvae	Rotifers	Larvae	Rotifers	Larvae	Rotifers	'I' Value
ASP	0.80 ± 0.09	1.34 ± 0.07	14.47 ± 0.45	13.83 ± 0.97	4.99	4.94	
GLU	1.95 ± 0.12	2.44 ± 0.08	15.87 ± 0.27	14.07 ± 0.62	5.82	5.37	
ASN	0.01 ± 0.01	1.44 ± 0.03	U	U	0.01	0.47	
SER	1.49 ± 0.03	3.86 ± 0.03	16.62 ± 0.08	14.91 ± 0.17	5.91	6.11	
GLN	0.34 ± 0.02	1.89 ± 0.04	U	U	0.11	0.62	
GLY	3.23 ± 0.08	5.83 ± 0.29	19.13 ± 0.13	16.28 ± 1.11	7.30	7.20	
ALA	3.55 ± 0.17	1.50 ± 0.03	U	U	1.52	0.49	
TYR	2.53 ± 0.21	4.96 ± 0.08	17.09 ± 0.41	14.79 ± 0.31	6.41	6.42	
HIS*	1.43 ± 0.04	2.21 ± 0.01	17.54 ± 0.14	15.43 ± 0.10	6.19	5.74	92.7 %
THR*	1.16 ± 0.01	3.64 ± 0.02	16.37 ± 0.02	14.75 ± 0.13	5.73	5.98	104.5 %
ARG*	1.41 ± 0.14	6.91 ± 0.12	18.24 ± 0.53	15.78 ± 0.38	6.42	7.38	115.1 %
TRP*	3.29 ± 0.03	3.03 ± 0.01	17.19 ± 0.05	15.32 ± 0.01	6.69	5.97	89.3 %
MET*	4.24 ± 0.20	6.68 ± 0.01	43.10 ± 0.59	38.10 ± 0.07	15.46	14.57	94.3 %
VAL*	1.08 ± 0.05	2.55 ± 0.06	17.10 ± 0.24	15.47 ± 0.52	5.93	5.86	98.9 %
PHE*	1.89 ± 0.01	3.28 ± 0.06	16.94 ± 0.03	15.02 ± 0.38	6.15	5.96	96.8 %
ILE*	0.97 ± 0.08	2.45 ± 0.02	17.26 ± 0.43	15.44 ± 0.21	5.95	5.82	97.8 %
LEU*	0.50 ± 0.03	3.24 ± 0.04	17.38 ± 0.26	15.37 ± 0.28	5.84	6.06	103.7%
LYS*	2.93 ± 0.08	7.60 ± 0.23	9.29 ± 0.08	7.82 ± 0.33	3.99	5.02	125.8 %

TABLE 1 - Free, bound and total amino acid profiles of first-feeding mangrove jack larvae and rotifers, *B. plicatilis*, reared on the microalga *N. oculata*. The chemical score indices ('I', refer to text) for total essential amino acids have been included to provide some indication of the value of rotifers as a protein source. Values represent means (± s.d.) of two replicate samples. Asterisks represent essential amino acids. 'U' represents undetected.

Fatty Acid	Pre-hatch Eggs (day 0)	50% Y.A 0% O.A. (day 1)	100% Y.A. 65% O.A. (day 2)	Rotifers Fed *N. oculata*
16:0	20.08 ± 0.04	20.85 ± 0.89	21.56 ± 2.23	28.1
16:1n7	2.47 ± 0.24	2.77 ± 0.18	2.58	29.3
18:0	9.78 ± 0.49	9.30 ± 0.06	10.20 ± 0.41	4.4
18:1n7	-	-	-	4.1
18:1n9	16.91 ± 0.51	17.13 ± 0.35	12.98 ± 1.81	17.1
18:1n6	3.47 ± 0.35	3.34 ± 0.10	2.53 ± 0.01	-
18:2n6	3.22 ± 0.27	3.77 ± 0.05	2.69 ± 0.32	2.1
20:1n9	-	-	-	2.6
20:4n3	-	-	-	4.1
20:4n6	1.91 ± 0.18	2.37 ± 0.43	2.92 ± 0.42	-
20:5n3 (EPA)	5.33 ± 1.02	3.70 ± 0.56	4.21 ± 0.48	3.0
22:5n3	2.57 ± 0.15	2.95 ± 0.42	2.34	2.2
22:6n3 (DHA)	29.67 ± 0.12	29.54 ± 0.87	31.88 ± 1.40	-
Σn-3 HUFA	37.57	36.19	38.43	9.3

