# INVESTIGATIONS INTO THE LARVAL REARING OF TWO SOUTH AFRICAN SPARID SPECIES

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# TABLE OF CONTENTS

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# ABSTRACT

The most significant bottleneck to the development of marine finfish culture is a reliable production of juveniles for growout. This is due to the small size at hatch and delicate nature of the pelagic larvae produced by most commercially desirable species. However, over the last 30 years, improved larviculture techniques have been developed to the extent that many species are being successfully cultured worldwide. These techniques were applied to two endemic species as a preliminary step towards establishing marine finfish aquaculture in South Africa.

Adult roman *Chrysoblephus laticeps* and carpenter *Argyrozona argyrozona* (Pisces: Sparidae) were caught in the Tsitsikamma National Park. Both species responded to injection with pituitary extract, HCG and LHRHa, and were succesfully stripped up to 48 hours after injection. Fish were stripped twice, the second stripping producing better quality eggs. *Chyrysoblephus laticeps* also spawned naturally after injection with LHRHa. The fertilised eggs were incubated and the larvae reared in a fully recirculating seawater system. One batch of *A. argyrozona* and three batches of *C. laticeps* were reared through metamorphosis on a diet of enriched rotifers and *Artemia*, and inert foods, following commonly used rearing procedures. Both species followed developmental patterns of other cultured larvae, displaying typical critical stages; high mortalities at first-feeding and cannibalism from 26-30 days after hatch resulted in survival rates ranging from 0.1-0.5%. Growth, survival, size of gape at first-feeding, and ease of weaning onto an inert diet of *C. laticeps* was comparable to other species being reared for the first time, indicating some potential as a candidate species. The numbers of *A. argyrozona* larvae reared were insufficient to make comparisons with other studies. The adults also proved to be susceptible to physical damage while in captivity and were, therefore, considered unsuitable for aquaculture

The thesis describes the spawning procedure, the systems developed and the larval rearing process. The critical stages of first-feeding, swim bladder inflation, settlement and cannibalism are discussed and the development of the larvae described. The ontogeny of both species is described in detail. Both species displayed typical sparid developmental patterns, but differed with respect to pigmentation, head spination and morphometrics.

# CHAPTER 1. INTRODUCTION

The general decline in the catch per unit effort of the South African linefishery is well known (Guastella & van der Elst 1990; Bennett 1991). This, combined with an increased demand for fresh linefish in the local restaurant trade and an international demand for new linefish species (Sweetman 1992), contributes to the economic potential of marine finfish aquaculture in South Africa. Furthermore the suitability of marine fish rather than freshwater species is reinforced by other factors. There is a tradition of eating marine as opposed to freshwater species in the country; and South Africa has an extensive coastline with access to both cold and warm oceans, supporting a rich endemic fish fauna (Smith & Heemstra 1991).

Investigations into the aquaculture potential of several endemic marine species are currently being made (Harris & Cook 1995; Deacon & Hecht in press; Irish & Hecht in prep.). However, before production techniques for any potential aquaculture species can be established, a reliable source of sufficient fry is essential (Hecht 1985). Most desirable finfish species have a planktonic larval stage. These larvae are small, vulnerable to changes in water quality and have specific nutritional requirements, all of which contribute to mortalities in excess of 99.98% per brood in the wild (Marliave 1980). Because of these high mortalities, the bottleneck to commercial production of marine finfish worldwide has been an unreliable or insufficient supply of fingerlings to seed grow-out facilities (Jones & Houde 1981).

A number of species are reared to harvest on a commercial basis from wild caught seed. The supply of wild caught fry is, however, unreliable and represents a risk of introducing disease. The need for establishing larval rearing techniques has long been acknowledged; some of the first documented successes were achieved in the 1960s with the rearing of clupeid larvae beyond metamorphosis. In these early studies, eggs were procured by dissection from the gonads and the larvae were reared in flow-through aquaria using wild caught zooplankton as live food (Schumann 1967; Blaxter 1968, 1969). With this available technology, larviculture remained at an experimental level, being hindered mainly by the lack of a suitable, small first-food organism which could be cultured on a large scale. The discovery of the brackish water rotifer *Brachionus plicatilis* and its adaption to mass culture in seawater by Ito in 1960 (Tsujigado & Lee 1993)

allowed for improved production of a variety of species both in the Far East and Europe (Theilacker & McMaster 1971; Tsujigado & Lee 1993).

Japanese larviculture advanced rapidly during the 1960s, partly in response to an acute demand for a farmed source of marine finfish, but also as a result of significant government funding and involvement with restocking programs (Kafuku & Ikenou 1983; Morizane 1993; Ungson 1993). Still, survival rates of under 10% in pilot operations were considered acceptable (Fujita 1979) and the majority of the fry reared in Japan in the 1970s were still wild caught (Wada & Mitsuda 1976).

The discovery of the importance of a functional swim bladder and the development of techniques to optimise inflation improved larval survival enough to lay the foundation for the first truly commercial production of red sea bream *Pagrus auratus* in Japan (Wada & Mitsuda 1976; Kitajima *et al.* 1981). Although the benefits of adding phytoplankton to the water was well known (Alserson & Howell 1973), recognition that marine larvae required long-chained *n*-3 polyunsaturated fatty acids, and the development of techniques for live food enrichment with cultured microalgae were the next important technological discoveries (Fujita 1979). Nutritional technology was further enhanced by the establishment of techniques for enriching live foods with artificial sources of essential *n*-3 fatty acids (Watanabe *et al.* 1983a,b), which resulted in a significant improvement in fatty acid levels of live foods (Hirayama & Funamoto 1983; Sweetman 1992; Fernandez-Reirez *et al.* 1993). Simultaneous improvements were being made in the area of egg production by hormone injection of both wild caught adults (Wada & Mitsuda 1976) and captive broodstocks (Zohar & Gordin 1979). These advancements facilitated the culture of a variety of species in countries outside of Japan and successful preliminary investigations were made into the rearing of species such as dolphin *Corypheana hippurus* in the United States (Hassler & Hogarth 1977), silver-black porgy *Acanthopagrus cuvieri* and spotted grouper *Epinephalus tauvina* in the Middle East (Hussaine & Higuchi 1980; Hussaine *et al.* 1981), rabbitfish *Siganus guttatus* in the Philippines (Juario *et al.* 1985), white sea bream *Mylio berda* in Hong Kong (Mok 1985), two-banded sea bream *Diplodus vulgaris* in Yugoslavia (Jug-Dujakovic & Glamuzina 1988), and the commercial farming of gilthead sea bass *Sparus aurata* and sea bream *Dicentrarchus labrax* in the Mediterranean (Jones & Houde 1981). By the end

of the 1980s the culture of black sea bream *Acanthopagrus schlegeli* (Fukuhara 1987) and *P. auratus* larvae was established in Japan (Foscarini 1988) and sea bass *S. aurata* and sea bream *D. labrax* in Europe (Anonymous 1987; Minkoff 1990). It was mainly due to the commercialapplication of larviculture techniques which led to the growth of the industry and improvements in culture techniques during the 1980s. Commercial scaling enabled the use of much larger tank sizes (eg., from approximately 500$\ell$ to 2-10$m^3$ (Minkoff 1990)) which was found to enhance larval survival (Foscarini 1988).

The most exciting development of the early 1990s was the successful rearing of coldwater species such as American Atlantic halibut *Paralichthys californicus* (Gadomski *et al.* 1992), Norwegian Atlantic halibut *Hippoglossus hippoglossus* and Atlantic cod *Gadus morhua* (Huse *et al.* 1992; Tilseth *et al.* 1992; Ottera 1993). The prolonged, delicate yolk-sac stage of these species necessitated advances in the areas of incubator design and optimum yolk utilisation (Stickney & Wu-Liu 1991; Lein & Holmefjord 1993; Opstad & Bergh 1993; Reitan *et al.* 1993a), which have since been applied with success to other species (Sweetman 1992).

As the market became more receptive to farmed linefish and advances in live food culture and spawning methods improved, many new species were reared during the early 1990s and the decade has been characterised by an explosion in larviculture research worldwide. Based on their experience with barramundi *Lates calcarifer*, Australia has entered the list of successful larviculture nations with the rearing of snapper *P. auratus* (later discovered to be the same species as the Japanese red sea bream), mulloway *Argyrosomus hololepidotus* (Battaglene & Talbot 1994), golden snapper *Lutjanus johnii* (Schipp & Pitney 1995) and yellowfin bream *Acanthopagrus australis* (Cowden, unpublished data). More recently, species such as pink dentex *Dentex gibbosus* have been successfully reared in Spain (Fernandez-Palacios *et al.* 1994), silver sea bream *Rhabdosargus sarba* and Mangrove red snapper *Lutjanus argentimaculatus* in the Philippines (Emata *et al.* 1994) and the brown-marbled grouper *Epinephalus fuscoguttatus* in Singapore (Chao *et al.* 1993). The Japanese have expanded their commercial production to include the coral trout *Plectropomus leopardus* (Masuma *et al.* 1993), Japanese parrotfish *Oplegnathus fasciatus* and Japanese flounder *Paralichthus olivaceus* (Morizane 1993). A noticeable trend in the 1990s is a move toward more technologically demanding groups such as

members of the Lutjanidae and Serranidae, indicating an improvement in available techniques, a diversification of countries involved in larviculture and the international demand for alternative species.

Hygiene, pathology and the bacterial microenvironment of live food cultures and larval rearing vessels have emerged as the next major challenges to larviculture (Colorni 1989; Nicolas *et al.* 1989; Harboe *et al.* 1994). The use of sterilised recirculating systems, batch culture and antibiotics in European hatcheries (Dahl-Madsen 1992; Sweetman 1992; Harboe *et al.* 1994; Hernandez-Cruz *et al.* 1994) contrasts strongly with the use of larger systems and the employment of probiotics (the use of beneficial bacteria as a prophylactic) in the Far East, mostly outside of Japan (Sorgeloos *et al.* 1993a). This difference in approach is typical of a deviation in technology from the increased efficiency, control and mechanisation of European and Japanese technologies towards more natural rearing methods. Success using extensive fertilised pond culture has been achieved with species such as red snapper *Lutjanus argentimaculatus* in Thailand (Sighagraiwan & Doi 1993) red drum *Sciaenops ocellatus* in the United States (Henderson Arzapalo 1992) and barramundi *L. calcarifer* in Australia (Rutledge & Rimmer 1991). The advantages of a more organic system approach using natural sunlight, greenwater and environmental control of spawning are being recognised (Caddell *et al.* 1990; Ako *et al.* 1994; Naas *et al.* 1995), and because of the simplified technology involved, it holds appeal for application in South Africa.

Several other aspects integral to larviculture development which have been the subject of ongoing research deserve mention, although fair review of these topics falls outside the scope of this introduction. These are live food production and broodstock manipulation. The techniques for culturing *Brachionus plicatilis* and *Artemia* have improved significantly in recent years and represent one of the most important advancements to the development of larviculture technology (Sorgeloos & Leger 1992; Hino 1993; Sorgeloos *et al.* 1993a, b). Spawning through hormone manipulation has been practised since the 1930s (Zohar 1990), but it was only by the late 1980s that research into the use of LHRH analogues and other gonadotropins significantly improved egg production (Ngamvongchon *et al.* 1987; Harmin & Crim 1992). The end of the 1980s and beginning of the 1990s saw advancements in hormone therapy and delivery systems through

pellet implantation (Carolsfeld *et al.* 1988; Garcia 1989; Zohar *et al.* 1990b; Lee *et al.* 1993; Matsuyama *et al.* 1993). The importance of a nutritionally adequate broodstock diet to egg quality and spawning was another important breakthrough (Boyer & van Touver 1993).

The species mentioned above belong to a variety of families and come from a diversity of countries with varied histories, intensity and success of marine aquaculture. As the list of species increases, subtle differences in culture requirements, particularly between families, improve larviculture techniques in general. However, except for deviations into extensive culture, the overwhelming majority of species discussed above have been reared using the same basic set of techniques. These usually involve hormone manipulation of a captive broodstock, first-feeding with *Brachionus*, progression onto enriched *Artemia* and weaning onto a formulated diet. Larvae have been typically reared in cylindro-conical, black rearing tanks (usually 1000 - 5000$\ell$), with low exchange rates of water, skimmed of lipids to promote swim bladder inflation. This uniformity was an indication that rearing technology is highly transferable, and it was on this assumption that the techniques used in this study were based. The possibility that the literature represents only research for which there has been a certain degree of success, does exist. The technology may therefore not be as transferable as it appears. Those species for which success has been achieved, and which have gone on to become commercially produced, may be examples of species whose biology was suitable for culture under established rearing techniques rather than evidence of transferability of culture techniques. Evidence for this exists in two forms. Firstly, of the species listed above, very few are produced in any significant quantity, and a uniform technology is employed to culture the larval stages. Secondly, Mediterranean hatcheries have resorted to importing Japanese red sea bream *P. auratus*, rather than developing local species (Sweetman 1992). Besides the marketing aspect of a unique species, concern regarding the impact of alien introductions into South Africa's aquatic environment has led to a virtual prohibition of foreign species imports, highlighting the need for research into indigenous species as potential candidates for aquaculture (de Moor & Bruton 1988). The purpose of this project was, therefore, to apply established techniques to endemic species as a first step towards establishing marine finfish culture in South Africa.

Previously, investigations into marine finfish larviculture in South Africa were conducted on three species: the southern striped mullet *Liza richardsonii* (Bok 1989), santer *Cheimerius nufar* (Garratt *et al.* 1989) and galjoen *Dichistius capensis* (van der Lingen 1994). The techniques used during these investigations were similar to those used during this study. Similar rates of survival were acheived with *L. richardsonii* and *C. Nufar* to those acheived during this study. Only several *D. capensis* were reared through the larval period. The projects did not result in further research or upscaling, the primary reason for which appeared to be lack of funding.

This project is the application of a policy decision and commitment by the Earth Marine and Atmospheric Technology (EMATEK) division of the Council for Scientific and Industrial Research (CSIR), having recognised the potential of the industry, to furthering the establishment of marine finfish aquaculture in South Africa.

To identify species which would serve as models for larviculture research, a preliminary study was carried out. Several species including the spotted grunter *Pomadasys commersonnii*, the bronze bream *Pachymetapon grande* and the fransmadam *Boopsoidea inornata*, were screened according to the availability of broodstock, the ability of the adults to withstand the stresses of captivity and artificial spawning techniques, as well as fecundity and market potential. Eventually, two species were selected; the carpenter (silverfish, doppie) *Argyrozona argyrozona* and the roman *Chrysoblephus laticeps*. *Argyrozona argyrozona* is a sparid endemic to South Africa (Smith & Heemstra 1991). It is a carnivorous species that grows to a maximum length of 90cm (Nepgen 1977; van der Elst 1988) and is important to both the commercial and recreational linefishery. Carpenter were abundant in the Tsitsikamma National Park (TNP) where they were easily caught on hook and line. Although the early larval stages were described by Gilchrist (1904, 1916) this study presents the first description of artificial spawning and larval rearing. *Chrysoblephus laticeps* is another endemic species (Smith & Heemstra 1991) easily caught in the TNP. It is a benthic, carnivorous species (Buxton 1984) growing to a maximum size of 40cm (Buxton 1993) and has been well researched due to its importance to local fisheries (Smale & Buxton 1985; Buxton 1989; Hecht & Tilney 1989; Buxton 1990; Penney 1990). Although *C. laticeps* have been observed to spawn in captivity (Buxton 1990) and several

individuals have been reared from eggs in the past (Brownell 1979a,b), this study describes the first hormonally induced spawning of the species and the first attempts at rearing the larvae for aquaculture purposes.

In order for any larvae to be reared it was necessary to have a supply of fertilised eggs. The first step was thus to spawn the fish. Because of the lack of laboratory facilities and the abundance of *A. argyrozona* and *C. laticeps* in the TNP, it was decided to depend on wild caught adults rather than maintain a broodstock. The methods used to capture ripe adults of both species, their manipulation with hormones, stripping and egg fertilisation are described in Chapter 2. The larvae which hatched out needed to be accommodated in a suitable environment. This is the first time an attempt has been made to rear marine larvae at the Department of Ichthyology and Fisheries Science (DIFS), Rhodes University, and the appropriate systems needed to be developed. A food production protocol had to be established to feed the larvae. The design and construction of the system and methods of live food culture are described in Chapter 3. To understand the factors responsible for larval mortality and to monitor the artificial culture techniques, it was necessary to observe the development of the larvae. Aspects of growth and the development and behaviour of the larvae are noted in Chapter 4. An integral knowledge of the biology of the animals being cultured was an important part of establishing culture techniques. A complete developmental life history series for both species is therefore presented in Chapter 5.

# CHAPTER 2. SPAWNING

## Introduction

Ensuring a source of fertilised eggs is problematic to the rearing of marine finfish larvae. The lack of a captive broodstock means procuring these eggs from wild caught adults, and some knowledge of the physiology behind the production of eggs and the role played by hormones in reproduction is therefore necessary. Ovarian development and maturation progresses in a series of phases which are controlled by the neuroendocrine system. In wild fish, the perception and integration of photoperiodic, thermal, nutritional, social and other environmental influences by the nervous system induces the secretion of releasing hormones by the hypothalamus, which in turn controls the activity of the pituitary gland. Stimulation of the gonadotropic cells in the pituitary causes the synthesis of one or more gonadotropic hormones which act upon receptors in the follicular layers of the developing ovary. The ovary secretes a suite of steroid hormones which drives the development of the eggs through the stages of oogenesis, including primary and secondary growth, vittelogenesis, ovulation and atresia (Hoar *et al.* 1983; Bromage & Camaranatunga 1988). Fish in captivity do not receive the environmental stimuli to which they are normally exposed in the wild; as a result, oocyte maturation and ovulation are not completed (Zohar *et al.* 1989a). The lack of ovulation in farmed or captive fish is probably due to the failure of the pituitary to release gonadotropin (GtH) (Zohar *et al.* 1989a). However, egg development can be manipulated artificially with the use of hormones.

There are four categories of hormone therapy involving artificial gonadotropins. These therapies have been used to both induce ovulation and to bring on spawning behaviour:

1) Application of pituitary extracts or hypophysation. This has been widely employed in fish farming due to the low cost and convenience involved, but the precise gonadotropin content of pituitary extract is difficult to quantify.

2) Application of purified fish gonadotropins. This is an expensive technique and purified preparations are not readily available.

3) Application of mammalian gonadotropins. Hormone preparations such as humanchorionic gonadotropin (HCG) and luteinizing hormone (LH) are readily available and have been used for a variety of species in experimental and commercial operations. HCG has been used to induce ovulation for stripping (Tucker 1994) and has induced natural spawning in commercially produced species such as gilthead seabream *S. aurata* (Zohar & Gordin 1979), orangemouth corvina *C. xanthulus* (Prentice *et al.* 1989) and red sea bream *P. auratus* (Battaglene & Talbot 1992). Natural fish hormones, however, display a high degree of species specificity (Zohar *et al.* 1990b), and although HCG has worked well for some species, it has been ineffective in others.