TABLE 2 - Fatty acid composition (% total fatty acids) of mangrove jack eggs, and larvae at two stages of development. Also included is the profile of rotifers, *B. plicatilis*, reared on the unicellular alga *N. oculata*. Values represent the mean of two replicates, except rotifer results due to an analytical error. Dashes represent values less than 1%. Y.A. represents yolk absorption; O.A. represents oil globule absorption.

species such as the dolphin fish, *C. hippurus* (Ostrowski and Divakaran, 1989, 1991; Ako *et al.*, 1991), gilthead seabream, *S. auratus* (Mourente and Odriozola, 1990b; Rodriguez *et al.*, 1994), rainbow trout, *O. mykis* (Watanabe *et al.*, 1978), red seabream, *P. auratus* (Watanabe *et al.*, 1984a,d), Atlantic cod, *Gadus morhua* (Ulvund and Grahl-Nielsen, 1988), and Dover sole, *Solea solea* (Dendrinos and Thorpe, 1987).

It is widely believed that n-3 HUFAs play a critical role in the phospholipid fraction of biomembranes, and are essential dietary components for many marine fish as they cannot be synthesised from shorter-chain fatty acids (Owen *et al.*, 1975; Kanazawa *et al.*, 1979, 1982, 1985; Yamada *et al.*, 1980; Watanabe, 1982). Recent studies have suggested that the roles of the two major HUFAs, DHA and EPA (20:5n3), are distinct (Kraul *et al.*, 1993; Mourente *et al.*, 1993; Watanabe, 1993), and the requirements for DHA and EPA are species-specific, some species requiring both and others only one or the other, or specific ratios of the two (Lubzens *et al.*, 1989; Sargent, 1991; Mourente *et al.*, 1993; Watanabe, 1993).

Heming and Buddington (1988) suggested that changes in the biochemical composition of eggs or yolksac larvae during development may indicate nutritive requirements during early feeding stages. The fatty acid profile of eggs and early larval stages in the present study did not change dramatically over time (Table 2). The major change was a drop in oleic acid (18:1n9), which is indicative of this fatty acid being catabolised as an important energy source for these larvae. Ako *et al.* (1991) and Ostrowski and Divakaran (1991) reported similar findings for dolphin fish larvae, and Mourente and Odriozola (1990a) for gilthead seabream larvae over this period. Rodriguez *et al.* (1994) similarly reported a reduction in oleic acid, but also a significant fall in 16:0 when studying gilthead seabream larvae, while Vazquez *et al.* (1994) recorded reductions in oleic, 16:0, 16:1n7 and 18:1n7 fatty

acids during yolk absorption in the Senegal sole, *Solea senegalensis*, and Rainuzzo *et al.* (1994) found that turbot larvae, *Scophthalmus maximus*, did not demonstrate a preferential utilisation of any particular fatty acids. It appears that fatty acid utilisation varies considerably between species. The fact that the polyunsaturated fatty acids 20:5n3, 22:6n3 and 20:4n6 (arachidonic acid) were conserved in the present study is consistent with the suggestion that these fatty acids play an important role in the early develoment of marine fish larvae (Rainuzzo *et al*, 1994; Vazquez *et al.*, 1994).

Sharp falls in DHA were recorded soon after this period (between days 2 and 20) in four larval species by Watanabe (1993). This is thought to be associated with the rapid development of the neural system (Bazan, 1990), and indicates the timing of DHA usage. Unfortunately, sampling in the present study did not span the time when this would be expected to occur in mangrove jack larvae.

As stated previously, the fatty acid profile itself may allude to the fatty acid requirements of mangrove jack larvae, and the best dietary fats should mimic the fatty acid profile of the egg or larva of the species concerned (Falk-Petersen *et al.*, 1986). The fatty acid profile presented here would suggest DHA to be a most important n-3 HUFA to mangrove jack, as it is quantitatively the major fatty acid present (Table 2). Furthermore, the absence of the DHA precursor, 18:3n3, in larval mangrove jack (Table 2) is consistent with the likely inability of these larvae, like most other marine larvae, to elongate and desaturate shorter-chain fatty acids (Yamada *et al.*, 1980; Watanabe, 1987).