4) Stimulation of hormone secretion from the fish's own pituitary. This ensures the correct type and dose of GtH and has been achieved by administering gonadotropin releasing hormone (GnRH) which works directly on the pituitary. The use of GnRH or its analogues is advantageous because they can be easily synthesised, exhibit a low degree of species specificity, are non-immunogenic and only require low dosages (Zohar *et al.* 1989b). Their efficiency may also be enhanced by removing inhibitory dopamine or gonadotropin release inhibitory factor (GRIF) (Peter 1983) with dopamine antagonists such as pimozide and domperidone (Zohar 1990b) (although the benefits of these antagonists for marine fish are disputed), and by manipulating the dose through the use of implanted pellets (Carolsfeld *et al.* 1988). Pituitary extracts, HCG and GnRH were all chosen for the present study.

Spermatogenesis in captive males usually proceeds normally without the use of artificial hormones (Zohar *et al.* 1989a; Stickney & Wu-Liu 1991). It has been suggested that this might be stimulated by pheromones secreted by females in the tank (Suquet *et al.* 1992). Hormonal manipulation of males has often been neglected for this reason. Injections of pituitary extract (Courtois *et al.* 1986), HCG (Kobayashi *et al.* 1986) and LHRH-A (Ngamvongchon *et al.* 1987) have, however, been shown to increase milt volume, number of spermatozoa, enhanced spermiation and testicular hydration of a variety of freshwater fish within a short time of injection. Male winter flounder, *Pseudopleuronectes americanus* as an example of a marine species, responded to a single injection of GnRH-A with an increase in steroid hormone levels

in the blood plasma within 12 hours, which lasted for several days (Harmin & Crim 1993). Males were, therefore, injected along with females in order to improve fertilisation.

Several authors have found that progress in breeding research has been hampered by both unreliable larviculture using fertilised eggs obtained from artificial spawning methods, and an impractical dependence on wild-caught adults for egg production (Halls 1992; Chao *et al.* 1993). However, the injection and stripping of wild-caught adults during the spawning season has been used successfully for obtaining fertilised eggs in a number of recent projects (Stickney and Wu-Liu 1991; Battaglene & Talbot 1992, 1994). Similar techniques were adopted in this study to provide eggs and sperm for larval rearing.

## Materials and methods

*Broodstock capture*

*Argyrozona argyrozona* and *C. laticeps* were caught by handline in the Tsitsikamma National Park (TNP), a marine sanctuary situated on the Southern Cape coast between Natures Valley ($34^0$ 59'S, $23^0$34'E) and Oubos-strand ($34^0$04'S, $24^0$12'E). Barbless hooks were used to facilitate easy removal from the jaw and minimise damage to the fish. After capture the swim bladder was deflated by inserting a hypodermic needle through the body wall (Battaglene & Talbot 1992). A maximum of four captured fish were placed into 80$\ell$ plastic bins for transport to shore, where they were transferred into plastic pools (5000$\ell$ capacity). Fresh seawater was continuously pumped into the pools, the tanks were covered with shadecloth in order to reduce stress and the fish were left to recover for approximately two hours before hormone injection.

In a preliminary investigation, a dose of 0.18m$\ell$ 2-phenoxyethanol.m$\ell$.$\ell^{-1}$ was found to be sufficient to anaesthetize *A. argyrozona* for transport to shore. *Chrysoblephus laticeps* did not panic during transportation, and during a preliminary investig ation several died as a result of anaesthasis. *Chrysoblephus laticeps* were therefore not anaesthetized.

*The use of hormones*

Cannulation of fish, especially by inexperienced workers, has been shown to damage and eventually cause the atrophication of the ovary (Shehadeh *et al.* 1973). This type of sampling was therefore restricted to a minimum. Because of the difficulty involved in capturing sufficient numbers of fish, all were injected with hormones, regardless of their state of maturity. Sex was determined at time of stripping.

The procedure for injecting fish with pituitary homogenate followed that outlined by Britz (1991) for sharptooth catfish *Clarias gariepinus*. Pituitaries were removed from *C. laticeps* and *A. argyrozona* during the natural spawning season when the levels of pituitary gonadotropic hormone (GtH) were the highest (Peute *et al.* 1986). Whole pituitaries were removed and preserved in 95% ethanol. When needed, pituitaries from fish of the same approximate weight as the ones being injected, were removed from the ethanol and placed on a paper towel for a

minute in order to allow the alcohol to evaporate. The number of pituitaries used matched the number of fish being injected, and each species was injected with pituitaries removed from conspecifics. The glands were homogenised with a small amount of sterile saline ($\pm$0.5m$\ell$) in a glass homogenizer (Caddell *et al.* 1990). The homogenate was further diluted with saline to a concentration of 1 gland.m$\ell^{-1}$. One m$\ell$ of solution was injected intramuscularly near the dorsal fin.

HCG was administered following the methods of Battaglene & Talbot (1992) for red sea bream *P. auratus*. Males and females were injected with 1000IU of the hormone per kilogram body weight, estimated to the nearest half kilogram.

In an attempt to improve fertilisation and hatch rate, Aquaspawn$^{R}$ a commercially available GnRH-analogue was used during the second season. Aquaspawn$^{R}$ contains 100$\mu$g of LHRHa and 500$\mu$g of the dopamine antagonist domperidone, in a 5m$\ell$ sterile saline solution. Aquaspawn$^{R}$ has been used to successfully spawn the southern mullet *Liza richardsonii*, the Clanwilliam yellowfish *Barbus capensis*, whitefish *Barbus andrewi*, largemouth bass, common carp and the sharptooth catfish (Bok 1989; A. Bok, Cape Nature Conservation, Amalinda, South Africa, pers. comm.). Aquaspawn$^{R}$ was administered at a dose of 1m$\ell$ per kilogram body weight of the fish, estimated to the nearest half kilogram.

*Stripping*

In order to strip the fish, the water level in the holding tank was lowered to facilitate capture with a hand net. Wrapping fish in a wet towel to protect the eyes had a calming effect and allowed them to be held firmly during the procedure. The abdomen was gently massaged on the ventro-lateral surface to free the eggs from the ovary. Stripping was facilitated by allowing the fish to flap freely in a tail down position while being held. Stripped gametes were washed into plastic bowls containing approximately 200m$\ell$ of seawater. Females were stripped until no further eggs could be expelled and the abdomen felt flaccid and empty. If the males did not strip well, the gonad was excised, chopped up and mixed with the eggs.

During the first season fish were stripped once after hormone injection; during the second season attempts were made to strip fish more than once. *Chrysoblephus laticeps* were

transported and injected with Aquaspawn$^{R}$ as described and were stripped after 26 hours. This time period was selected after *C. laticeps* were observed to spawn naturally, approximately 24 hours after injection (see below). After the first stripping, the fish were left for 24 hours and stripped again.

Unlike *C. laticeps*, *A. argyrozona* did not spawn naturally and ovulation time therefore had to be investigated. To do this, three of the fish were identified as female through cannulation (Shehade *et al.* 1973) and separated from the rest of the group. All the fish were injected with Aquaspawn$^{R}$ and left for twelve hours without being examined. Since eggs are only found free in the lumen of the ovary once they have been ovulated or released from the follicle (Zohar & Gordin 1979), it followed that if eggs were available for stripping ovulation must have taken place. The fish were removed at four-hour intervals, throughout the night, and stripping was attempted. If eggs were not forthcoming with gentle pressure on the abdomen the fish were assumed not to have ovulated. As soon as eggs were easily stripped from two of the three fish, the time was recorded. A similar technique for determining ideal stripping time has been used by several authors (Caddell *et al.* 1990; Stickney & Wu-Liu 1991; Harmin & Crim 1992).

*Fertilisation*

Fish spermatozoa can retain their fertilising capability for several hours if stored at low temperatures (Hogan *et al.* 1987; Chao *et al.* 1992). Wherever possible, males were stripped first in order to have milt available for fertilisation. This technique was easier with *C. laticeps* because of the sexual dimorphism displayed by the species (Buxton 1990). Once *A. argyrozona* were identified as male after stripping, the first dorsal spine was clipped for identification. Milt was stored in a glass container surrounded by ice and protected from direct sunlight to avoid damage (Don & Avtalion 1993). The temperature of the milt in the container was maintained at $\pm 4$°C. The milt of all the available males was mixed in this container and diluted with saline. If insufficient sperm was collected the most productive male was sacrificed and testes removed. The testes were then sliced open and added to the sperm mixture. Approximately 10m$\ell$ of this mixture was added to approximately 400m$\ell$ of stripped eggs. Eggs were fertilised by swirling the bowl continuously while small quantities of seawater were added. The bowl was then left to

stand for approximately 5 minutes to ensure fertilisation (Stickney & Wu Liu 1991). After fertilisation, *C. laticeps* and *A. argyrozona* eggs were placed in a bucket given strong aeration and a continuous flow of fresh seawater in order to oxygenate, mix and clean the eggs of gonadal material, blood and faeces. Five, 15mℓ samples of water containing eggs were taken from the bucket in order to ascertain fertilisation success. Fertilisation success was estimated by allowing the sample of mixed eggs to stand in a 20mℓ glass vial for approximately 10 minutes. Fertilised eggs floated due to the capacity of the embryo to regulate the osmotic balance of the yolk almost immediately after closure of the blastopore. Unfertilised eggs did not possess an embryo, could not osmoregulate and sank to the bottom after becoming isosmotic with the surrounding water (Holliday & Jones 1967; Alderdice 1988). Floating, fertilised eggs were decanted into a separate vial, counted and percentage fertilisation calculated (Ako *et al.* 1994). After taking the samples, the aeration was shut off allowing dead eggs to sink to the bottom where they could be siphoned out. The remaining live eggs were placed into a 70ℓ plastic drum. Transportation back to the larviculture facility was undertaken within 12 hours of fertilisation to minimise transportation mortality (Wada & Mitsuda 1976; Brownell 1979b; Quinitio *et al.* 1991; Stickney & Wu-Liu 1991).

## Results

Of the three *A. argyrozona* injected with Aquaspawn$^{R}$, for investigating time to ovulation, two of them spawned 18 hours after injection (Table 2.1).

**Table 2.1** Results of investigation into the time required for ovulation of *A. argyrozona* females after being injected with 5m$\ell$.kg$^{-1}$ Aquaspawn$^{R}$.

| Time from injection (hours) | Eggs forthcoming with gentle preassure on the abdomen (yes or no) | | |
|---|---|---|---|
| | Fish #1[a] | Fish # 2[b] | Fish # 3[c] |
| 12 | no | no | no |
| 14 | no | no | no |
| 16 | no | no | no |
| 18 | yes | yes | no |
| 20 | yes | yes | no |
| 22 | yes | yes | yes |

a=fish weight=771.7g
b=fish weight=788.1g
c=fish weight=779.9g

A total of twelve succesful spawnings were achieved between November 1993 and January 1995. A group of over 100 *A. argyrozona* and *C. laticeps* were caught and manipulated during this period. Of these, 14 female and 16 male *C. laticeps* were succesfully spawned, producing a total of 1 140 000 eggs. Ten female and 14 male *A. argyrozona* were injected and succesfully stripped, producing a total of 1 390 000 eggs. The remainder of the fish either died as a result of handling stress, were immature individuals or could not be spawned for other unknown reasons. The data presented in Tables 2.2 and 2.3 does not include these animals and because facilities for incubation were limited, data for hatching success have been pooled.

The sex and wight of succesfully spawned *C. laticeps* as well as the amount of eggs each female produced and the fertilisation and hatching success of those eggs are summarised in Table 2.2. From the eggs spawned during the first season, a batch of *C. laticeps* were reared to 21

days after hatch (DAH). All three spawnings of the second season were reared through metamorphosis. The sex and wight of succesfully spawned *A. argyrozona* as well as the amount of eggs each female produced and the fertilisation and hatching success of those eggs are summarised in Table 2.3. Larvae hatched from eggs produced during the first season were reared through to the juvenile stage, but none of the larvae from the eggs spawned during the second season survived beyond first-feeding (7 days). During the first season both *A. argyrozona* and *C. laticeps* were difficult to strip and fertilisation success was poor. In the case of *A. argyrozona* injected with pituitary extract and stripped 12 hours after injection the fish could not be effectively stripped and the gonads had to be dissected to facilitate fertilisation.

During the second season a longer time period between injection and stripping improved results. Although the number of eggs produced variously increased or decreased between strippings, eggs from the second stripping were of better quality, both in terms of fertility and hatching success for both species.

**Table 2.2** Information pertaining to the spawning of *C. laticeps*. Numbers in parenthasis indicate hours between injection and spawning/stripping. Numbers in curled brackets are pooled results.

| Spawning event | Sex of fish | Mass of fish (g) | Number of eggs produced | | % Fertilisation | | % Hatching success | |
|---|---|---|---|---|---|---|---|---|
| | | | First stripping | Second stripping | First stripping | Second stripping | First stripping | Second stripping |
| First season | ♀: | 755.5[H] | 7700(12) | - | 11.3 | - | | |
| | | 891.3[H] | 6300(12) | - | 13.0 | - | {26.0} | - |
| | ♂: | 1003.8[H] | | | | | | |
| | | 1043.3[H] | | | | | | |
| | | 1447.6[H] | | | | | | |
| | | 1576.4[H] | | | | | | |
| Second season | | | | | | | | |
| First spawning | ♀: | 546.4[P] | 41000(24) | - | 19.9 | - | | |
| | | 620.3[A] | 32000(24) | - | 24.3 | - | {49.4} | - |
| | | 678.1[A] | 54000(24) | - | 17.9 | - | | |
| | | 771.7[A] | 20000(24) | - | 14.7 | - | | |
| | | | 22800* | | 32.2 | | 54.4 | - |
| | ♂: | 909.6[A] | | | | | | |
| | | 974.9[A] | | | | | | |
| | | 1189.9[P] | | | | | | |
| Second spawning | ♀: | 527.3[A] | 13600(25) | 13100(48) | 14.2 | 19.6 | | |
| | | 599.5[A] | 50400(25) | 39000(48) | 18.6 | 13.9 | | |
| | | 663.3[P] | 22000(25) | 15000(48) | 15.0 | 32.4 | {60.2} | {65.1} |
| | | 678.1[A] | 94400(25) | 7100(48) | 16.2 | 17.7 | | |
| | | 955.9[A] | 70200(25) | 20000(48) | 17.0 | 28.1 | | |
| | ♂: | 914.6[A] | | | | | | |
| | | 1053.7[P] | | | | | | |
| | | 1211.9[P] | | | | | | |
| | | 2009.5[A] | | | | | | |
| Third spawning | ♀: | 700.8[A] | 52300(25) | 92000(48) | 23.6 | 75.1 | | |
| | | 763.6[A] | 84000(25) | 127300(48) | 30.2 | 89.3 | {18.5} | {54.1} |
| | | 918.1[A] | 104800(25) | 177400(48) | 26.9 | 91.2 | | |
| | ♂: | 994.1[P] | | | | | | |
| | | 1223.0[A] | | | | | | |
| | | 1268.2[P] | | | | | | |
| | | 1447.6[A] | | | | | | |
| | | 1457.5[A] | | | | | | |

*=Natural spawning
H=Human Chorionic Gonadotropin administered at a dose of 1000IU.kg$^{-1}$
P=Pituitary homogenate administered at a dose of 1m$\ell$.kg$^{-1}$
A=Aquaspawn$^{R}$ administered at a dose of 0.5m$\ell$.kg$^{-1}$

**Table 2.3** Information pertaining to the spawning of *A. argyrozona*. Numbers in parenthasis indicate hours between injection and spawning/stripping. Numbers in curled brackets are pooled results.

| Spawning event | Sex of fish | Weight of fish (g) | Number of eggs produced | | % Fertilisation | | % Hatching success | |
|---|---|---|---|---|---|---|---|---|
| | | | First stripping | Second stripping | First stripping | Second stripping | First stripping | Second strip. |
| First season | ♀: | 752.4[H] | 72000(12) | - | 6.2 | - | | |
| | | 1047.7[H] | 50000(12) | - | 5.6 | - | {34} | - |
| | ♂: | 855.3[H] | | | | | | |
| | | 891.7[H] | | | | | | |
| | | 1007.0[H] | | | | | | |
| | | 1089.4[H] | | | | | | |
| Second season | | | | | | | | |
| First spawning | ♀: | 739.4[P] | 37000(18) | 156000(36) | 8.4 | 96.5 | | |
| | | 862.5[A] | 57800(18) | 1714000(36) | 4.3 | 54.2 | {0.0} | {74.1} |
| | | 2223.6[A] | 156000(18) | 344666(36) | 6.0 | 89.7 | | |
| | ♂: | 545.9[A] | | | | | | |
| | | 640.5[A] | | | | | | |
| | | 701.2[A] | | | | | | |
| | | 827.0[P] | | | | | | |
| | | 848.2[A] | | | | | | |
| | | 998.5[P] | | | | | | |
| Second spawning | ♀: | 720.1[P] | 7033(18) | 7000(36) | 0.0 | 0.0 | | |
| | | 745.8[P] | 23900(18) | 2067(36) | 0.0 | 0.0 | | |
| | | 848.2[A] | 30966(18) | 24066(36) | 0.0 | 0.0 | {0.0} | {6.0} |
| | | 1015.1[A] | 24000(18) | 10200(36) | 0.1 | 0.0 | | |
| | | 1424.1[A] | 22634(18) | 52000(36) | 0.0 | 15.2 | | |
| | ♂: | 711.2[A] | | | | | | |
| | | 790.8[A] | | | | | | |
| | | 1466.7[P] | | | | | | |
| | | 1571.8[A] | | | | | | |

H=Human Chorionic Gonadotropin administered at a dose of 1000IU.kg$^{-1}$
P=Pituitary homogenate administered at a dose of 1m$\ell$.kg$^{-1}$
A=Aquaspawn$^{R}$ administered at a dose of 0.5m$\ell$.kg$^{-1}$

## Discussion and observations

The lack of a regular supply of good quality seed was the most important obstacle to the success of the study. The factors influencing the supply of eggs were primarily of a logistical nature that were beyond the control or scope of the project. They included the following: *C. laticeps* and *A. argyrozona*, as with many of the temperate sparids, were seasonal spawners having spring/summer breeding seasons. The gonosomatic index of *C. laticeps* populations monitored for two years in the Tsitsikamma National Park (TNP) showed a distinct peak rising in September and climaxing from October through to January (Buxton 1990). *Argyrozona argyrozona* have only been found with ripe gonads during spring and summer months (Nepgen 1977). The spawning season in any one year was further restricted by oceanographic variables. *Chrysoblephus laticeps* caught in the TNP during the 1994/95 season, for example, had active gonads only from mid November to mid January. This meant that fertilised eggs could only be collected within a potential three-month period. This three-month period was further restricted by bad weather and administrative difficulties. The time spent collecting seed during a field trip was compromised by the need to take care of larvae from previous spawnings and the need for eggs to be transported to the laboratory as soon after fertilisation as possible.

Egg production and fertilisation rate was improved between the first and second seasons. A variety of factors were considered responsible for the poorer results achieved during the first season.

1) Spawning was attempted late in the season for both species. The size and quality of the eggs and the survival of first-feeding larvae is known to decline in numerous species as a season progresses (Knutsen & Tilseth 1985; Hay 1986; Blaxter 1988; McEvoy & McEvoy 1991).