Table 2 also shows the fatty acid profile of unenriched rotifers to deviate most dramatically from that of mangrove jack larvae in DHA content, which only occurred in trace amounts in unenriched rotifers, but at 31.9% in first-

feeding mangrove jack larvae. This results from deficiencies in DHA in the alga *Nannocloropsis oculata* (Eustigmatophyceae), which rotifers were raised on (Mourente *et al.*, 1990; Dunstan *et al.*, 1993). Analytical results for rotifers presented here are derived from earlier work with yellowfin bream (Chap. 4, Table 1), but the comparison is considered valid as culture conditions other than natural photoperiod changes, were the same. Other analyses of rotifers raised on this alga (e.g. Watanabe *et al.*, 1983; Dendrinos and Thorpe, 1987; Rimmer and Reed, 1990; Mourante *et al.*, 1993) have shown a similar fatty acid profile, showing low DHA content. This indicates the need to heavily enrich rotifers with DHA-rich oils before using as an early food for mangrove jack larvae. This finding is relevant to the final greenwater/wild zooplankton trial in the present study, where rotifers were not enriched prior to their introduction to the tank. Even in the present trials where rotifers were enriched, their nutritional value declines in the larval rearing tank over time without continued enrichment (e.g. Lubzens *et al.*, 1989; Rainuzzo *et al.*, 1989). *Artemia* nauplii, particularly Great Salt Lake strain, tend to be deficient in DHA, but contain a variable amount of EPA, depending on strain (Leger *et al.*, 1986, 1987).

Although copepods are considered to be nutritionally complete for marine fish larvae (Tucker, 1992a), oyster trochophores are considered to be similar nutritionally to rotifers (Kuronuma and Fukusho, 1984). Lim *et al.* (1985) reported 80% mortality between days 4-7 to be common in the larval rearing of *L. johni*, and attributed this to poor nutritional value of mussel trochophores when given alone, as 60-70% of larvae accepted the trochophores but still appeared emaciated.

Researchers from Thailand and the Philippines have observed a second mortality peak amongst mangrove jack larvae at about day 18-20, corresponding with a period when larvae become particularly sensitive to

stress of any kind (Doi and Singhagraiwan, 1993; Emata *et al.*, 1994). This is very likely the result of an n-3 HUFA deficiency, which often manifests itself at this time and in this manner, when maternally-derived HUFA has been exhausted (Dhert *et al.*, 1992). At this time any dietary HUFA inadequacy will become apparent. This often results because the second live food given, brine shrimp, is naturally deficient in DHA (Webster and Lovell, 1990; Stottrup and Attramadal, 1992). This syndrome again implies a need for high levels of HUFA in the diet of mangrove jack larvae, particularly DHA. Tucker (1992a) recommends a dietary n-3 HUFA content of 2-4% dry food weight, including at least 1% EPA and 1% DHA, for marine fish larvae whose specific requirements have not been tested.

In conclusion, a high dietary n-3 HUFA requirement, particularly of DHA, is indicated from fatty acid analysis of eggs and yolksac larvae of mangrove jack. However, while the n-3 HUFA content of the diets offered in the present rearing trials were likely to be below requirements, the heavy mortality observed in the very early larval stages is unlikely to be due to n-3 HUFA deficiency as high levels of maternally-derived n-3 HUFA were detected in the eggs. This should have at least been sufficient to sustain the larvae through the first one or two weeks of life as essential fatty acids can be strongly conserved for several weeks when in shortage in the diet (Koven *et al.*, 1989; Dhert *et al.*, 1991). Kraul *et al.* (1989, 1992) have similarly concluded for dolphin fish that early survival is not related to fatty acid nutrition. Rather, other aspects of egg quality, and broodstock nutrition and stress, appear to be.