2) HCG and pituitary homogenate were used as manipulating hormones. HCG has shown a high degree of biological specificity (Zohar *et al.* 1989b); despite success with other species, *C. laticeps* and *A. argyrozona* might not have been suited to HCG manipulation. Crude pituitary extracts that vary in their gonadotropic potency will not yield optimum results.

3) The time allowed for ovulation between injection and stripping was only 12 hours. Subsequent observations and investigations into the time required by *C. laticeps* and *A. argyrozona* from hormone injection to ovulation suggest that 12 hours is insufficient for ovulation to be completed. This was especially evident in *A. argyrozona* where none of the fish could be stripped, and of those only two out of nine females contained ovulated eggs. It was possible that those fish from which the eggs were dissected were actively engaged in spawning when caught and therefore contained a certain amount of naturally ovulated eggs.

4) Poor fertilisation due to the fact that eggs were stripped into a small amount of seawater. Fertilisation of the stripped eggs was occasionally subject to delay because the eggs of more than one female were added into the same bowl before being fertilised. Also, sperm was not stored during the first season and the first male caught out of the holding tank did not always produce sufficient sperm.

Adequate water is essential to the fertilisation process in that it prepares the egg for fertilisation by moving into the perivitelline space and lifting the capsular membrane. Water also activates the spermatozoa, and provides a medium through which they can "swim" to the micropyle (Blaxter 1988). For this reason, gametes were stripped into a relatively small quantity (100 - 200m$\ell$) of seawater (Battaglene and Talbot 1994). However, water has been shown to have the effect of hardening or toughening the capsular membrane in order to prevent over-absorbtion of water and to protect the developing embryo. A minute hole in the capsular membrane, termed the micropyle, is situated at the animal pole of the egg and remains permeable to sperm for a short period after the hardening of the membrane. Depending on temperature, the micropyle remains open from 0.5 - 1 minute. If sperm is not added to the eggs within this period the micropyle becomes impermeable and the eggs cannot be fertilised (Smith 1957).

In the second season, spawning success was improved by using the following techniques:

1) Both species were spawned early in the breeding season.

2) Aquaspawn$^{R}$ was used to induce ovulation.

3) The time allowed between injection and stripping was at 24 hours for *C. laticeps* and 18 hours for *A. argyrozona*.

4) Eggs were stripped into a dry bowl and water was gradually added after the addition of the milt suspension.

Based on the experience acquired during the first season, more spawnings were possible during the second season. Although not empirically tested, several important observations were made during the season which contributed to the knowledge of captive spawning of *C. laticeps* and *A. argyrozona*.

1) *Chrysoblephus laticeps* caught at the beginning of November 1994 with a view to using them as broodstock were kept in a flow-through system for three weeks and were fed to satiation once daily with diced pilchards and squid. Cannulation of the females in late November did not extract eggs or ripe gonadal tissue. When dissected, the gonad appeared to be in a state of regression. *Chrysoblephus laticeps* caught at the same time, as part of a tag and release program, were sacrificed in order to compare the state of their gonads. Their ovaries were ripe (Buxton & Clarke 1986) and contained hydrated eggs. Gonad deterioration was assumed to be a result of captivity. The condition of the gonad has been known to be adversely affected by poor diet (Tyler & Dunn 1976), high stocking density and other stress related to captivity (Blaxter 1988; Stickney & Wu-Liu 1991). *Chrysoblephus laticeps* in the holding tanks did not have access to their natural diet and were exposed to higher stocking densities than in the wild. Because these stressors can effect the quality of eggs and hatched larvae (Blaxter 1988) it was decided to catch adults from the wild rather than maintain a broodstock.

2) *Chrysoblephus laticeps* were observed to spawn naturally in their holding tank, early in the second season. The spawning behaviour was similar to that observed by Buxton (1990), but lacked any rushing or circling behaviour. This may have been due to the presence of other fish in the tank and the inability of the male to establish a territory. Natural spawning of *C. laticeps* in captivity had important implications.

i) The spawning of *C. laticeps* observed by Buxton (1990) in the Port Elizabeth museum was not an isolated event.

ii) A single application of LHRHa + domperidone was sufficient to induce natural spawning of *C. laticeps*.

iii) The relatively shallow depth (approximately 1.5m) of the holding tank provided sufficient depth for *C. laticeps* to complete their spawning ascent.

iv) It indicated that ovulation occurred 24 hours after injection and that fish should be stripped just prior to this if natural spawning is not desirable.

3) *Chrysoblephus laticeps* caught during mid December 1995, injected and stripped following the usual protocol, produced few, opaque eggs. When dissected, the ovaries appeared to have regressed to what appeared to be the developing stage, although during the previous field trip they were in the active stage as classified by Buxton & Clarke (1986). The water temperature was colder than usual, measuring 9°C at the surface. This low water temperature was due to an upwelling event. Upwelling in the Tsitsikamma area is characterised by an offshore transport of surface water and a replacement of this warmer water by much colder subsurface water. There are an average of nine of these events in the Tsitsikamma per annum and 81% of these events occur between November and April (Hanekom *et al.* 1989). Water temperatures had risen to 19°C during the following field trip, about a week later. The ovaries of fish caught at this time were active and the fish were stripped normally.

Conversely, *A. argyrozona* caught in early January 1995 were adversely affected by high water temperatures (21.5°C). The fish exhibited a continuous stress pattern, were observed to lose stability while in the holding tank and shed scales very easily when stripped, resulting in several mortalities. Although females injected with hormone produced large quantities of eggs, the majority were opaque.

Photoperiod and temperature have been found to be the most important factors influencing the annual reproductive cycles of marine percoid fishes (de Vlaming 1972a). Gonadal regression due to changing temperature both on a seasonal basis eg. *C. laticeps, Chrysoblephus cristiceps* (Buxton 1990), and during the spawning season eg. the longjaw goby *Gillichthys mirabilis* (de Vlaming 1972b) have been recorded. The effect of cold water on *C. laticeps* had interesting implications. Tilney and Buxton (1994) recorded a decline in egg and larval abundance following upwelling events and suggested that either the eggs and larvae were carried offshore by surface Ekman transport or were killed by the sudden decline in water temperatures. Based on the observations noted above, the possible cessation of spawning due to colder temperatures during upwelling events may have contributed to the reduction of egg and larval abundance at these times. The implications to aquaculture were that *C. laticeps* broodstock

would have to be held at temperatures higher than at least 9°C and *A. argyrozona* at temperatures lower than 22°C in order for spawning to take place.

4) Slight swelling and lightening of the area surrounding the vent of female *C. laticeps* was a good indication that the female contained large numbers of hydrated, ovulated eggs. Good fertilisation and hatching was only achieved from eggs which were translucent, flowed easily from the female and had an orange tint to them. Usually, fish displaying the symptoms described above produced good quality eggs and were easily stripped. However, several fish, despite displaying these symptoms, could not be stripped conventionally. When stripping was continued longer than usual and pressure to the abdomen was applied more firmly, the eggs were eventually expelled. The cause of blockage appeared to be a plug of eggs which had accumulated at the vent. These eggs were opaque and tended to stick together in clumps. After the plug was expelled, normal, translucent eggs were easily stripped. The egg plug was only observed when fish were stripped for the first time. On subsequent strippings the plug was absent. Similar clumping of eggs after hormone injection have been observed in the orangemouth corvina *C. xanthulus* (Prentice and Colura 1984) and has been described as typical of partially ovulated eggs in serranids (Tucker 1994). Microscopic observation revealed a large percentage of these eggs ($22.6 \pm 0.2\%$ ($x \pm$ Standard Deviation)) to be larger than normal eggs and irregularly shaped. An overdose of hormone or injection just before ovulation had caused overhydration of the egg (Tucker 1994).

5) The quality of eggs produced from a second stripping was better than that from eggs obtained from an initial stripping. It has been well documented that fish injected with hormones are able to ovulate subsequently with no further manipulation. Red sea bream *P. auratus* (Singhagraiwan & Doi 1993) and gilthead sea bream *S. aurata* (Zohar & Gordin 1979) were stripped for 5-6 consecutive days after an initial injection. Successive natural spawnings have been achieved for four consecutive nights with *L. johnii* (Singhagraiwan & Doi 1993) and 100 consecutive days for *S. aurata* (Zohar & Gordin 1979). The improvement of egg quality witnessed during this project has several possible explanations.

i) The ideal time to ovulation may have been longer than the 24 hours indicated by natural spawning for *C. laticeps* and the 18 hours indicated by trials with *A. argyrozona*. Poor egg quality of red sea bream *P. auratus* eggs, for example, has been attributed to premature

stripping after hormonal injection, as opposed to better quality achieved with spontaneous spawning (Singhagraiwan & Doi 1993).

ii) The eggs expelled during the first stripping may have been ovulated by the fish while still in the wild. The time allowed for ovulation after injection allowed those eggs to overripen (Hay 1986; Stickney & Wu Liu 1991; Battaglene & Talbot 1992). After these overripe eggs were eliminated by the first stripping, the eggs which had developed subsequently were at the correct stage of development for fertilisation. This was supported by the fact that difficulties with stripping were only encountered in the first stripping attempt, and that behind the plug of opaque eggs, clear, healthy-looking eggs were found.

iii) Enzymes of the pituitary, kidney and liver have been shown to rapidly degrade GnRH (Zohar 1990a; Zohar *et al.* 1989a; Goren *et al.* 1990). It was therefore unlikely that injected GnRH was active beyond the first induced ovulation or that it was the direct cause of subsequent ovulations. Zohar and Gordin (1979) observed GtH surges in the blood plasma of *S. aurata* at 24-hour intervals (following the natural spawning regime of serially spawning fish), after an initial gonadotropin surge occurred as a result of hormone injection. They postulated that the subsequent surges resulted either from an evoked endogenous circadian rhythm of the pituitary gland or from feedback mechanisms occurring between non-hypophyseal hormones involved in the process of maturation and ovulation, and the pituitary gonadotrophs. Zohar and Gordin's (1979) observations imply that ovulation of *C. laticeps* and *A. argyrozona* eggs subsequent to that induced by hormone, were under the direct control of the fish's own neuroendocrine system. If eggs were being ovulated as a result of natural hormone surges, this would have had a positive effect on egg quality (Caddell *et al.* 1990; Halls 1992).

6) There was no apparent difference in ease of stripping or visible egg quality correlating to the different hormones used.

## Conclusion

The difficult task of obtaining seed for larval rearing has provided useful information on the manipulation and spawning of *C. laticeps* and *A. argyrozona.*

1) Within their spawning season, *C. laticeps* spawn naturally in captivity, 24 hours after receiving a single dose of Aquaspawn$^{R}$. 2) *Chrysoblephus laticeps* and *A. argyrozona* caught from the wild and having ovaries in the active stage can be induced to ovulate and can be stripped after hormone therapy.

3) The lag between injection and ovulation lies between 24 and 48 hours for *C. laticeps* and between 18 and 36 hours for *A. argyrozona.*

4) Both species can be stripped more than once after a single hormone injection, with the subsequent stripping likely to provide better quality eggs as reflected by better fertility and hatch rate.

5) The ovaries of *C. laticeps* caught during the spawning season and kept in captivity for two weeks may regress, probably due to the stresses of captivity.

6) A tank size of 5000$\ell$ and 1.5m depth is sufficient for *C. laticeps* to complete their spawning ascent.

7) Active ovaries of *C. laticeps* may regress as a result of exposure to the cold water temperatures (<9°C) caused by upwelling. *Argyrozona argyrozona* are easily stressed and suffer mortalities in water temperatures above 21°C. Further experimentation is required to validate this observation.

The need for eggs from which to rear larvae was the priority of this phase of the study. The methods used for assesing the induction of spawning through hormone treatment was, therefore compomised. The observations made and experience gained during the study will, however, lay the basis for future research. Areas which will be explored in the near future include the following:

1) A detailed study on the time required for ovulation after injection with Aquaspawn$^{R}$.

2) The performance of Aquaspawn$^{R}$ will be compared with pituitary homogenate and HCG.

3) The optimum dose of the best hormone which produces the best results will be determined.

4) The optimum number of injections of the best hormone will be tested.

# CHAPTER 3. SYSTEMS DESIGN, CONSTRUCTION AND FUNCTION, INCLUDING NOTES ON LIVE FOOD CULTURE AND FEEDING

## Introduction

Many designs for marine hatcheries have been used successfully in overseas countries to rear a variety of species. However, simply transferring a design is often impractical due to differences in local infrastructure, expertise, materials and general environment. It was therefore necessary to design and construct unique systems which would suit local conditions and situations.

The DIFS's distance from the sea made a recirculating, as opposed to a flow-through system, necessary. Recirculating systems offer direct control over water quality parameters and are becoming more popular in foreign marine finfish hatcheries (Wada & Mitsuda 1976; Mok 1985; Dahl-Madsen 1992). This is the first time, however, that marine larvae have been reared in a recirculating system in South Africa.

Marine pelagic larvae hatch and go through their early life stages lacking or with poorly developed organ systems, which in post larval-fish are used to regulate their internal environment (Holliday & Jones 1967). The body surface fulfils many of these functions in early larvae and the performance of the skin as a regulatory organ is enhanced by a large surface area to body volume ratio, increased by an expanded medial finfold and covered by a layer of skin only two cells thick (Roberts *et al.* 1973). As a consequence, larvae are very sensitive to water quality. The stable environment provided by the large volume of the open ocean permits this primitive physiology (Blaxter 1992). However, the smaller volumes and higher stocking densities of culture conditions can result in potentially harmful fluctuations of water parameters, to which larvae are more sensitive than more developed fish (Von Westernhagen 1988; Blaxter 1992; Little *et al.* 1993).Physical parameters of seawater include temperature, density, colour, turbidity and suspended solids, while chemical parameters include salinity, ionic composition, dissolved gasses, salts and molecules, and dissolved and particulate organic matter (Alzieu 1990).

Temperature is one of the most important variables for all aquatic organisms (Alzieu 1990). Besides influencing water chemistry, temperature directly affects poikilothermic organisms by influencing metabolic rate (Blaxter 1992). Because of their small size, the metabolic rate of larvae is affected more acutely and they are therefore more stenothermal than adults of the same species (Rombough 1988). Despite this, most larvae thrive under a variety of temperatures, provided those temperatures remain relatively constant (Blaxter 1992). The range of acceptable temperatures (specific temperature range) has important implications for larval rearing. Higher temperatures lead to a decrease in developmental time due to an increase in metabolic rate, enhancement of enzyme function (Clarke *et al.* 1992) and an increase in the animal's spontaneous activity (Fuiman & Ottey 1993); consequently, larvae have been reared at temperatures above those normally encountered in the wild (Laurence 1973; Gadomski *et al.* 1992; Miranda *et al.* 1992). However, the efficiency with which yolk is transformed into body tissue is better at the lower end of the specific temperature range, resulting in larger larvae at first-feeding (Laurence 1973; Blaxter 1988). Energy deficits are also built up quicker in warmer water (Laurence 1973). Lower temperatures, therefore, increase the time required before exogenous feeding is necessary, the time for larvae to find their first-food organism and the time to irreversible starvation (Doi *et al.* 1993).

Another consideration influencing the management of water temperature is its effect on water chemistry. Marine larvae are more sensitive to acute ammonia toxicity at lower temperatures (Sampaio & Minillo 1995), whereas the dissolved oxygen capacity of seawater diminishes with increasing temperature (Alzieu 1990). The ideal situation would be to hatch and raise larvae through first-feeding at lower temperatures and gradually increase the temperature as they develop. For the purposes of this study larval survival was considered more important than rate of development, so an optimum temperature was selected as one which *C. laticeps* and *A. argyrozona* larvae encounter in the wild. Data were available for mean monthly temperatures of the surface water in the TNP during 1986 sea temperatures varied between 15 and 19.5°C with an average of 17.3°C over the summer spawning season (Buxton 1990) while ambient water temperatures at the DIFS during the 1994/95 summer season ranged from 15 to 34°C with an average of 24°C. Water in the hatchery therefore needed to be cooled.

Despite seawater having a low affinity for oxygen the surface layers of the oceans, in which marine larvae are usually found, generally have a high level of dissolved oxygen (DO) (Alzieu 1990). Maintaining sufficiently high levels of DO is therefore both problematic and essential under culture conditions. Before branchial gas exchange with opercular movement develops toward the end of the larval period, gas exchange is purely cutaneous and relatively less efficient, despite compensatory mechanisms such as medial fin folds or fanning of enlarged pectoral fin buds (Rombough 1988; Brownell 1979b). In addition to these physiological restrictions, there are several external factors to which larvae under culture conditions are indirectly vulnerable at low concentrations of DO: 1) A maximum density of live food, which has its own oxygen demand, is required at first-feeding. 2) Larvae are sensitive to the shearing forces of oxygenation techniques (Chatain & Ounais-Guschmann 1990). 3) The toxicity of several substances is increased with a decrease in DO (Spotte 1992).

Both the eggs and larvae of marine fish have the ability to osmoregulate (Holliday & Jones 1967). The ionic concentration of eggs is initially maintained by the cells of the blastoderm and in larvae by means of chloride cells on the skin (Alderdice 1988). Many marine larvae have been found to be tolerant of a wide range of salt concentrations (Lein & Holmefjord 1993; Provencher *et al.* 1993). The most important aspect of salinity to larval culture is the effect that it has on buoyancy. Eggs and larvae tend to maintain their internal salinity at levels slightly below that of the seawater in which the eggs were ovulated (Alderdice 1988). If eggs or larvae are exposed to higher density seawater they become negatively buoyant and *vice versa* (Reitan *et al.* 1993b). Problems arise when abrupt changes to salinity cause larvae to either sink to the bottom or float on the surface of the tank (Wada & Mitsuda 1976). Oxygen saturation has also been found to decrease with increasing salinity and the proportion of un-ionised ammonia to decrease (Alabaster & Lloyde 1982; Alzieu 1990).

The pH of the open ocean is relatively constant at approximately 8.3 (Petit 1990). Marine fish are tolerant of higher pH values than freshwater species and are generally cultured under more alkaline conditions (Brownell 1979b). An increase in pH by a factor of one increases ammonia toxicity by a factor of ten, highlighting the need for well buffered water (Petit 1990; Spotte 1992; Tudor *et al.* 1994).

Ammonia is produced as an end product of nitrogen metabolism - as excreted by fish, their invertebrate food and bacteria (Brownell 1979b; Hawkins & Anthony 1981; Alzieu 1990). The un-ionised form of ammonia - $NH_3$ is the most toxic form (Spotte 1992). Comparisons with freshwater larvae have revealed that marine larvae have a higher resistance to ammonia toxicity (Sampaio & Minillo 1995), but within marine groups, levels of resistance vary widely. It is desirable to maintain ammonia levels as low as possible. The toxicity of ammonia can be decreased by increased temperatures (Sampaio & Minillo 1995) and high oxygen saturation (Petit 1990; Wajsbrot *et al.* 1991).

The larviculture systems were designed to provide the best possible physical parameters for the rearing of marine finfish larvae, within the strictures of available resources. Consideration was given to keeping the designs simple, easy to construct, inexpensive and easy to maintain; all systems were made out of plastic in order to avoid metallic contamination (Von Westernhagen 1988).

Along with their physical environment, the nutrition of developing larvae is an important aspect influencing growth and survival. Traditionally, marine larvae have been reared on algae and zooplankton (Kanazawa & Teshima 1989; Nellen *et al.* 1981). Due to the financial, space and time costs involved with the rearing of live foods, a great deal of research has been done to find suitable inert replacement diets (Watanabe & Kiron 1994). The larval stages of numerous species have been successfully reared on inert diets, including for example, the red sea bream *P. auratus*, Japanese flounder *Paralichthyes olivaceus* (Kanazawa *et al.* 1989), silver sea bream *Sparus sarba* (Leu *et al.* 1990) and sea bass *Lates calcarifer* (Southgate & Lee 1993). However, before an inert diet can be used, the precise nutritional requirements of the species concerned needs to be known (Beck 1979; Kanazawa *et al.* 1989; Vazquez *et al.* 1994) and larvae seem to still perform better when provided with live food as at least part of their diet (Appelbaum 1985; Watanabe & Kiron 1994; Kolkovski & Tandler 1995). Live foods were therefore chosen for this study.

## Description of systems

*The recirculating filter system*

The system was designed according to Hirayama *et al.* (1988) and Spotte (1992) to accommodate a potentially larger biomass of fish than originally anticipated. This could obviate possible sublethal accumulations of toxic metabolites (Hirayama *et al.* 1988) and act as a buffer to changes in physical or chemical parameters. After construction the system was flushed with fresh water for three weeks and drained before filling with seawater. At the end of the first season, the system was disinfected with chlorine for one week, flushed with fresh water twice over two weeks and left dry for three months before being refilled with seawater for the second season. The recirculating system is illustrated in Figures 3.1 and 3.2.

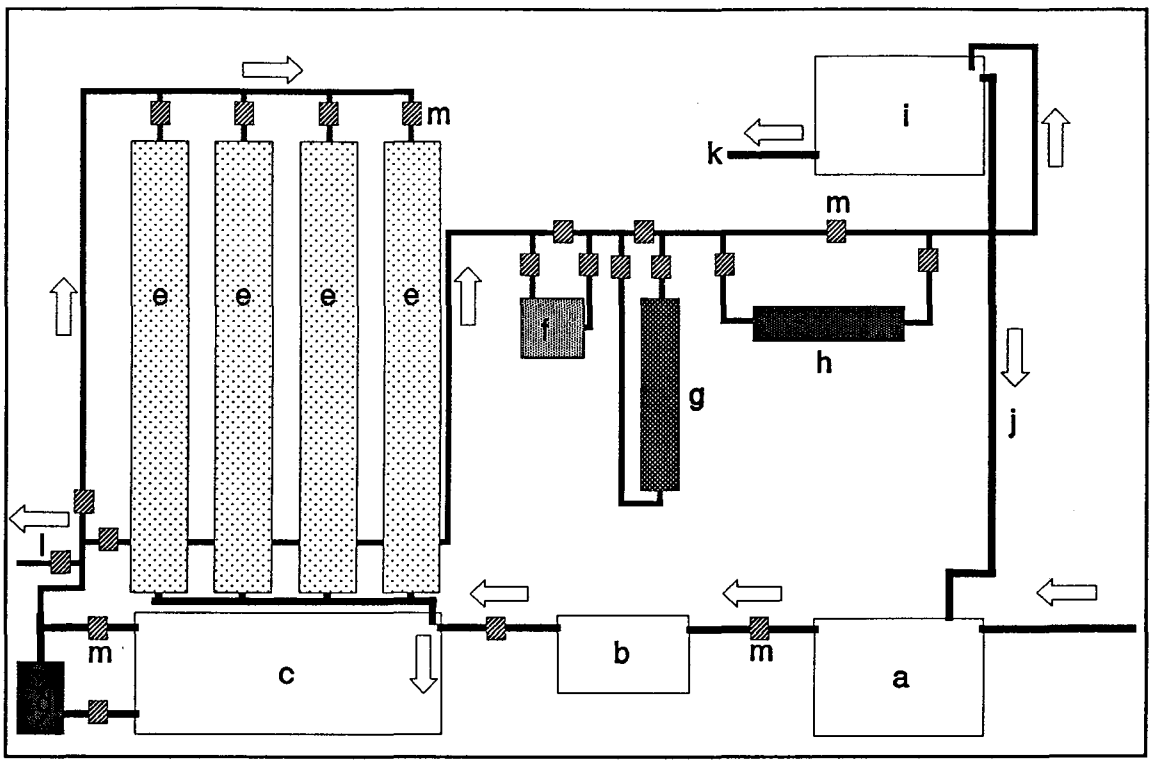



**Figure 3.1** Recirculating filter system. a=450ℓ settlement tank; b=80ℓ combined buffering, submerged filter; c=1000ℓ sump; d=1.1kw swimming pool pump; e=trickle filters (h.=1.7m x r.=80mm); f=10$\mu$m pressurised cartridge filter; g=GAC filter; h=900mm long UV sterilisation unit; i=450ℓ header tank; j=overflow drainage; k=supply to rearing tanks; l=hose to live food room; arrows=direction of water flow; striped blocks (m)=valves.

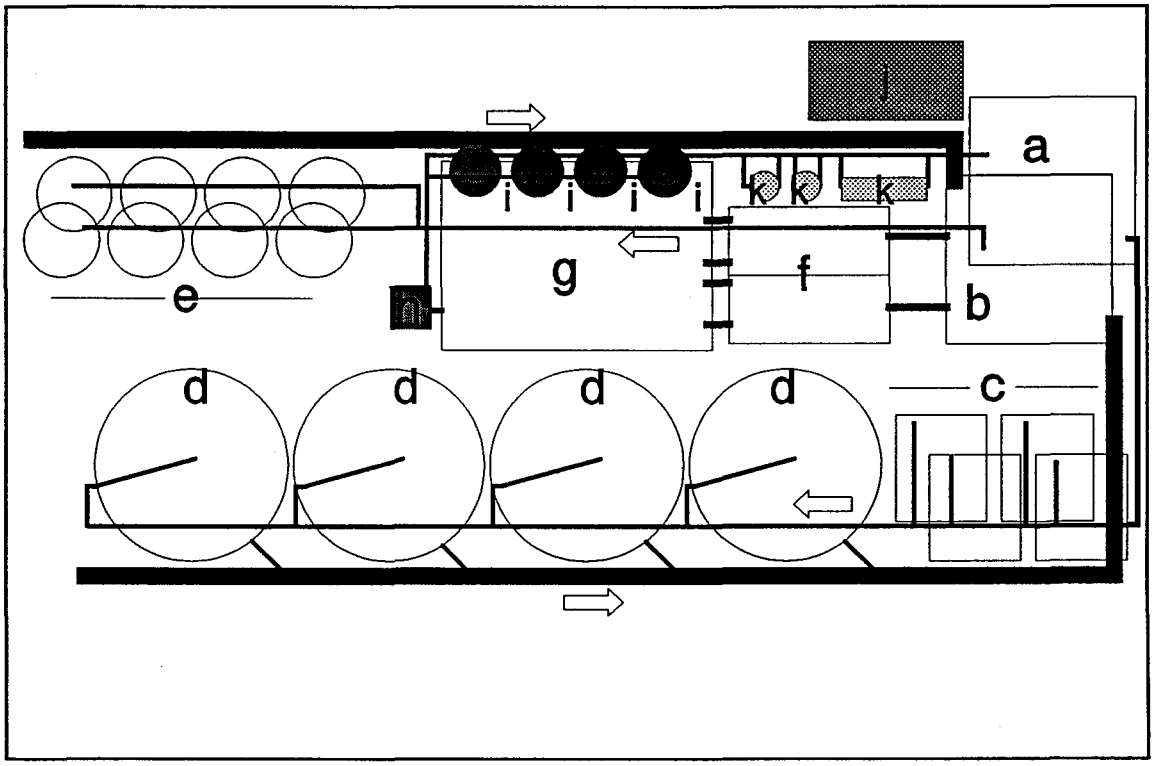



**Figure 3.2** Plan of the hatchery. a=450ℓ header tank; b=450ℓ settlement tank; c=incubators; d=400ℓ rearing tanks; e=50ℓ rearing tanks; f=80ℓ submerged filters; g=1000ℓ sump; h=pump; i=trickle filters; j=air conditioning unit; k=pressure filters; arrows=direction of water flow; room measurements=7x2.3m.

The components and characteristics are discussed in the order of labelling.

Water was drained from the rearing tanks via 110mm PVC piping into a 450$\ell$ settlement tank.

Two parallel (only one depicted in Figure 3.1) submerged filters had the bottom half of each box filled with crushed oyster shell to serve as a pH buffer (Spotte 1992). The shell was loosely packed in shadecloth bags for easy removal and cleaning. The top half of the filter boxes were filled with nylon pillow stuffing, serving as a primary mechanical filter and a submerged biological filter. Inspection of the literature has shown that systems which use a combination of submerged and trickle-type (described below) biological filtration have performed better in terms of ammonia conversion than when either of these filter types was used alone (H. Kaiser, Department of Ichthyology and Fisheries Science, Rhodes University, pers. comm.). In order to replenish the buffering capacity of the oyster shell, the flow to one of the boxes was shut down quarterly and the contents removed, cleaned and dried in the sun for several days (Spotte 1992).

A 1000$\ell$ sump was chosen in order to increase the volume of the system and to act as a reservoir. This was large enough to accommodate the contents of the gravity-drained header tank in case of a power failure, plus the contents of rearing tanks.

The pump (1.1kw "Badu", Speck pumps, Johannesburg) was fitted with an overflow back to the sump which allowed it to operate at full capacity.

Trickle filters ensured that nitrifying bacteria were separated from the surrounding air with only a thin layer of water as opposed to submerged biological filters. Nitrifying bacteria consume large amounts of oxygen and biological filters are thus more efficient when DO levels are high (Hawkins & Anthony 1981). Four PVC pipes (1.7m high x 160mm diameter, 34.18 x $10^3 cm^3$ each) were filled with filter medium in the form of PVC pipe offcuts sawn into 2-3cm lengths. Water was sprayed onto the top of the trickle filters through shower heads to promote even distribution. Each column received 3.3$\ell.min^{-1}$ of pressurised seawater, representing 61% of the total pump delivery. This water was pumped through the trickle filters in a continuous loop. The remaining 39% of the pump's output was bled off through a series of pressurised filters described below.

A pressurised cartridge filter, removed particles larger than 10$\mu$m from the water. The cartridges were cleaned once every six months.

A granular activated carbon (GAC) filter was designed for the system for adsorption of dissolved organics (Spotte 1992). The filter was made from a 1.13m length of 110mm-diameter PVC pressure piping filled with activated carbon. Water welled up through the GAC to prevent compaction of the granules. The GAC was replaced at regular intervals and reactivated by soaking in a bath of concentrated (40%) HCl for four hours.

A 900mm ultraviolet (UV) lamp was installed to control the number of unattached microbes in the system, to kill bacteria and viruses and to oxidise organic compounds into simpler constituents (Spotte 1992). Prefiltration enhanced the effectiveness of the irradiation process and so the filter was placed after the GAC filter. The UV tube was replaced at the beginning of each season.

A single exposure of pathogens to UV light is sometimes ineffective if the pathogens are again exposed to visible light after irradiation (Liltved & Landfald 1993). The header tank was darkened and the water vigorously ($1.27\ell.min^{-1}$) aerated there in order to dissipate any excessive dissolved nitrogen which could have resulted in gas bubble disease.

Pressurised air was supplied by a blower and was delivered via 25mm PVC piping which was looped around the entire hatchery. Air was tapped off the main supply line through plastic nipples (1.5mm internal diameter) of the type usually used for garden irrigation systems and was used to agitate eggs in the incubators, maintain good mixing within the larval rearing tanks and to operate the lipid skimmers.

The most inexpensive and convenient method of cooling the water was with the use of a wall-mounted 3500 watt air conditioning unit. Windows were insulated with 30mm polystyrene sheets. Sealing the windows was also necessary in order to prevent insects from entering the hatchery through cracks.

Tanks were illuminated with a single "GEC Ahlstrom", 36 watt, cool white fluorescent tube, 1m from the water surface, delivering 452 lux at the water surface.

The recirculating filtration system performed adequately. The only problem arose when additional water was needed for post metamorphosis juveniles. Because of the resistance offered by pressurised filters, the maximum delivery to the header tank was 8.24$\ell$.min$^{-1}$. When more was required by the fish, the header tank drained faster than it could be filled. The main site for slowing down the water flow was the GAC filter. The majority (51.5%) of the time taken for water to pass through the pressurised loop was taken up by water pushing through the carbon column. The pressure and UV filters consumed 27% and 21.6% of the time respectively. An additional supply line was thus installed which branched off just before the series of pressurised filters and increased flow to $\pm$28.0$\ell$.min$^{-1}$.

For practical reasons only the water parameters which posed the most immediate danger to larvae were monitored. Temperature was measured using a mercury thermometer, DO was measured using an oxygen probe, salinity using a hand refractometer, pH using an electronic pH tester and ammonia with an aquarium-type test kit (Tetra). Readings for DO, temperature, pH and salinity in the larval rearing vessels are presented in Figures 3.7 - 3.12. Flow rates of air and water, combined with ammonia readings are presented in Figures 3.13 - 3.18. Water quality was monitored for the first 40 DAH in the 400$\ell$ rearing vessels. Water quality was monitored in the 50$\ell$ tanks until the number of surviving larvae fell below ten.

Water temperatures in the hatchery were maintained as close as possible to 18°C. However, as water passed through the filters, it was heated by the pump, UV lamp and friction against the inside of the pipes, as much as three degrees above the temperature of ambient air. Tanks receiving water from the system were thus warmer than static tanks. There was a 1°C drop in temperature every 12 hours in a static 400$\ell$ tank and every 6 hours in 50$\ell$ tanks. This decrease in temperature with reduced flow increased ammonia toxicity during first-feeding and created a discrepancy in temperature between incubators and rearing tanks. Whereas water in the rearing tanks before the introduction of yolk-sac larvae was circulated at $\pm$6.0m$\ell$.min$^{-1}$, flow rate through the incubators was restricted to below $\pm$200m$\ell$.min$^{-1}$, above which eggs would get sucked against the outlet mesh. The incubators were also positioned closer to the air conditioning unit than the rearing tanks and had a much smaller volume. This resulted in a 1-2°C difference in temperature when stocking larvae.

Salinity of the main system was maintained below 37‰ due to regular water changes. Fresh water was added when necessary to counteract the effect of evaporation. One instance did arise when due to an accidental overflow the system had to be topped up with fresh water and salinity in the system was reduced to approximately 30‰. This affected a batch of newly hatched *A. argyrozona* adversely, causing the whole population to sink down to the bottom of the tank where most of the larvae died. Older fish in the system were unaffected by this event, probably due to a better ability to cope with changes in salinity (Lein & Holmefjord 1993; Provencher *et al.* 1993). When the surviving larvae were placed into more saline water they resumed normal swimming behaviour.

pH levels were kept well within tolerance levels of marine larvae and did not fluctuate above 8.3 or below 7.9.

Ammonia levels in the main filtration system did not register above zero measured by test kit. However, in the rearing tanks, ammonia levels increased during first-feeding and reached toxic levels in most of the tanks (Figures 3.13 - 3.18). Low flow, high densities of live food and high larval mortalities contributed to these high readings. Low DO levels due to low aeration and reduced temperatures contributed to the toxicity of the ammonia (Emerson *et al.* 1975). As soon as flow was resumed and aeration increased, both ammonia and DO levels improved.

*Rearing vessels*

Three different vessels were used during the first spawning season: 15$\ell$ aquariums, 150$\ell$ fibreglass funnels and 50$\ell$ round fibreglass tanks with conical bottoms (Figure 3.3). The walls of all the vessels were matte black (Ostrowski 1989). All tanks used in the first season were supplied with upwelling water from bottom inlets and were drained via banjo filters at the water surface. Banjo filters were simply constructed from $\pm$50mm-wide sections of 110mm PVC piping, the open ends of which were covered with nylon screens ranging from 100 - 500$\mu$m mesh size.

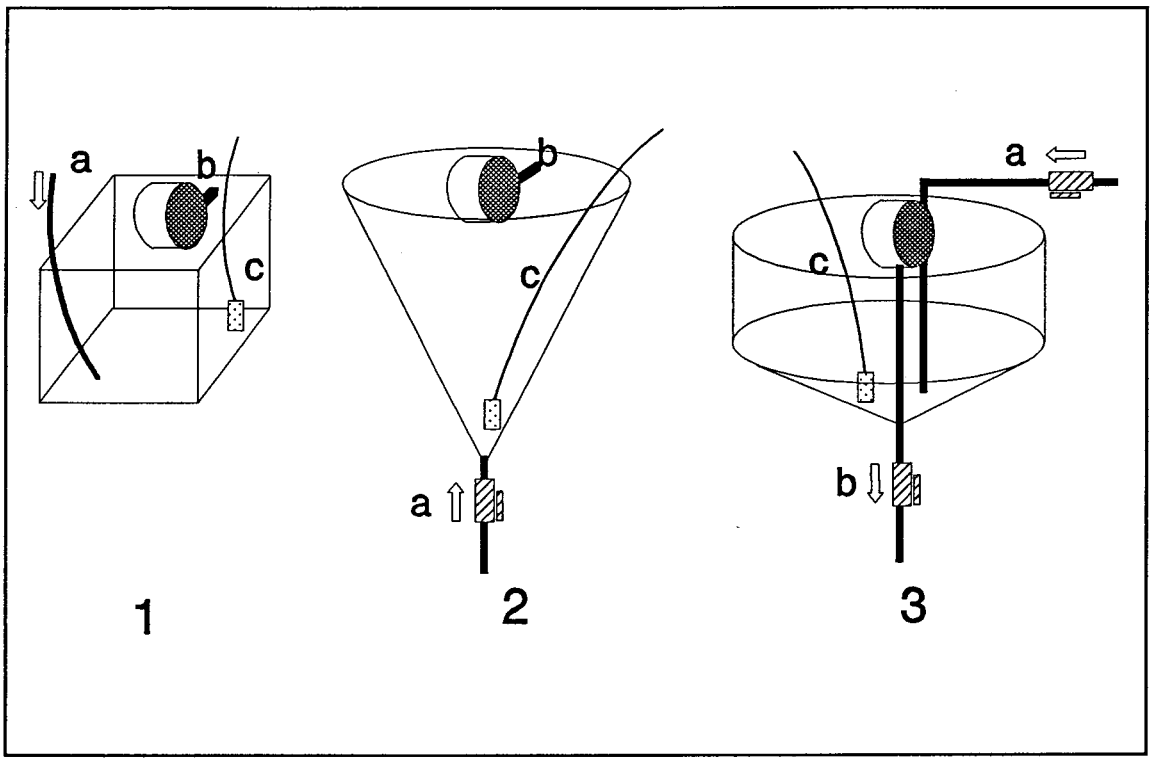



**Figure 3.3** Basic rearing vessel design. 1=15ℓ aquarium; 2=150ℓ conical tank; 3=50ℓ round tank; a=water supply; b=water drainage (banjo filter); c=aeration; arrows=direction of water flow.