(iii) egg and larval quality

All larvae used in the rearing trials described were obtained through hormone-induced spawning of captive broodfish (Chapter 6). Egg quality is widely regarded as being inferior from hormone-induced spawnings than from natural spawnings, due to stress imparted on the fish during the hormone administration procedure, the presumably unnatural action of the hormone, and, if hand-stripping is carried out, due to the difficulty in estimating correct timing to avoid the post-ovulatory decline in egg viability. Moreover, Ako *et al.* (1994) found significantly lesser amounts of most fatty acids (including EPA and arachidonic acid) and essential amino acids in hormonally-induced than naturally-spawned eggs from the milkfish, *Chanos chanos*. Researchers in Japan have noted superior egg quality from completely spontaneous spawnings of red seabream than from spontaneous hormone-induced spawnings or strip-spawnings (Foscàrini, 1988). Lim *et al.* (1985) attributed the frequent total mortality of larval *L. johni* between days 4-7 to poor egg quality arising from difficulty in correctly timing strip-spawnings. Survival through this period has been improved with the ability to achieve spontaneous spawnings.

Billard *et al.* (1981) reviewed the effects of stress on teleost reproduction, and cited numerous examples of stress having adverse effects on egg quality. The hormone-induction process in the present study involved the capture and anaesthetisation of each female fish three separate times - administration of primary and resolving hormone doses, and strip-spawning. This handling and concurrent stress are likely to have had deleterious impacts on egg quality. Furthermore, broodfish in the present project were held at high density (15-20 kg/m^3) and in small netcages (5 m^3). Frequent fighting occurred, sometimes resulting in the death of a fish. Kraul *et al.* (1992)

implicated stress caused by broodstock fighting to reduce egg viability in dolphin fish.

In addition, the diet of captive broodfish is likely to be inferior to that obtained in the wild where a wider variety of food types is available (Rainuzzo, 1993). The link between broodstock diet up to spawning and egg composition and quality has been dramatically demonstrated in many species of fish (e.g. Craik and Harvey, 1984b; Watanabe *et al.*, 1984a-d; Devauchelle *et al.*, 1988; Ming-Yih and Wu, 1990; Mourente and Odriozola, 1990b; Harel *et al.*, 1992), and it is known that the embryos of oviparous vertebrates are totally dependent on the nutrients stored in the yolk for successful early development (Mourente and Odriozola, 1990a). For example, concentrations of protein, vitamins A and E, and in particular fatty acid content and composition of eggs have all been shown to depend on levels in the broodstock diet of red seabream, *P. auratus* (Watanabe *et al.*, 1984a-d, 1985). Vitamin E, phospholipids, and astaxanthin (a carotenoid) were found to be particularly critical elements related to egg quality.

The natural diet of mangrove jack is likely to contain high levels of carotenoids derived from crabs, shrimps and various other crustaceans which form a major part of the natural diet of most lutjanids (Allen, 1985; Salini *et al.*, 1994). Tacon (1981) and Meyers and Chen (1983) have suggested that carotenoids impart (on broodfish) benefits such as disease-resistance, improved gonadal development, egg viability, larval survival and growth. Carotenoids and other pigments are certain to have been well below natural dietary levels in the present study as only fish and no crustaceans were used in the diet.

As the rapidly developing eggs and larvae of warmwater marine fish species, such as mangrove jack, appear to use lipid rather than protein as an

energy source (Vetter *et al.*, 1983; Ostrowski and Divakaran, 1991; Southgate *et al.*, 1994; Vazquez *et al.*, 1994), larval survival is likely to depend heavily on the lipid content of eggs, and so in turn heavily on broodstock dietary lipid intake and composition. Sufficient reserves of yolk and oil must be available to sustain larvae through the transition from endogenous to exogenous nutrition. In several species, egg quality can be correlated with egg total lipid or essential fatty acid content (Harel *et al.*, 1992; Rainuzzo, 1993), and this must be investigated for mangrove jack.

Poor egg quality was evident in several spawnings in the present study in which the percentage of eggs containing divided oil globules was up to 80%. This was reduced to less than 10% after 4 weeks of intensive feeding with frozen pilchards containing a vitamin/mineral supplement. The occurrence of multiple oil globules has been closely linked to poor egg quality resulting from protein, phosphorus, and EFA (essential fatty acid)-deficient test diets in red seabream (Watanabe, 1989). Clearly, investigations into the importance of carotenoids, free amino acids, phosphorus, peptides, other lipid classes and vitamins, and even hormone levels, in addition to EFAs, to mangrove jack larvae should be given high priority in future research.