The advantage of the 15ℓ aquariums was that the fish were more easily observed throughout the rearing process and the smaller volumes facilitated high food densities and easy maintenance. During the first season 23 *C. laticeps* were reared through first-feeding to ±14 days.

The 150ℓ black fibreglass funnels were not ideal rearing vessels. The fish were difficult to observe, the conical shape presented a large surface area to volume ratio and the tanks were difficult to clean. Several *C. laticeps* larvae were, however, reared to ±21 days after hatch in one of these funnels during the first season.

Both sizes of round fibreglass tanks were easily maintained and allowed for good observation of the larvae from above. Both *A. argyrozona* and *C. laticeps* were reared through metamorphosis in these tanks to 150 DAH and 134 DAH respectively, before being transferred to an outdoor rearing facility.

Although some success was achieved using 50ℓ round tanks during the first season, observations made during larval rearing during that season, combined with the fact that commercial hatcheries usually work with much larger volumes (Hussaine & Higuchi 1980; Fukuhara 1984; Minkoff 1990), prompted the installation of larger 400ℓ tanks. Because of the importance of tank size to cost and design as well as larval survival, the effect of tank size on survival was further investigated.

In order to investigate the effect of tank size on larval survival, *C. laticeps* larvae from each of the second season's spawnings were stocked into one 400ℓ and two 50ℓ round tanks. Stocking density was equivalent in both tank sizes and the larvae were reared according to the same protocol. Initial larval numbers at stocking were compared with number of survivors at the end of the rearing period. Results are presented in Table 3.1.

**Table 3.1** Survival of *C. laticeps* larvae through metamorphosis kept in two small (50l) and one large (400l) tank.

| Spawn | Number of yolk-sac larvae | | Stocking density (larvae.$\ell^{-1}$) | Post metamorphosis juveniles | | % survival in tank | |
|---|---|---|---|---|---|---|---|
| | large | small | both tanks | large | small | large | small |
| 1 | 18890 | 2361x2 | 47.23 | 24 | 12 & 0 | 0.127 | 0.51 & 0 |
| 2 | 21240 | 2655x2 | 53.10 | 40 | 4 & 1 | 0.188 | 0.15 & 0.04 |
| 3 | 72500 | 9062x2 | 181.25 | 148 | 3 & 1 | 0.155 | 0.03 & 0.01 |

Survival was better in the larger tanks in all but one case. Percentage survival and the number of larvae successfully reared through metamorphosis for the 400ℓ tanks was better than the 50ℓ tanks (Table 3.1). Statistical analysis of these results was not possible due to the difference in stocking densities between the spawnings, there was no replication of the treatments and the batches of larvae were from different spawnings.

The smaller water volumes in the 50ℓ tanks may have been less able to act as a buffer to DO, ammonia and changes in temperature. Although no substantial differences were, however, observed in water quality parameters between the two tank sizes (Figures 3.7 - 3.12)

it is possible that sublethal concentrations of ammonia accumulated which were not picked up by the test kit.

The smaller tanks had a larger wall surface area to volume ratio; it is well documented that contact between larvae and internal tank surface is undesirable (Jones *et al.* 1974; Marliave 1980; Brownell & Horstman 1987). However, the difference was relatively small - 1:21 for 400$\ell$ tanks as opposed to 1:15 for 50$\ell$ tanks - and the attraction of larvae to the wall of the rearing vessel was probably a more important influence on larvae contacting tank walls than was the extent of wall area. One observation made during the rearing process concerned the difference in hydrodynamics between the tank sizes. Currents caused by aeration, water flow and surface skimmers resulted in greater turbulence in the smaller tanks which may have hindered first-feeding and/or swim bladder inflation (Chatain & Ounais-Guschmann 1990; Naas & Mangor-Jensen 1990; Naas *et al.* 1995).

Water was supplied to the rearing tanks by means of gravity feed, ensuring equivalent flow to all tanks, allowing fine adjustment of water delivery, ensuring gentle homogenous flow and to lessen the risk of gas bubble disease. Yolk-sac larvae were provided with flow rates of $\pm 0.15\ell.min^{-1}$, allowing one complete turnover of the water within 44.44 hours in the 400$\ell$ tanks and 5.55 hours in the 50$\ell$ tanks. When the yolk-sac was absorbed and rotifers introduced after the third day after hatch, water flow was cut off completely for three to four days to enhance first-feeding and swim bladder inflation. During the second spawning water flow of $\pm 0.05\ell.min^{-1}$ was introduced at night a week after hatching. Water flow was introduced at night for two reasons: Larvae depend on light for hunting and nocturnal water flow would not interfere with feeding and to flush uneaten rotifers out of the system, ensuring only freshly enriched rotifers were available to larvae when fed the next morning. For the following week, nightly water flow was gradually increased to $0.16\ell.min^{-1}$. For the remainder of the rearing period, nightly water flow was increased steadily to a rate of $1.3\ell.min^{-1}$. After metamorphosis, flow rate was continuous at a level of $0.95\ell.min^{-1}$. Flow rates are presented in Figures 3.13 - 3.18.

Adjusting flow rate in the tank meant a compromise between water quality and mechanical stress to larvae, particularly in the early stages of development. Minimising water flow during the early life stages (usually for about the first 10 days after hatch) has been found

to be beneficial, to the extent that best survival has been recorded when water in rearing tanks was left completely static, despite an accumulation of metabolites (Kafuku & Ikenoue 1983; Lein & Holmefjord 1993). After first-feeding, it is desirable to introduce flow at the highest possible exchange rate in order to flush out the accumulated metabolites. The flow patterns resulting from this increased flow have a direct influence on larval distribution in the rearing vessel, which is an important factor influencing survival (Barahona-Fernandes 1979; Naas & Mangor-Jensen 1990; Opstad and Bergh 1993; Naas *et al.* 1995). Cripps and Poxton (1992) listed the factors influencing the flow patterns as volume and shape of the tank, nature and positioning of the inlets and outlets and the nature and velocity of the water being introduced. The hydraulic performance of the 400$\ell$ rearing tanks was examined in relation to different combinations of inlet and outlet designs in order to decrease turnover time and improve distribution, without disturbing the normal behaviour of the larvae.

Three different combinations of inlets and outlets were used during the rearing of *C. laticeps* (Figure 3.4).

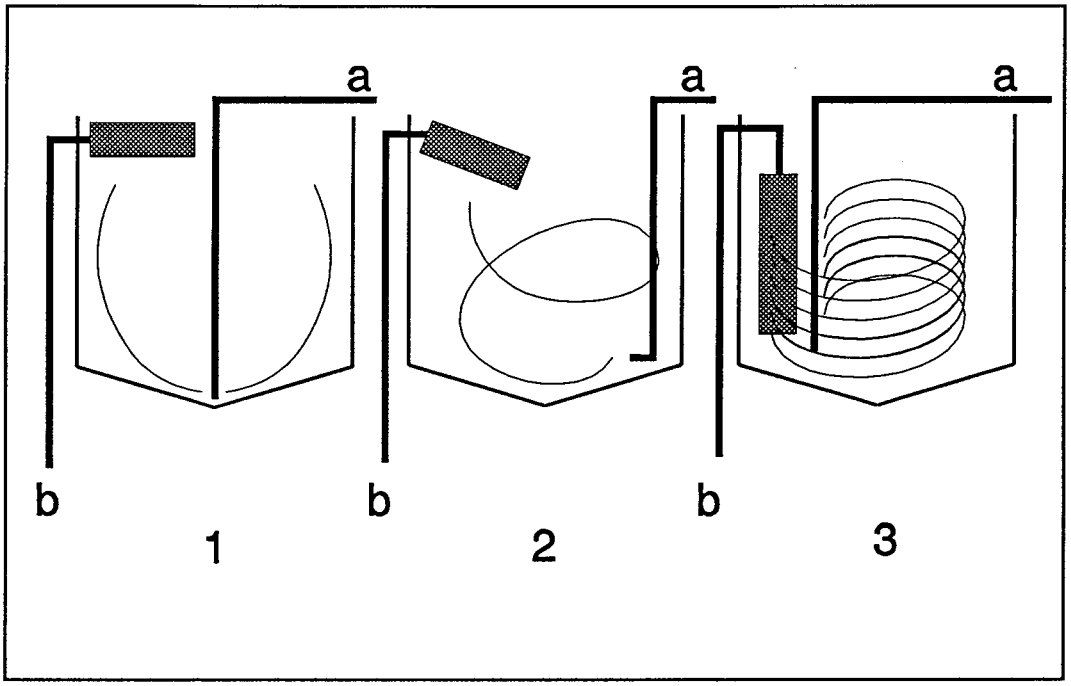



**Figure 3.4** Three different inflow/outflow combinations used for 400ℓ rearing vessels a=inflow; b=outflow; thin lines=approximate flow pattern; cross hatched blocks=mesh covered outlets.

1) Water was introduced into the apex of the sloping tank bottom to produce an even, upwelling flow. The outflow screen was positioned horizontally at the surface in an attempt to drain lipids. Larvae and rotifers concentrated in the upper layers of the water column and particularly around the outlet. Although a pattern of upwelling water flow within rearing tanks has been found to benefit larvae by keeping both the fish and their prey in the upper layers of the water column and away from the debris that accumulates at the bottom (Opstad & Bergh 1993; Moore *et al.* 1994), the upwelling tended to concentrate larvae around the outlet if water flows were above $\pm 150 m\ell.min^{-1}$. Larvae moved to other areas of the tank once the flow was reduced. The mesh covering the outlet did not allow lipids to escape and instead reduced the lipid skimming efficiency by trapping a certain amount of lipid around the outlets.

2) The inlet was placed at the bottom of the tank, against the wall, creating a circular, upwelling flow pattern. The outlet screen was tilted into the water column to expand drainage area. The circular, upwelling flow pattern in this design helped to concentrate debris in the conical bottom. This aided self cleaning and facilitated efficient siphoning. However, the flow pattern did not improve distribution of the fish or their live food. Larvae still concentrated around the outlet screen at higher flow rates. Tilting the screen into the water did, however, increase the effective surface area for water to flow out of the tanks and improved the efficiency of the skimmers. Because of this increased drainage area, flow could be increased to $300m\ell.min^{-1}$ (turnover time = 22.2 hours) without the larvae collecting around the outlet.

Water was introduced into the tank through holes drilled along the length of the inlet pipe, creating a circular flow. The outlet screen was positioned vertically in the water column and was a third longer than the other two outlet screens, presenting a larger surface area for drainage. The circular water current created with this design effectively concentrated debris in the bottom of the tank and improved larval distribution. Because water was both introduced and drained over a wide surface area there was less turbulence than the other designs and the larvae were less disturbed by the flow. Water flows of over $1.2\ell.min^{-1}$ could be introduced without bunching the larvae or disturbing feeding behaviour. A circular flow pattern, as compared with an upwelling one, has been found to benefit the growth and survival in the larvae of walleye *Stizostedion vitreum* (Barrows *et al.* 1993a; Moore *et al.* 1994). Stickney & Wu Liu (1991) have found that contact with the walls of the tank can prove fatal to larvae and circular tanks with peripheral current flows will keep larvae away from the sides of the container (Marliave 1980). Upwelling currents have also been known to inhibit swim bladder inflation (Barrows *et al* 1993b).

All three designs were applied to the same tank and the consequent distribution of the larvae observed. Flow patterns within the tanks were traced by introducing water at a different salinity and temperature into the tanks and observing the turbulent pattern of mixing. Water was introduced to all tanks through 20mm-diameter PVC piping via gravity feed from the header tank. Drainage screens were made from lengths of 110mm-diameter PVC pipes. Sections were cut out of the pipes, to form a frame onto which 100$\mu$m plankton mesh was siliconed during the

rotifer feeding stage, and 500$\mu$m mesh during the *Artemia* feeding stage. After weaning onto inert feed the outlet screens were removed. There was a tendency for the outlet screens to become blocked within several days of use. Initially the screens were changed with new ones once a week while the old ones were cleaned and sterilised. To reduce maintenance the stiff plankton meshing was replaced with nylon stocking stretched over the frame. This system had several advantages. 1) Used stockings were inexpensive. 2) As holes in the stocking blocked up, the stocking stretched inwards, expanding the mesh size and minimizing the need for replacement of the screen. 3) The mesh size of stocking material was smaller than 100$\mu$m which meant that smaller food organisms could not escape.

*Incubators*

The pelagic eggs of marine larvae were incubated in separate containers from those in which the larvae were reared so that infertile or dead eggs did not contaminate the larval rearing environment (Devauchelle *et al.* 1986). Two types of incubators were used, 15$\ell$ and 60$\ell$ glass aquaria, and 10$\ell$ plastic cones. These are diagrammed in Figure 3.5.

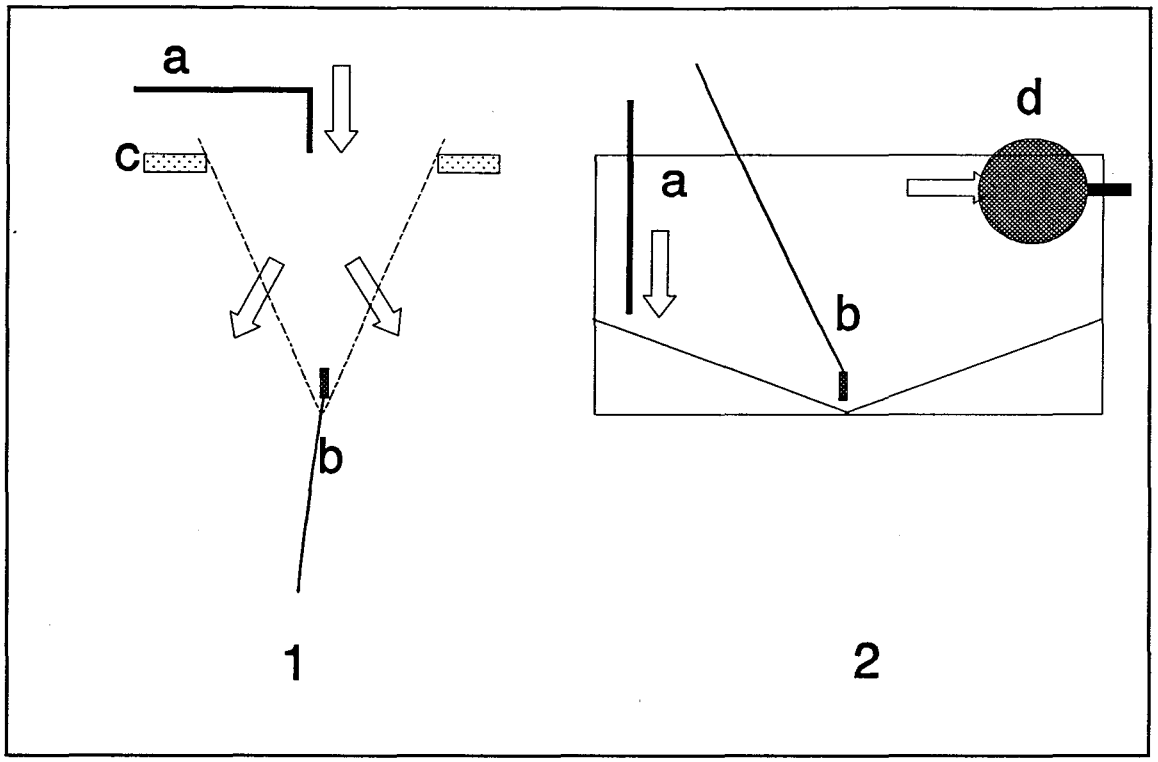



**Figure 3.5** Cross sections of two incubator designs. 1=plastic cone; 2=glass aquaria; a=water supply; b=aeration; c=polystyrene collar; d=drainage; arrows=direction of water flow.

The glass aquaria were supplied with filtered seawater delivered through standard aquarium hosing. Water was drained at the surface via 100$\mu$m banjo filters and vigorously aerated (400 m$\ell$.min$^{-1}$). Sixty-litre glass aquaria were modified with panels of glass siliconed into the aquarium, forming a false bottom which sloped to the middle. The depth of the 15$\ell$ aquaria (20cm) proved too shallow for efficient separation of floating (fertilised) and sinking (unfertilised) eggs, causing live eggs to be siphoned out along with dead ones and dead eggs to be circulated among live ones, increasing bacterial buildup.

The depth provided by the 60$\ell$ glass aquaria improved separation of dead from live eggs; simplifying the siphoning procedure and the sloping bottom improved the function of these incubators by concentrating dead eggs in the V of the slope, making siphoning more efficient and mixing better. There were, however, several drawbacks to the use of these aquaria. Between batches of eggs they needed to be moved for cleaning and sterilisation.

The glass was easily damaged during this process. The tanks were too heavy to be moved when full and hatched larvae had to be transferred to rearing tanks using a jug. The transfer process was a potential cause of stress.

The plastic cones had 50mm-diameter holes drilled in the sides, covered by 500$\mu$m mesh. The necks of the cones were fitted with a polystyrene collar which allowed them to be floated in the rearing tanks. Air was introduced into the bottom of the cones in order to agitate the eggs. Filtered seawater was introduced into the top of the cones; it flowed out through the mesh and drained out of the rearing tank. The plastic cone incubators were less ideal because it was not possible to observe the eggs during incubation, impeding removal of unfertilised eggs. Dead eggs were also inevitably transferred into the rearing tanks along with hatched larvae. Overall, the incubator designs appeared to be feasible. Due to their physical structure and physiology, pelagic eggs have been found to be resistant to both physical and chemical stresses (Blaxter 1988; Barnabe 1990).

*Surface skimmers*

Food offered to marine larvae needed to be enriched with lipids containing essential fatty acids. When introduced to the water in the rearing vessels these lipids floated to the surface, forming a film on the meniscus and inhibiting swim bladder inflation (Chatain & Ounais-Guschmann 1990). Several methods have been used to remove surface films including fine surface sprays (Barrows *et al.* 1993b), aeration (Barahona-Fernandes 1979), siphoning (Chapman *et al.* 1988), sprinklers, hydrojets and hydrophillic food emulsifiers (Chatain & Ounais-Guschemann 1990). The use of a blower-type surface skimmer was an effective means of removing surface film oil without creating disruptive surface currents or other undesirable side effects and has been extensively used by commercial hatcheries (Foscarini 1988; Chatain & Ounais-Guschmann 1990). Blower-type skimmers work by blowing air onto the water at an acute angle to the surface and into the neck of a floating trap. This sets up a current which draws surface water along with floating lipids into the trap while excess water flows underneath the trap. Although the principles behind the functioning of surface skimmers are simple, perfecting a design which worked efficiently proved to be quite difficult.

The design which functioned best is illustrated in Figure 3.6. The trap was made from thin gauge, 25mm PVC electrical conduit piping. Once bent into a spiral the ends of the pipe

were sealed with 25mm nylon stop ends. Thin gauge piping was used because it was easier to bend and made the trap lighter and it thus floated higher in the water. When thick gauge PVC pressure piping was used, the trap would float too low in the water. At high internal surface tensions, lipids would escape over the top of the pipe, particularly if the trap was splashed. Air was supplied through two aquarium hoses which were positioned approximately 5cm above and 10cm outside of the mouth of the trap. The hoses were attached to a fixed horizontal pipe so that their angle and position could be adjusted. The trap was attached to the horizontal pipe with a loose fitting anchor so that it could swivel according to the water level in the tank.

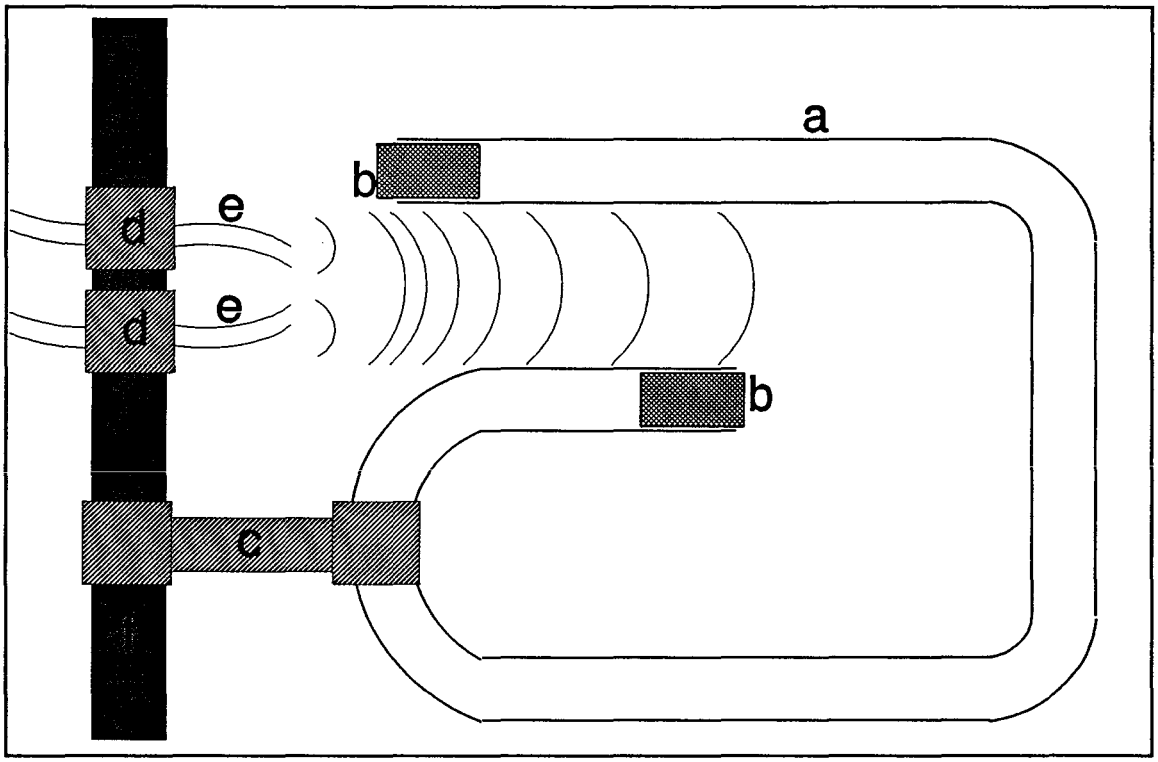



**Figure 3.6** Overhead view of lipid skimmer. a=pvc piping trap; b=stop ends; c=pivoting anchor; d=adjustable clamps; e=air supply tubes; f=horizontal pipe; (dimensions=30x22cm).

*Food and feeding*

Live food was cultured in a room adjacent to the hatchery in seawater extracted from the recirculation system. The water was sterilised in batches of 25$\ell$ over 6 hours with 0.125g granular chlorine, giving a $Cl^-$ concentration of 1mg.$\ell^{-1}$. Chlorine was deactivated with 0.375g sodium thiosulphate per litre.

Two species of algae were cultured, *Chlorella* spp. and *Isochrysis galbana* (Tahitian strain), according to established techniques (Richmond 1986). The algae were grown in glass carboys and plastic bags suspended from a frame supplied with fluorescent light (450 lux). Algae was also cultured in outdoor plastic pools (1300$\ell$) into which rotifers were stocked. The pools were filled with 35‰ seawater and fertilised with 0.05g.$\ell^{-1}$ superphosphate and 0.02g.$\ell^{-1}$ ammonium phosphate, following the recipe of Doi *et al.* (1994). The pools were vigorously aerated (3.6$\ell$.min$^{-1}$) and topped up with tap water to allow for evaporation.

The water in the outdoor pools turned green after 2-3 weeks, depending on weather conditions. The organisms making up the greenwater consisted of a mixture of *Chlorella* spp. and photosynthetic bacteria. Occasionally the species composition changed in the pools to a diatom dominated one, making the water go brown; otherwise the algal culture lasted approximately one month before the algae turned yellow and died or were too heavily contaminated with rotifers. Once the pond had turned green it was either seeded with rotifers or used as a source of algae for the indoor rotifer cultures. Towards the end of the second season, it was impossible to keep rotifers from contaminating the greenwater ponds and eventually clearing the water. After contamination no amount of fertilisation could reestablish the algal culture.

The cultured rotifers *Brachionus plicatilis* were a local (L) strain from the Sundays River estuary isolated by a tropical fish hobbyist in Port Elizabeth. Rotifers were cultured indoors in 20$\ell$ suspended plastic bags, 30$\ell$ plastic tubs and 150$\ell$ fibreglass funnels, and outdoors in 1300$\ell$ plastic pools. Rotifers were stocked at $\pm$10 individuals.m$\ell^{-1}$ along with 2-3$\ell$ of microalgae (*Isochrysis galbana* or *Chlorella* spp.). Thereafter 500mg of fresh baker's yeast was added twice a day. Rotifers were also cultured in the outdoor algal pools.

The most convenient container for indoor culture of rotifers proved to be the disposable plastic bags as they could be harvested easily and did not need to be sterilised. Indoor cultures performed better at temperatures above 28°C and salinities below 24‰. Rotifers were harvested from the bags within 10±4.3 days at 28.5°C, having reached maximum density of 120±25 rotifers.m$\ell^{-1}$.

Rotifer densities in the pools averaged 50±25 rotifers.m$\ell^{-1}$. This population could sustain daily harvesting of approximately two million rotifers per day without adversely affecting the population, as long as ambient, daytime temperatures remained above approximately 24°C. As soon as the weather became overcast and temperatures in the pond dropped below 15°C for longer than five days densities quickly dropped to less than 1 rotifer.m$\ell^{-1}$. Rotifers cultured in the ponds were a bright pink-orange colour as opposed to the pale pink colour of rotifers cultured indoors, suggesting a better nutritional value.

Marine fish have an essential requirement for the (*n*-3) highly unsaturated fatty acids (HUFA) eicosapentanoic (EPA, 20:5*n*-3) and docosahexanoic (DHA, 22:6*n*-3) (Kanazawa 1993; Koven *et al.* 1992; 1993), in ratios that are species specific (Vazquez *et al.* 1994). It is an area of research into larval rearing which has contributed significantly to the development of larviculture and has enjoyed a great deal of attention in the literature. Commercial enrichment media were used to bring the cultured live food up to the correct nutritional value needed to support normal development (Fernandez-Reirez 1993).

After harvesting through 60$\mu$m floating mesh sieves, approximately five million rotifers were placed into a 15$\ell$ plastic bucket of sterilised seawater, to which was added 0.53g of liquid "Super Selco" (INVE) homogenized with seawater for five minutes. Another 0.53g was added after 3 hours. After 8 hours of enrichment, rotifers were cleaned of excess lipids before being fed to larval fish. Initially, rotifers were transferred into a cleaning apparatus where they were flushed in fresh seawater for 15 minutes. Cleaning was improved by transferring the rotifers into a 2$\ell$ glass container of fresh seawater illuminated from the side. The rotifers were left for 15 minutes during which time healthy rotifers concentrated in the middle of the water column, away from dead rotifers and debris on the bottom and lipids on the surface. They were then siphoned onto a 60$\mu$m mesh. Rotifers were introduced into the trap of the surface skimmers in order to

avoid contamination of the surface with any remaining enrichment lipids. Rotifer density was monitored to maintain the recommended 15 rotifers.m$\ell^{-1}$ (Tandler & Mason 1983; Gadomski *et al.* 1992).

Great Salt Lake *Artemia* cysts (Bio Marine Brand) were hatched in 2g batches each day in 2$\ell$ plastic bottles. The bottom was cut off the bottles, the neck painted black and an airstone placed into a hole drilled into the lid. Before hatching, the cysts were hydrated for 3 hours in 300m$\ell$ of fresh tapwater; 20m$\ell$ of liquid bleach was added in order to disinfect and decapsulate the cysts. The mixture was then vigorously ($\pm 6\ell.min^{-1}$) aerated for approximately five minutes, or until the cysts changed from grey-brown to orange. They were rinsed under running tapwater in a 100$\mu$m mesh sieve until the smell of chlorine disappeared (approximately 10 minutes). The cysts were then returned to the bottle and incubated at $\pm$23°C for 24 hours in 25‰ seawater. Hatched nauplii were harvested by shutting off the air flow and letting the unhatched cysts fall to the bottom of the incubator. Because the neck of the bottle was dark, the nauplii swam towards the lighted surface and could be siphoned with ease onto a 100$\mu$m mesh (Verischele *et al.* 1990). Only about 60% of the cysts hatched after 24 hours. The remainder were harvested at 48 hours. *Artemia* were enriched using similar techniques as those used for rotifers, except that 4.5g of Super Selco was used in the 15$\ell$ bucket and they were only enriched once. *Artemia* were offered to the fish as soon as the fish began to chase copepods in the tank. After weaning was complete, enriched *Artemia* were fed twice a day, in order to maintain a density of 5.m$\ell^{-1}$.

A population of the ubiquitous estuarine copepod *Acartia longipatella* (Jerling & Wooldridge 1994) was introduced into the outdoor pools and into the recirculating system along with seawater transported from the Kowie River. Densities in the rotifer cultures was minimal making up approximately 0.5% of the population. The copepods reproduced once they had been introduced to the rearing tanks with the live food, feeding primarily on detritus on the bottom and sides of the tank.

A variety of inert food was offered to the fish for weaning. Prepared diets included "Lansy W3" (INVE) and salmon starter pellets (70% protein), minced frozen fish (gurnard *Chelidonichthys* sp., roman *C. laticeps* and pilchard *Sardinops ocellatus*) and frozen mussel *Mytilus* sp. Inert food was offered as long as the fish consumed it within five minutes.

Once weaned onto inert diets, the juveniles were fed to satiation three to four times daily. The process of weaning larvae and juveniles onto inert diets differed markedly between the species. *A. argyrozona* did not wean easily and although fish occasionally accepted pieces of fish flesh they lost condition as soon as live feeding was stopped. *Chrysoblephus laticeps* larvae weaned onto inert diets very quickly, but only if live foods were excluded. After regular feedings of fish flesh or pelleted foods, juveniles accepted the new diet within two days of initial feeding.

# Conclusions

*Recirculating system*

The problems of restricted water flow through the pressurised filters limited the amount of water available to the rearing vessels. This problem was solved by diverting a portion of the flow around these filters. However, the effect of this diversion on organic carbon and bacterial levels was not monitored. In a larger scale operation, this kind of problem would be best solved by placing a number of filters in parallel or using filters with larger volumes in order to improve water flow. Cooling the water with air conditioning can create discrepancies in temperature which can be otherwise avoided by using submerged chiller units or heat exchange.

*Water quality*

Because of the demand that marine larvae have for high water quality, investment in an up-to-date water quality laboratory, dedicated to the analysis of contaminants in seawater would be essential to large-scale larviculture research. Crude water quality analysis did provide an indication of certain trends. Water in the filtration system itself did not appear to deteriorate, no matter how much the system was loaded. (Subsequent stocking of the system with approximately eight kilograms of adult ornamental fish (*Abudefduf sordidis*) did not produce any deterioration in water quality). Problems with water quality emerged when water exchange and aeration needed to be reduced in the rearing vessels during first-feeding. The concentration of dissolved ammonia increased substantially during this period due to high densities of live food and larval mortality. The toxicity of the ammonia increased due to low water temperature (as a result of static water) and low levels of DO. The benefits of reduced water flow during first-feeding must be weighed against the toxic effects of accumulating metabolites under experimental conditions. Large tank volumes and refined inlet and outlet designs can ease this problem.

*Rearing vessels*

The rearing vessels were positioned in the hatchery according to space requirements. Accessibility to the tanks for cleaning and monitor the larvae is a vital component of hatchery design. Post-settlement larvae (see Chapter 4) were also disturbed by activity near tanks placed at foot level (Figure 3.2).

*Incubators*

The design of an incubator may advantageously include the following points: 1) They should be made of a lightweight, transparent, resilient material such as perspex or clear PVC. 2) They should be cylindrical with the water column being high enough to allow efficient separation of dead from live eggs. 3) They should have a sloping or conical bottom fitted with a tap for easy removal of dead eggs. 4) They should be light enough to be carried and small enough so that hatched larvae can be poured from the incubator directly into the rearing tank. (A similar design made from 1m long and 20cm in diameter plastic pipes and sealed at one end have been used to successfully incubate halibut *H. hippoglossus* embryos (Stickney & Wu-Liu 91)).

*Surface skimmers*

Maximum flow rates of air delivered to the skimmers from the blower was 15.24$\ell$.min$^{-1}$. This flow is insufficient to operate skimmers of the type used in most modern hatcheries (Foscarini 1988). Air in these skimmers is blown into a triangular or spiral trap through a series of small holes drilled into a pipe positioned above the neck of the trap. This allows the neck to be the widest part of the trap, thus enhancing function. The problem of insufficient air supply to the lipid skimmers can be solved by using either larger-diameter delivery pipes (a minimum size would be $\pm$20mm diameter) or by providing air through a compressor rather than a blower.

*Food and feeding*

1) Production protocols must be established for the rearing of live foods. In order for larviculture technology to progress in South Africa, research will importantly need to include the establishment of efficient and least time consuming methods for the production of algae and live food.

2) Rotifers and algae cultured outdoors can perform better than those indoors. Considering the South African climate and reduced maintenance needed for outdoor cultures, any research into live food culture should include an investigation of semi-intensive, outdoor cultures.

3) The presence of copepods on the bottom of the tanks may be an asset to tank hygiene and larval nutrition. There is a constant search for new live food resources worldwide and local species should not be ignored as possible culture candidates.

4) *Argyrozona argyrozona* larvae are not easily weaned onto an inert diet whereas *C. laticeps* wean relatively easily, taking a variety of inert foods soon after introduction. This aspect alone may preclude *A. argyrozona* from being a good candidate species, but adds to the suitability of *C. laticeps* for culture.

5) Both *A. argyrozona* and *C. laticeps* can be reared from yolk-sac larvae to juveniles using the standard formula of enriched rotifers and *Artemia* followed by an inert diet.

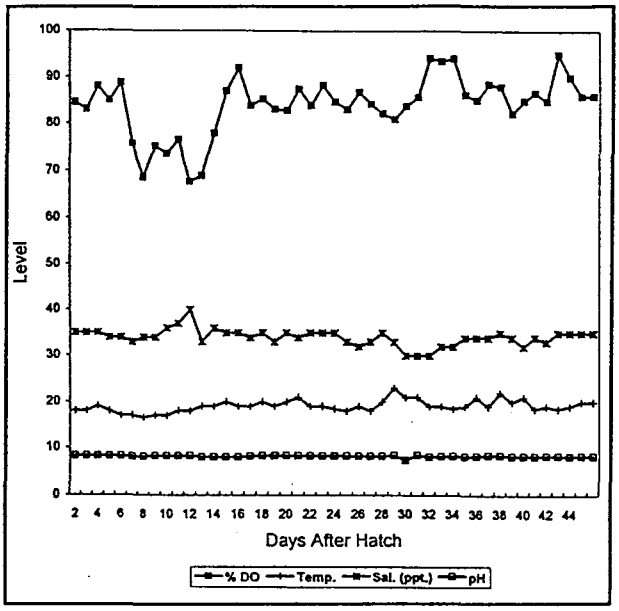



**Figure 3.7** Water quality parameters for *C. laticeps* spawned during the second season, first spawning, stocked into a 400ℓ tank. Larval survival=0.127%

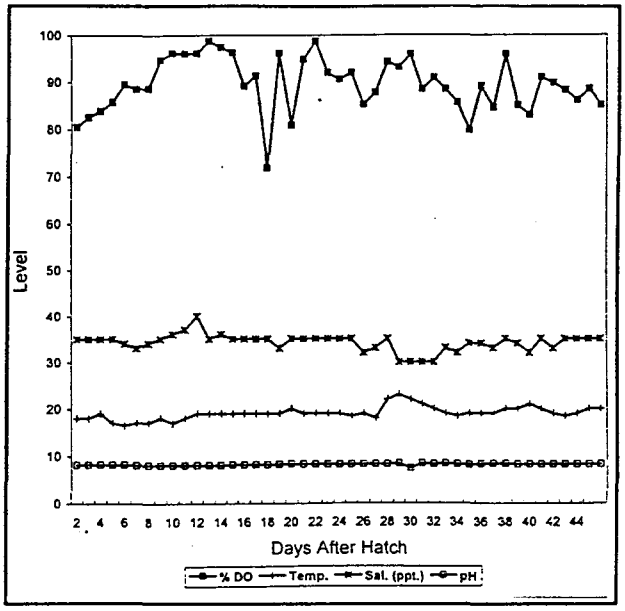



**Figure 3.8** Water quality parameters for *C. laticeps* spawned during the second season, second spawning, stocked into a 400ℓ tank. Larval survival=0.188%

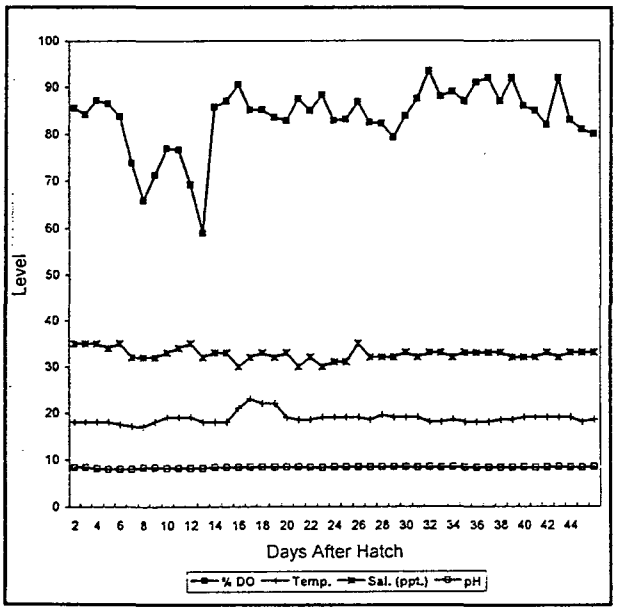



**Figure 3.9** Water quality parameters for *C. laticeps* spawned during the second season, third spawning, stocked into a 400ℓ tank. Larval survival=0.155%