In summary, the effects of hormone-induction, stress from captive conditions, and compromised diet, may singly or collectively have had significant impacts on egg quality and larval vigour in the present study. While hatch rate is often used as a gauge of egg quality, the hatch rates in the present study were generally good (> 80%). A truer test of egg quality may come from a detailed biochemical analysis of eggs from which a particular nutrient may prove to correlate with quality, allowing egg quality criteria to be established. A comparison of egg composition with naturally-spawned eggs would be invaluable, but presently remains impossible as the natural spawning sites for mangrove jack have not been identified.

(iv) the physical rearing environment

The temperature and salinity requirements of feeding larvae have not been tested for explicitly. However, based on results for yolksac larvae (Chapter 7), and natural conditions during the spawning season (Chapter 6, Fig. 1), lower salinities could prove beneficial. The survival of yolksac larvae was significantly better at 22 ppt than at 27 or 32 ppt. Whether or not this preference extends into the larval feeding phase needs to be investigated.

Light levels are of paramount importance to marine fish larvae as they are visual feeders with a pure cone retina (Blaxter, 1969, 1975; Hunter, 1981; Tucker, 1992a). Tandler and Mason (1983), for example, found survival and growth of gilthead seabream, *S. auratus*, to correlate positively with light intensity between 205 and 1370 lux. Optima appear to be very species-specific, but generally fall within the range of 500-2000 lux with artificial light (Tucker, 1992a). In all of the present trials, except the first clearwater/rotifer trial, a low light level of 250 lux was provided. This may be too low for mangrove jack larvae, preventing clear identification of prey organisms. In the first rearing trial, diffused sunlight was supplied for lighting, providing up to 1000 lux at midday. This trial was also the most successful, with larvae remaining to day 12. It seems reasonable to suggest that mangrove jack larvae may require several thousand lux, which would be consistent with natural conditions at the middle barrier reefs, if these are in fact the natural spawning sites.

Photoperiod is also a consideration as it dictates the number of hours available to larvae for feeding. Providing continuous illumination, while allowing maximum feeding time, appears to be stressful to some larvae and remains a controversial practice, benefiting some species and hindering others (Tandler and Mason, 1983, 1984; Tandler and Helps, 1985; Lubzens *et*

al., 1989). Doi *et al.* (1993) have made the point for mangrove jack larvae that as the 'window' of initial feeding opportunity is so short, it should not be allowed to coincide with nightfall which would shorten it further. In the present trials the potential benefits of an extended photoperiod were not investigated, but could well prove beneficial, at least while larvae are switching to exogenous nutrition.

Tank size can influence the success of larval rearing efforts. Lim *et al.* (1985) reported total mortality, or 'negligible survival', in tanks of 1 m^3 when attempting to rear *L. johni* larvae, and have since conducted larval rearing trials in 5 m^3 tanks with better results. A similar change has occurred in the red seabream industry in Japan, where 1-5 m^3 tanks have been discarded in favour of 50-100 m^3 tanks largely due to improved survival (Fukusho, 1991). Doi and Singhagraiwan (1993) state that survival of mangrove jack larvae was particularly poor and variable in 2 m^3 tanks, often resulting in 100% mortality, and they now use either small ponds or very large tanks (e.g. 190 m^3) for their rearing trials. Continuing poor survival rates amongst mangrove jack larvae, however, indicate that tank volume is not the only factor involved.

The present trials used rearing tanks of 0.2-1.5 m^3 capacity. These small volumes reduce the stability of water conditions, and increase the contact of larvae with unnatural tank walls, where harmful bacteria may reside. For these reasons, larger tanks are usually preferred. Recent mangrove jack larval rearing efforts in Thailand, in which low percentages of larvae (typically < 1%) have developed successfully to metamorphosis, have been carried out in large earthen ponds where these problems would be minimised.