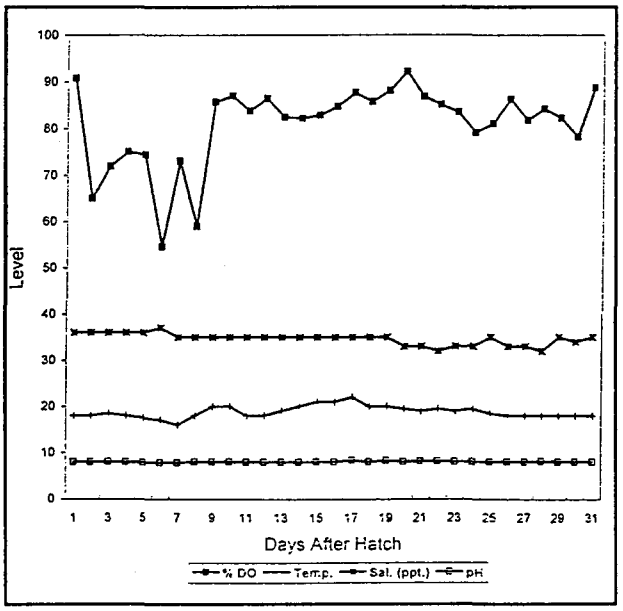



**Figure 3.10** Water quality parameters for *C. laticeps* spawned during the second season, first spawning, stocked into a 50ℓ tank. Larval survival=0.51%

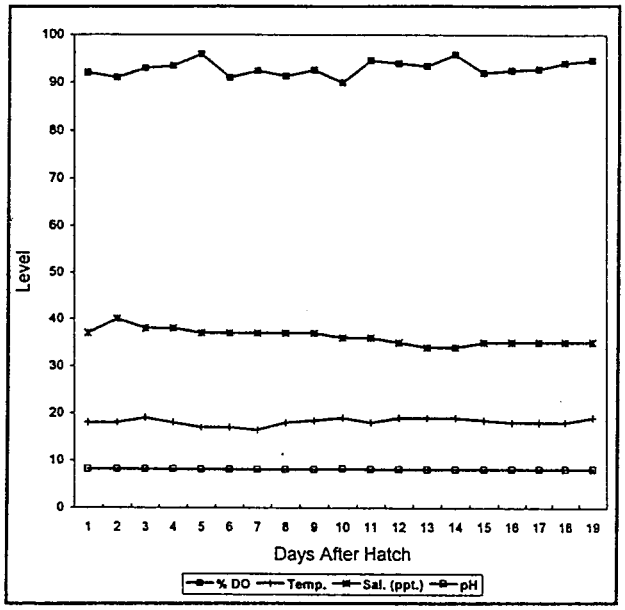



**Figure 3.11** Water quality parameters for *C. laticeps* spawned during the second season, second spawning, stocked into a 50ℓ tank. Larval survival=0.15%

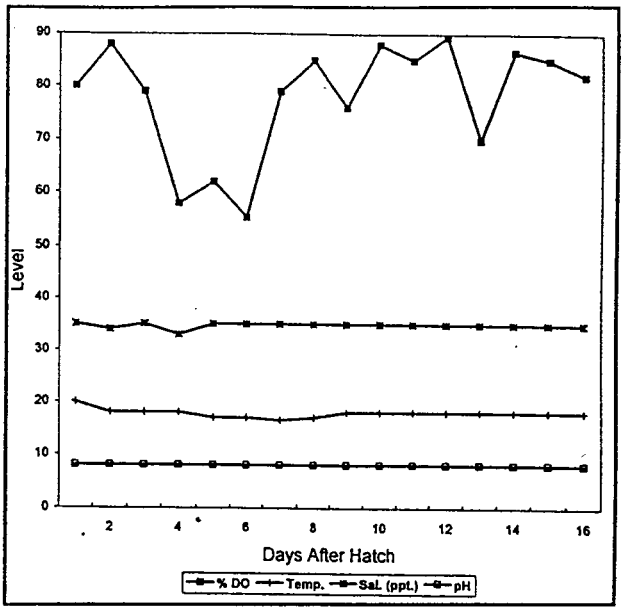



**Figure 3.12** Water quality parameters for *C. laticeps* spawned during the second season, third spawning, stocked into a 50ℓ tank. Larval survival=0.03%

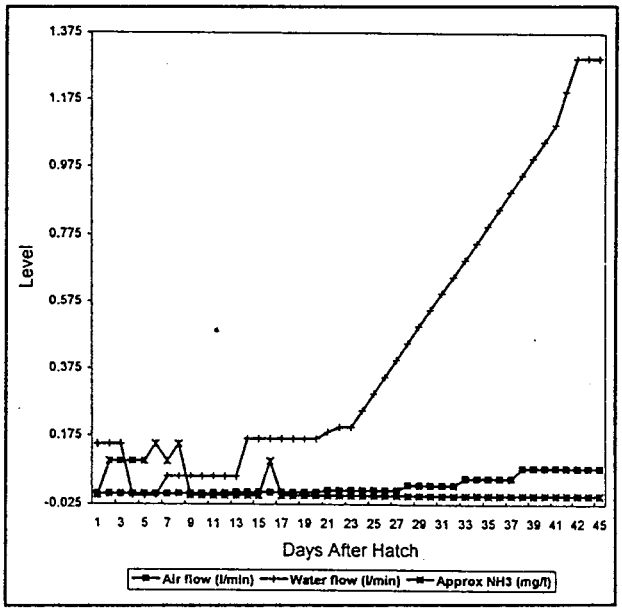



**Figure 3.13** Flow rate vs ammonia levels for *C. laticeps* spawned during the second season, first spawning, stocked into a 400ℓ tank.

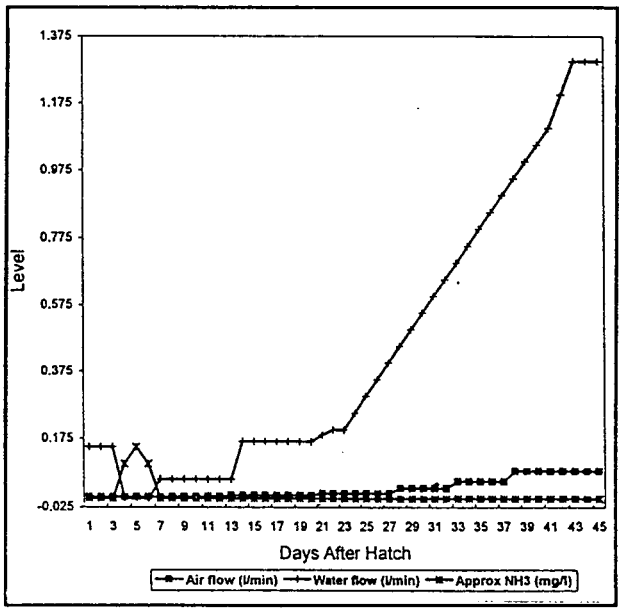



**Figure 3.14** Flow vs ammonia level for *C. laticeps* spawned during the second season, second spawning, stocked into a 400ℓ tank.

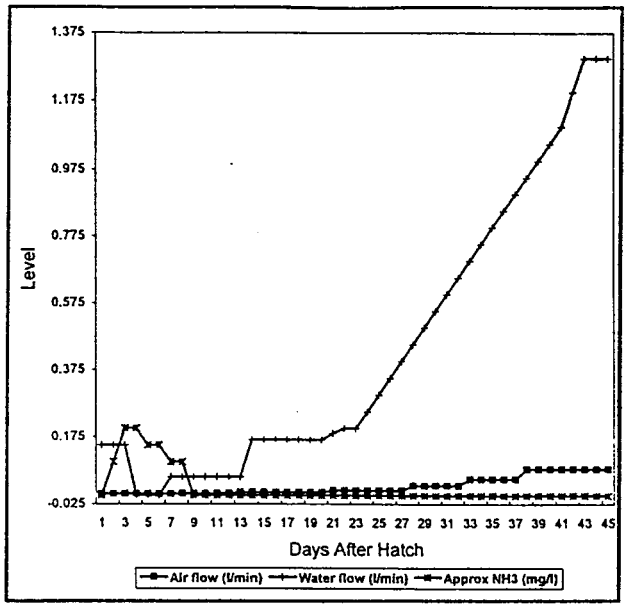



**Figure 3.15** Flow rate vs ammonia levels for *C. laticeps* spawned during the second season, third spawning, stocked into a 400ℓ tank.

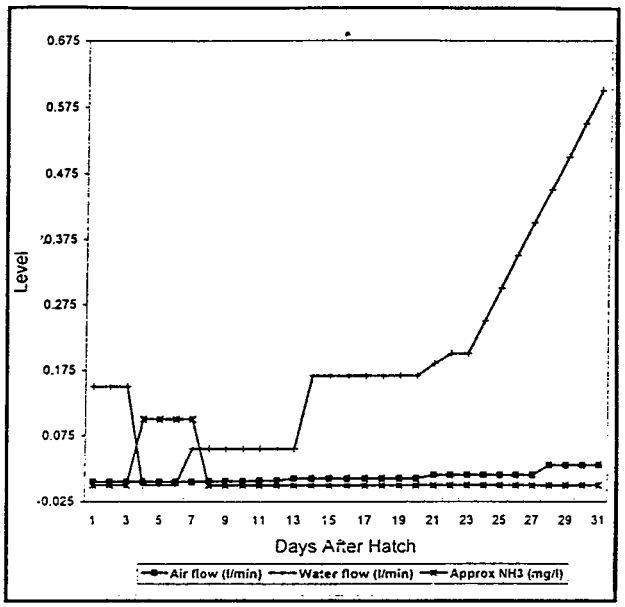



**Figure 3.16** Flow vs ammonia level for *C. laticeps* spawned during the second season, first spawning, stocked into a 50ℓ tank.

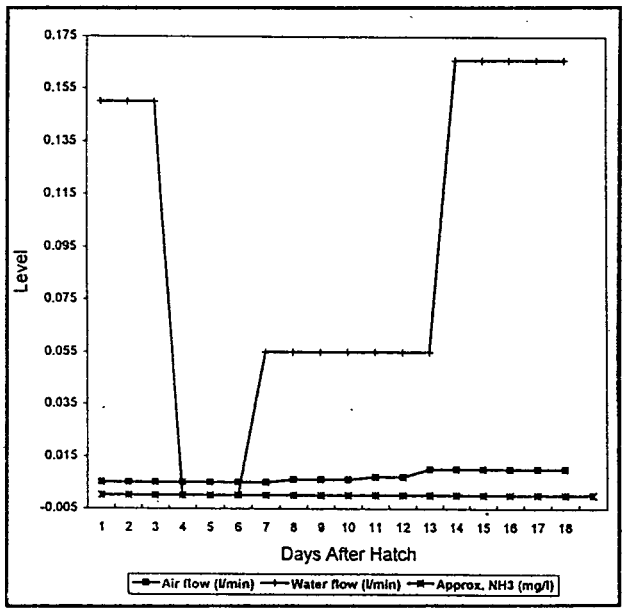



**Figure 3.17** Flow rate vs ammonia levels for *C. laticeps* spawned during the second season, third spawning, stocked into a 400ℓ tank.

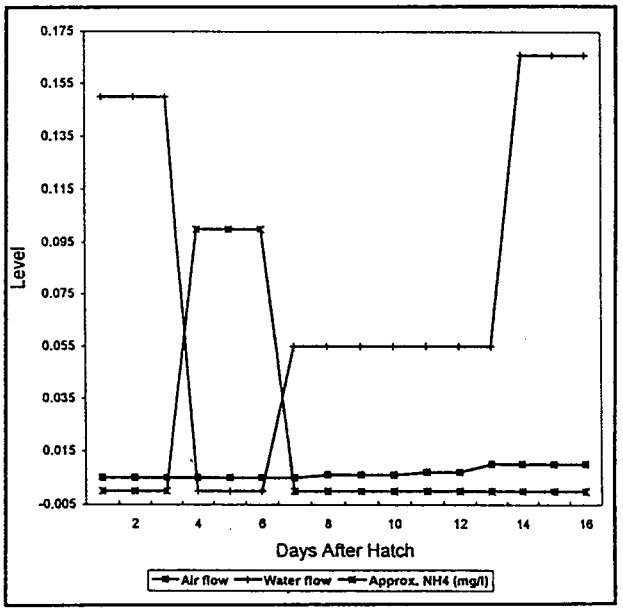



**Figure 3.18** Flow vs ammonia level for *C. laticeps* spawned during the second season, third spawning, stocked into a 50ℓ tank.

The data presented in figures 3.7-3.18 refers to that represented in tables 2.2; 2.3; 3.1.

# CHAPTER 4. CONSIDERATIONS ON LARVAL REARING AND DEVELOPMENT

## Introduction

Due to the complexities involved with marine larval fish rearing, and because of the lack of working knowledge on the subject in South Africa, it was considered essential to document all observations made during the rearing process. Statistical analysis of the results was hampered by a limited number of available larvae. However, by combining our observations with literature reviews it was hoped that a greater understanding of the aspects involved in larval rearing would be gained. It was also hoped that information here could be used to asses the aquaculture potential of the species reared.

Marine larvae hatch at a premature state of development and undergo constant changes in physiology as structures and organ systems are formed. Growth is therefore an important indicator of development and the less time larvae spend in the earlier, more delicate stages, the less vulnerable they are to mortality. Atlantic halibut *H. hippoglossus*, for example, have proved extremely difficult to rear in large numbers because they spend over 30 days in the delicate yolk-sac phase, during which there is comparatively little growth (Halls 1992). It is important when investigating any new species to determine what the pattern and rate of growth is and whether there are any phases where growth is either accelerated or depressed.

Whereas growth is important, survival of the larvae is ultimately the most important factor in larviculture. Although survival throughout the larval period of marine fishes is generally poor, there are stages where mortality increases due either to physiological demands on the larvae or an inability to provide acceptable artificial culture conditions (Blaxter 1988). These stages seem to be common to the majority of species with small pelagic larvae and can result in massive mortalities (May 1974; Blaxter 1988). Under culture conditions they include, in chronological order: first-feeding, swim bladder inflation and cannibalism. One more stage, settlement, although often observed during the rearing process, has not been greatly discussed in the literature. Because this process was seen to have an influence on larvae in this study, it was included as an important stage in larval development.

*First-feeding*

Marine species which produce large quantities of gametes invest very little energy in the yolk of individual eggs (Heming & Buddington 1988). After the yolk-sac has been depleted, larvae are still relatively underdeveloped. At the time when first-feeding through active predation occurs, the young larvae possess a simple eye, primitive olfaction, no gustatory organs (Senoo *et al.* 1994), a poorly developed digestive system lacking a capacity for enzyme secretion (Kolkovski *et al.* 1993; Lam 1994) and an underdeveloped muscular and skeletal system (Kohno *et al.* 1983; Fukuhara 1984). The switch between an endogenous (yolk-sac) source of food to an exogenous (live prey) one is therefore one of the most delicate and crucial stages in the early life history of marine fish larvae, resulting in massive mortalities in the wild (May 1974; Seig 1992) as well as in culture conditions (Li & Mathias 1982; Polo *et al.* 1992).

Once larvae have exhausted endogenous energy supplied by the yolk, they need to encounter an appropriate food organism or the process of starvation begins. The effects of starvation are cumulative in that a deficit of energy starts to inhibit the ability of larvae to respond to stimuli. This prevents larvae from moving toward prey sources and hinders their hunting capacity (Olla & Davis 1992). The point at which larvae do not possess enough energy to hunt is termed the "point of no return" (Strussmann & Takashima 1992), and generally occurs between 2 and 3 days after depletion of the yolk-sac (Polo *et al.* 1992). Food should, therefore, be made available to larvae as soon as possible. Offering food early in development is, however, compromised by two factors. Firstly, the high light intensities required for first-feeding (Barahona-Fernandes 1979) elicits activity from larvae at a very early age, draining limited energy resources in yolk-sac larvae (Naas & Mangor-Jensen 1990; Olla & Davis 1992). Secondly, live food enriched with fatty acids is the major source of the oily film which prevents larvae from breaking the meniscus and inflating the swim bladder (see below) (Sorgeloos & Leger 1992). It was therefore important to determine when first-feeding occurred so that light and food could be provided at the appropriate time.

In order for larvae to utilise first-food organisms, they have to be large enough to be perceived, but small enough to fit into the mouth. The range between the two is restricted within tens of microns. Prey of an appropriate size is of primary importance for successful initiation of feeding (Dabrowski & Bardega 1984; Polo *et al.* 1992). A small gape has been one of the most serious barriers to the successful rearing of many species of marine larvae (Nellen *et al.* 1981; Pourriot 1990). One of the most significant advances in marine larviculture has been the discovery of the marine rotifer *Brachionus plicatilis* (which usually ranges in lorica length from 150-300$\mu$m) as an acceptable first-food organism (Minkoff 1990). After observing large mortalities related to first-feeding during the project, it was important to determine the exact size of the gape at first-feeding and how that gape size related to the size of the rotifer.

*Swim bladder inflation*

A functional swim bladder is essential to the normal development of fish larvae (Soares *et al.* 1994). As their density increases with development, larvae with uninflated swim bladders have to swim continuously to maintain their position in the water column, wasting large amounts of energy (Webb & Weihs 1986). This depresses their growth rate (Johnson & Katavic 1984) and increases their susceptibility to stress (Chapman *et al.* 1988; Battaglene & Talbot 1992), making it a major cause of mortality in many species of larvae (Villani 1990). Hyperinflation of the swim bladder on the other hand, forces larvae to the surface (Johnson & Katavic 1984; Soares *et al.* 1994) and an abnormal swim bladder causes spinal deformities (Jones 1994; Kafuku & Ikenoue 1983). Before measures could be taken to optimise swim bladder inflation, the processes involved had to be well understood.

Embryologically, the swim bladder of all fish develops as a pouch protruding from the foregut, or the pneumatic duct, which is retained by adults in physostomous (swim bladder open to the gut) species. In physoclistous fish (swim bladder seperate from the gut) fish, the proximal part of the pneumatic duct disappears early in development, whereas the distal part develops into a closed swim bladder (Steen 1970). Most commercially cultured marine larvae are physostomous for the initial period of their development, with the formation of the gas secreting glands only occurring once the pneumatic duct has closed (Kitajima *et al.* 1985).

Histological studies have shown that if the swim bladder is not inflated by the larvae gulping air at the surface, the bladder remains closed (Battaglene *et al.* 1994) or does not develop normally (Chatain 1986). Facilitating access to the surface before the closure of the pneumatic duct is therefore one of the most critical considerations during the rearing of most commercially produced species (Morizane 1993).

Abiotic factors which influence swim bladder inflation are species specific and include surface film, aeration, current, light, temperature and salinity (Barnabe 1990; Battaglene and Talbot 1993). Of these, light is considered to be one of the most important (Foscarini 1988). The swim bladders of sparid larvae are known to contract during the day and expand at night (Leis & Trnski 1989), serving to regulate vertical position in the water column, and studies have shown a direct link between light and swim bladder volume (Kitajima *et al.* 1985). Optimising swim bladder inflation has been maximised by keeping larvae in the dark before first-feeding (Barnabe 1990; Naas & Mangor-Jensen 1990; Battaglene & Talbot 1994) and maintaining a constant photoperiod thereafter (Battaglene *et al.* 1994).