Rosenthal and Alderdice (1976), in their review of sublethal effects of environmental stressors on marine fish eggs and larvae, concluded that larvae

undergoing the transition from endogenous to exogenous nutrition are particularly susceptible to environmental or pollutional stress. As this is the time that most mangrove jack larvae were observed to die, it remains a possibility that larvae at this stage are particularly sensitive to some component of the culture water. Nitrogenous compounds derived from metabolic wastes (ammonia, nitrite, and nitrate) are well known to be stressful or harmful to marine fish larvae at certain concentrations (Brownell, 1980). However, in all but the last greenwater/wild zooplankton trial, where total ammonia reached 0.5 mg/l, levels of these compounds were well within acceptable levels (Brownell, 1980; Yu-Han and Wu, 1990). Chronic and acute toxicity trials with various metabolic wastes and other unnatural substances present in rearing water are needed to reveal sensitivities to chemical stress.

In summary, the problems experienced in the larval rearing of mangrove jack appear to be quite complex and the result of several factors acting in concert. Mangrove jack seem innately difficult to rear through the transition to exogenous nutrition due to their small endogenous energy reserves, in particular the small oil globule, providing them with only a short 'window' of initial feeding opportunity. Doi *et al.* (1993) have suggested that the time available for mangrove jack larvae to complete the transition to exogenous nutrition may be as short as 12 h. This likely explains the universally-observed poor larval survival, whether in Australia, Thailand or the Philippines. This innate problem could be minimised, and survival maximised, by providing particular attention to broodstock diet, initial prey organisms and zootechnical aspects (correct salinity and light intensity and duration may prove particularly crucial). At this stage the major cause of mortality appears to be a failure of larvae to commence feeding, and consequent starvation. However, this also appears to be a secondary

consequence of suboptimal egg quality, prey availability, and rearing environment.

CHAPTER 9

Conclusion

This study has focused on the biological attributes of yellowfin bream and mangrove jack relevant to their consideration for aquaculture. A full assessment of the aquaculture potential of a species also requires careful examination of economic factors such as market acceptance and value, and production costs. A species may be ranked highly or poorly on the basis of either biological or economic factors. Generally, some degree of trade-off exists between the two, such that, for example, a greater tolerance is given to poor biological characteristics if the economic rewards are likely to be high. While economic factors may vary substantially with geographic location and time, the biological factors tend to be more rigid, although varying to some extent amongst populations. However, certain biological factors acting initially as constraints, such as poor larval rearing success, may be overcome through dedicated research.

This study aimed to assess the biological suitability of yellowfin bream and mangrove jack for aquaculture from the viewpoints of broodstock maturation and spawning, larval rearing, and growout performance. Unfortunately, poor larval rearing results for mangrove jack precluded growout assessment for this species. However, some data are available for mangrove jack populations from Thailand and the Philippines, from the growout of wild-caught juveniles. The following attempts to tie together biological and likely economic factors requiring consideration for each species.

The yellowfin bream, *Acanthopagrus australis*

The seabreams, or porgies, are given high market status in many countries, and command high prices. This is particularly true of many Southeast Asian, Mediterranean and Middle-Eastern countries. Unfortunately in Australia, the breams, while regarded as quality fish, do not attain the high prices they do overseas. Yellowfin bream currently sell wholesale at the Sydney fish markets for around A $4.50-6.50/kg whole. This may, in part, be due to their prevalence in coastal waters, ensuring a big take by recreational anglers and a stable supply from the commercial fishery. In contrast, the very successful red seabream and gilthead seabream aquaculture industries of Japan and Mediterranean Europe can be largely attributed to their high market status and declining wild stocks, combining to drive prices of both species to over US $14/kg in 1992.

This study has shown that, biologically, bream are exceptional in the area of hatchery operations. Mature wild broodfish were readily available throughout the spawning season, and quickly adapted to captive conditions. These fish responded well to hormone induction, reliably yielding high quality eggs with good fertilisation and hatching rates. Fecundity was high at approximately 1.6 million eggs/kg. Multiple spawnings on consecutive nights were possible through use of slow-release hormone pellet implants.

Larvae, while small, were equipped with a large yolksac and oil globule, which may be largely responsible for the very high (> 80%) first-feeding success observed under greenwater conditions. On a simple diet of rotifers and brine shrimp, a remarkable survival of 75% to metamorphosis was attained. At this stage, fish were easily weaned, and did not show the asynchronous growth reported for gilthead seabream. The larval stage was short at less than 30 days, offering savings on labour and live food

production. Limited aggression and tail-nipping was observed in the two months following metamorphosis, but not thereafter.

Juveniles readily adapted to netcage conditions and accepted dry pellet food. Good survival and food conversion was observed through growout, although growth was moderate, with fish requiring approximately 25 months from hatch to reach a mean size of 252 g. Yellowfin bream may offer feeding advantages for aquaculture if high carbohydrate utilisation can be demonstrated, as has been shown for some other sparids. Disease resistance through growout was high.

This project has shown, then, that the induced spawning, larval rearing and netcage culture of yellowfin bream is biologically feasible, although the moderate growth rate and market price may, in the short term, hinder the development of an industry based on it. Slower growth translates to slower investment turnover, increased production costs and increased risk of accidental losses. However, this situation could be reversed through exploitation of export markets where higher prices are achievable, and through experimentation with methods of increasing growth rate, as outlined in Chapter 5. These two areas, therefore, should be given high priority in future research. Also, it would be beneficial to further investigate the superiority of the greenwater larval rearing technique for yellowfin bream, and to develop an artificial food for the growout of this species based on further research into nutritional requirements.

The mangrove jack, *Lutjanus argentimaculatus*

In contrast with the yellowfin bream, the major problems relating to the profitable aquaculture of mangrove jack have been identified at the level of larval production. This species is very highly priced and valued throughout Southeast Asia, which has largely been the driving force for research into its

aquaculture. Currently in Australia, however, mangrove jack does not attain the same high prices despite its exceptional edible qualities. Whole fish currently sell for A $3.50-4.50/kg wholesale at the Brisbane fish markets. Profitability of mangrove jack culture in Australia, then, would mostly rely on its export potential, or would necessitate improving its market status in Australia. This is quite achievable given its highly palatable white flesh.

However, several important biological constraints exist, that have prevented the establishment of large-scale culture everywhere it has been attempted. This is despite over a decade of research into this species in Thailand, and major investigations in other countries. These constraints occur in the areas of broodstock maturation and spawning, and particularly in larval rearing. At present, the small mangrove jack aquaculture industry in Southeast Asian countries still relies almost wholly on wild-caught juveniles.

At present, poor larval rearing success presents a bottleneck in the production of juvenile mangrove jack. Consistently poor survival rates, particularly under intensive culture conditions, have been common to rearing attempts in Thailand, the Philippines, and Australia. Survival rates exceeding 1% have been recorded using 'semi-intensive' techniques, which at this stage appear to be the most promising. Reasons for the poor survival have not been fully elucidated, but appear to stem from innately meagre endogenous energy reserves, poor egg quality, and offering of inappropriate initial food organisms, all contributing to very poor first-feeding success. The latter two considerations are likely to be all the more critical, given the apparent innate difficulty in rearing these larvae.

In terms of fecundity and response to hormone administration, mangrove jack have proven to be good, although the quality of eggs obtained in this way has remained dubious. The possibility of suboptimal egg quality

will persist until egg quality criteria are developed, the broodstock diet is modified in accordance with these findings, and induced spawning techniques are developed which minimise broodfish stress. Ultimately, spawning techniques based on environmental manipulation and spontaneous egg release, rather than hormone induction and strip-spawning, are required to this end.

The culture of wild-caught juveniles has provided growout performance data, which appears favourable. Growth is rapid, with fish in Thailand reaching over 500 g in 9-10 months on trash fish, with survival rates over 90% (Doi and Singhagraiwan, 1993). Food conversion, tolerance of crowded netcage conditions, and disease resistance were all high. Surprisingly, the effects of aggression and cannibalism are said to be minimal, despite mangrove jack being piscivorous and equipped with sharp incisors. While juveniles apparently wean quite easily onto minced trash fish, acceptance of dry artificial food is unknown. These growout attributes, coupled with export market potential, create a scenario for profitable aquaculture, subject only to the availability of juveniles.

Given the high market status, price, and demand for mangrove jack, and favourable growout characteristics, aquaculture potential and continuing research into the species remain strong. Priority should be given to future research into improving spawning techniques and egg quality, and providing appropriate initial food organisms and physical rearing conditions for the larvae. It is hoped that this will lead to the development of specific larval rearing practices suited to the large-scale culture of the species, and allow the establishment of an aquaculture industry based on it.

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