Compromising this need for reduced light is the nature of the larval eye in the early stages of development. Due to the simplified structure the eye, early teleost larvae require light at high intensities to feed (Blaxter 1986). Increased photoperiod increases foraging time and volume searched, and reduces the chance of underfeeding, particularly during the inefficient first-feeding phase (Blaxter & Staines 1971; Tandler and Mason 1983). This reasoning has been confirmed in several studies, where in the presence of sufficient food, growth and survival have been enhanced by providing larvae with constant illumination (Tandler & Mason 1983; Tandler & Helps 1985; Duray & Kohno 1988). Even though it is generally acknowledged that longer photoperiods can benefit larvae through extended feeding times, exposure to extended periods of high light intensity has also proven detrimental (Barahona-Fernandes 1979; Dowd & Houde 1980). Marine larvae have been reared under a variety of photoperiods and light intensities, indicating a certain species specificity. The influence of light regime on the balance between optimising either first-feeding or swim bladder inflation makes inquiry into photoperiod one of the most important areas of investigation when establishing rearing protocols.

*Settlement*

In cultured species where settlement is noticeable and permanent, such as the gastropods and flatfishes, the phenomenon has been the subject of numerous studies (Purchon 1968; O'Hahn 1989; Gadomski *et al.* 1992; Tong *et al.* 1992). However, it has not been discussed in much depth by roundfish larviculturists, being confined to anecdotal descriptions of the event (Hussaine & Higuchi 1980; Fukuhara 1985; Foscarini 1988; Battaglene & Talbot 1994; Senoo *et al.* 1994). This lack of attention may be because settlement has not been assumed to have any direct effects on mortality or because the process is difficult to quantify. In contrast to this, settlement in the wild is considered an important process, being directly responsible for the recruitment of young of the year to their adult habitat (Setran & Behrens 1993). The success or failure of the process thus regulates population sizes and community structure in many marine ecosystems (Robertson *et al.* 1988; Shenker *et al.* 1993).

The importance of settlement from a culture perspective, relates to the developmental stage at which it occurs. Metamorphosis either coincides with settlement, or is closely associated with it (Fukuhara 1984; Kaufman *et al.* 1992; McCormick 1993). Both *A. argyrozona* and *C. laticeps* started metamorphosing into juveniles only once they had settled. The importance of this coincidence may have been underestimated in the past. Even if the two processes are not linked, the phenomenon of settlement is a distinct one where fish often exhibit behaviour or coloration which are not apparent at any other phase of their life histories (Kaufman *et al.* 1992). Whereas before settlement, the entire population is distributed throughout the water column, during settlement, the same population concentrates into a relatively smaller volume at the tank bottom. Competition for food (which is still distributed throughout the column) and space must intensify and density must in effect increase. Another aspect is the importance of the substratum needed by the fish at settlement (Hussain & Higuchi 1980).

*Cannibalism*

Cannibalism is found throughout the animal kingdom, occurring in some form, in at least 30 families of fish (Dominey & Blumer 1984). Hecht and Pienaar (1993) suggest that carnivorous fish have a propensity towards cannibalism should the environment be conducive to it, and even those species which rarely demonstrate cannibalism in nature may exhibit it when reared under intensive conditions (Braid 1981). Mortality resulting from coeval, sibling cannibalism in fish under culture conditions may range from 15-90% (Hecht & Appelbaum 1988). Cannibalism is a serious handicap which needs to be understood and controlled by culturists (Hecht & Pienaar 1993). After first-feeding, cannibalism was one of the most important factors influencing survival of *C. laticeps* larvae.

## Observations on the development of laboratory reared *A. argyrozona* and *C. laticeps* larvae

*Growth*

Growth was estimated by randomly removing larvae from the rearing vessels throughout development, preserving them and measuring them as described in Chapter 5. A growth curve for *C. laticeps* was generated by combining samples from all three spawnings.

The growth of *A. argyrozona* larvae is not presented in detail because of insufficient sample size, but the growth of *C. laticeps* during the larval stages is presented in Figure 4.1. Figure 4.2 plots growth for both the larval and juvenile stages. The fitted exponential growth curve for the larval phase is presented in Figure 4.3. Growth through the larval stages followed an exponential pattern, typical of cultured sparid larvae (Hussain *et al.* 1981; Mok 1985; Leu 1994). Comparing growth with three other sparids cultured under similar circumstances with *C. laticeps* in this study, revealed that the growth of *C. laticeps* was not significantly different (Table 4.1), although comparisons of this type should be made with caution due to differences in rearing parameters. Egg size is mentioned because of its direct influence on growth (Knutsen & Tilseth 1985).

**Table 4.1** Comparison of growth between *C. laticeps* and some other cultured sparid larvae.

| Species | *Chrysoblephus laticeps* | *Acanthopagrus latus* | *Dentex gibbosus* | *Acanthopagrus schlegeli* |
|---|---|---|---|---|
| Temp. range (°C) | 17-22 | 18-25 | 19-22 | 18.8-25.9 |
| Growth equation | $y=1.9024e^{0.0382x}$ | $y=1.730e^{0.0349x}$ | $y=2.321e^{0.03975x}$ | $y=2.83+0.15x+0.00247x^2$ |
| Correlation coefficient | $r^2=0.897$ | $r^2=0.957$ | $r^2=0.923$ | $r^2=0.978$ |
| Egg diameter | 0.76±0.018mm | 0.83±0.02mm | 0.184±0.02mm | 0.90±0.027mm |
| Source | This study | Leu & Chou in press | Fernandez-Palacios *et al.* 1994 | Fukuhara 1987 |
| Days after hatch | | Body Length (mm) | | |
| 10 | 2.787 | 2.452 | 3.454 | 4.572 |
| 20 | 4.084 | 3.477 | 5.140 | 6.818 |
| 30 | 5.984 | 4.929 | 7.648 | 9.553 |
| 40 | 8.768 | 6.987 | 11.38 | 12.782 |

y=total length in mm

x=days after hatching

Inspection of the growth curves available in the literature reveal that the slope of the exponential curve tends to be more vertical in those species for which the technology is well established, for example the black sea bream *A. schlegeli* and red sea bream *P. auratus* in Japan (Fukuhara 1987; Foscarini 1988). This suggests that the growth rate of *C. laticeps* could be enhanced with improvements in culture technique.

Specific growth rate was calculated using the following equation:

SGR = (lnBLt - lnBL0)/t x 100

Where BLt = Length of larvae at time t; BL0 = Length of larvae at time 0; t = time in days.

The growth of *C. laticeps* was comparatively slow for the first 20 DAH with samples indicating a population specific growth rate (SGR) of 3.54mm.day$^{-1}$. SGR for the next 20 days increased

to 5.88mm.day$^{-1}$. During the early stages of development, growth was relatively constant, increasing from ±1.87mm BL at 2 DAH to 3.8mm BL at 20 DAH. At 20 DAH, length decreased slightly relative to age due to measured BL changing from notochord length to standard length due to flexion of the notochord (see Chapter 5). The most impressive feature occurred after 26 DAH. Whereas length increased a total of 2.48mm in the first 26 DAH, it increased 7.68mm in the next 15 days.

Since temperature and other environmental parameters remained constant throughout the rearing period, the sharp increase of growth after 26 days could have been a result of either a physiological change such as enzyme activity or gut development, or a change in diet or source of prey (Lindberg & Doroshov 1986; Mookerji & Rao 1994; Moyano *et al.* 1995). In order to investigate the first possibilities, extensive histological and enzymatic studies would be necessary. There were insufficient numbers of cultured larvae to conduct such studies. The life history event which coincided most closely with the increase in growth was cannibalism, which was first observed on day 26 and lasted for two weeks, ie., to day 40, a period which coincided with the period of maximum growth. Standard deviation in length increased from an average of 0.39mm before day 26 to 1.20mm after day 26 in larvae and increased further as juveniles developed. An increase in growth due to the ideal nutritional balance of siblings and increased growth depensation (difference within a population) have been observed to occur as direct results of cannibalism (van Damme *et al.* 1989; Hecht & Appelbaum 1988; Li & Mathias 1982).

At the end of the rearing process, *C. laticeps* juveniles were weighed and measured before being transferred to a grow-out facility. Length and weight of juveniles harvested at 134 DAH are presented in Table 4.2.

**Table 4.2** Final length and weight of *C. laticeps* juveniles, 134 DAH.

| Spawning | *n* | Standard Length | Body Weight | Photoperiod |
|---|---|---|---|---|
| First | 24 | 38.4 ± 4.5 mm | 4.0 ± 1.91g | 24 hours |
| Second | 40 | 36.6 ± 5.8 mm | 2.0 ± 1.19g | 12 hours |
| Third | 148 | 35.1 ± 5.5 mm | 2.1 ± 0.9g | 18 hours |

Daily growth over the entire rearing period was estimated at $0.27 \pm 0.039 mm.day^{-1}$. There was no discernable difference in growth between the three photoperiods.

Comparing growth of *C. laticeps* shows that fish reared during this study displayed substantially slower growth than that projected for juveniles i

n the wild (Lang & Buxton 1993). Whereas at four months after hatch *C. laticeps* was estimated by Lang and Buxton (1993) to have a length of 65-69mm, fish cultured in this project had grown to an average length of only $32.4 \pm 4.68 mm$. This difference was a further indication that the conditions under which *C. laticeps* were cultured, were below optimum and that growth rates could be enhanced with an improvement in rearing conditions.

*The timing of major events during the development of* A. argyrozona *and* C. laticeps

The developmental stages of *C. laticeps* and *A. argyrozona* are described in Table 4.3. The lengths of *A. argyrozona* larvae at each stage do not include standard deviations because of insufficient larval numbers available for sampling. It was also not possible to detect when swim bladder inflation occurred for this species. Due to the heterogenous distribution of larvae in the rearing vessels, quantification of survival at each stage of development would have meant the removal of large numbers of larvae. Calculating mortality by siphoning dead fish off the bottom of the tanks was not possible during the early larval stages because larvae often foraged near the bottom. Survival at each stage could, therefore, not be calculated, but was visually estimated.

**Table 4.3** Major events during the development and culture of *A. argyrozona* and *C. laticeps* larvae. BL=notochord length in preflexion larvae; SL=Standard length in post flexion larvae; DAH=Days after hatch.

| Event | *A. argyrozona* | | *C. laticeps* | | |
|---|---|---|---|---|---|
| | Time | Approx. Length | Time | Length | Approx. Mortality |
| Hatching @ 19.5°C | 26-36 hrs. | ±2.4mm BL | 26-30 hrs. | 1.76±0.04mm BL | Low |
| Yolk depletion | 2-3 DAH | ±2.48mm BL | 4-5 DAH | 2.22±0.25mm BL | Low |
| Oil globule depletion | 3-4 DAH | ±2.5mm BL | 5-7 DAH | 2.39±0.23mm BL | Low |
| First-feeding# | 3-4 DAH | ±2.5mm BL | 4-7 DAH | 2.41±0.25mm BL | Moderate |
| Bladder inflation# | * | * | 4-6 DAH | 2.40±0.25mm BL | Moderate |
| Major mortality[1#] | 8-9 DAH | ±3.0mm BL | 9-11 DAH | 2.59±0.33mm BL | High |
| Copepods & *Artemia*[2] | 14 DAH | ±3.5mm BL | 20-22 DAH | 4.10±0.80mm SL | Moderate |
| Weaning onto *Artemia*[3] | 15-17 DAH | ±3.7mm BL | 24-29 DAH | 4.49±0.72mm SL | Low |
| Settlement[4] | 16-30 DAH | ±5.5-7.5mm BL | 17-32 DAH | 5.08±1.11mm SL | Low |
| Cannibalism | 26-29 DAH | 7.5-8.6mm SL | 26-30 DAH | 4.87±0.98mm SL | High |
| Weaning[5] | 26-* | 8.6-10.6mm SL | 23-30 DAH | 4.68±0.83mm SL | Low |
| Metamorphosis[6] | 29-35 DAH | 8.6-10.6mm SL | 26-45 DAH | 9.62±2.29mm SL | Low |

#=Larvae fed on enriched *Brachionus plicatilis*.

1=Where most (approximately 90%) of the larvae died, presumably due to failure of first-feeding.

2=The first time the larvae pursued copepods which was also the first time *Artemia* were introduced.

3=The time when most of the fish were feeding primarily on *Artemia*.

4=The time after first larvae were seen to be exclusively at the bottom of the tank to the time when larvae started returning to the water column.

5=Refers to the transition from live to inert food and was measured from the first time inert food was introduced to the fish till the time that they were all feeding readily on inert food, and when *Artemia* were no longer introduced.

6=The change from larval to juvenile form as described in Chapter 5.

*=Not observed.

High mortality = 15-25% mortality per day.

Moderate mortality = 5-15% mortality per day.

Low mortality = 0-5% mortality per day.

Time to hatch and resorption of the yolk at these temperatures were not significantly different to other cultured sparid species, and the difference between *A. argyrozona* and *C. laticeps* was negligible (Wada & Mitsuda 1976; Hussaine & Higuchi 1980; Garratt *et al.* 1989; Fernandez-Palacios 1994; Cowden, unpublished data). It was significant that the oil globule lasted through first-feeding in both species, ensuring a source of energy beyond depletion of the yolk-sac.

*First-feeding*

The large majority of the mortalities suffered by both *C. laticeps* and *A. argyrozona* larvae in this study occurred at, or shortly after the time that first-feeding should have occurred (Table 4.3). A small number of larvae ($\pm$15) of both species were not transferred from the incubators with their siblings and were not fed. All of these larvae died at the same time as the mass mortality observed in the rearing tanks, confirming that this mortality was a result of unsuccessful first-feeding.

In order to determine when first-feeding occurred, seven larvae were removed from the rearing vessels each day and examined. First-feeding was determined by direct observation under a dissecting microscope. This observation was enhanced after the specimens were cleared and stained, making the gut contents (particularly the mouthparts of *Brachionus*) more visible. Dissection of gut contents proved to be unreliable. The first signs of food in the gut of at least 50% of the larvae examined appeared in *A. argyrozona* at 3 DAH and in *C. laticeps* at 4 DAH. However, it was not until 5 and 7 DAH respectively that the larvae were seen to be actively feeding on rotifers in the tanks and more than 75% of the larvae observed had a full gut so that first-feeding could be confirmed.

Rotifer length was measured from the tip of the ciliary crown to the base of the foot (Pourriot 1990). Adult amictic females of the *Brachionus plicatilis* cultured had a length of 260$\pm$20$\mu$m. The width of the lorica was 64.9$\pm$8.5% of the length, spanning 150-200$\mu$m. The female egg measured 75$\pm$0.005$\mu$m. Approximately 65% of the females carried eggs when harvested. The rotifers were identified as L-strain because of measured lengths being between 150-300$\mu$m, and the twin egg carried outside of the elongated lorica (Jug-Dujakovic & Glamuzina 1988).

The size of the gape of *C. laticeps* was measured by observing unpreserved larvae end-on under a dissecting microscope. The gape was taken as the mouth width (Shirota 1970; Blaxter 1988). The gape of first-feeding *C. laticeps* measured at 4-5 DAH was 333.03±14.4$\mu$m.

The ideal prey size for first-feeding larvae has not been conclusively determined in the literature. The recommended size of the food particle has been estimated at values ranging from 50-75% (Shirota 1970) to 70-90% (Iizawa in Barnabe 1990) of gape size, with the malleability of the food being a contributing factor (Kolkovski & Tandler 1995). Using even the least conservative estimates, only a small portion of the rotifers were available to first-feeding *C. laticeps* larvae. The gape at first-feeding was however not particularly small compared with other commercially cultured sparids eg. 154-182$\mu$m for *Diplodus vulgarus* (Jug-Dujacovic & Glamuzina 1988) and 320$\mu$m for yellowfin porgy *Acanthopagrus lates* (Leu & Chou in press), suggesting that with the appropriately sized first-food, first-feeding of *C. laticeps* could be improved. Although *B. plicatilis* has been used successfully as a first-food organism for a variety of species, several species have been found to require smaller first-food organisms such as the nauplii of certain copepods (Chao *et al.* 1993; Singhagraiwan & Doi 1993; Doi *et al.* 1994), or smaller strains of *B. plicatilis* such as the S-type which measure from 80-160$\mu$m (Jug-Dujakovic & Glamuzina 1988).

First-feeding was also influenced by water currents at the surface of the tank produced by aeration and surface skimmers. These had the effect of clumping first-feeding *C. laticeps* larvae into dense concentrations at the water surface. This clumping caused tactile stress, inducing frequent fright responses and prevented the larvae from feeding (Hecht *et al.* in press). Similar observations have been made for several other species (Barahona-Fernandes 1979; Naas & Mangor-Jensen 1990; Opstad & Bergh 1993; Naas *et al.* 1995). Sparids posses free neuromasts at hatching and startle responses were probably an escape response from perceived invertebrate predators (Pankhurst *et al.* 1991). It was assumed that first-feeding would therefore have been enhanced by having a more homogenous distribution of the larvae within the tank. In order to achieve this, aeration was increased (Barahona Fernandes 1979). This had positive effects on larval distribution but increased water turbulence, which disturbed the feeding behaviour of early larvae. Feeding for early *C. laticeps* larvae was a relatively complicated procedure, comprising three distinct phases.

1) Searching for the prey: Larvae appeared to be very selective in their choice of individual prey organisms. They searched for prey amongst high densities, even bumping into

several and withdrawing, before singling one out. This search mechanism implied one of two possibilities, that the fish were selecting rotifers small enough for ingestion, or that the fish were only able to recognise prey under the correct light conditions.

2) Aiming at the prey: Larvae drew themselves into an S posture and followed the movement of the prey with their heads. The S posture is common to many marine larvae (Senoo *et al.* 1994).

3) Capture and ingestion: The larvae thrust themselves at the prey by straightening the body out and swallowing the organism immediately. If this process was disturbed at any of these stages, the attack was aborted, or larvae missed their target. High turbulence has been observed to significantly reduce predation efficiency at or just following first-feeding with less than 10% of strikes being successful (Chatain & Onais-Guschemann 1990; Dutton 1992), and the mortality of halibut *H. hippoglossus* larvae has been found to increase with increasing air bubbling, probably due to shearing forces to which larvae are vulnerable (Opstad & Bergh 1993). Positioning of airstones and hydrodynamics within the rearing tank, to distribute larvae evenly without disturbing their feeding behaviour or causing them to bunch at the surface, could be an important consideration in future research (see Chapter 3).

*Swim bladder inflation*

In order to promote swim bladder inflation, larvae were kept in complete darkness with reduced air and water flow until the yolk sac had been resorbed. Surface skimmers were installed into the rearing vessels from hatch and were kept in operation throughout the larval stages.

Determining the exact moment of pneumatic duct closure was impossible due to insufficient samples for histological examination and the fact that detection of the swim bladder was extremely difficult in larvae smaller than 2mm BL. However, an understanding of the function of the early swim bladder and how it effects buoyancy gives a clue to when inflation

was likely to have occurred. The pelagic eggs of sparids are slightly positively buoyant. Hatched yolk-sac larvae are neutrally buoyant due to the balance of negatively buoyant yolk with a positively buoyant oil globule. The combination of a resorbed oil globule and increased density due to somatic growth results in first-feeding larvae having negative buoyancy (Kitajima *et al.* 1993). They therefore need to inflate the swim bladder as soon after absorbtion of the oil globule as possible (Chatain 1986). Swim bladder inflation therefore usually coincides with yolk depletion and the transition from endogenous to exogenous feeding (Battaglene & Talbot 1994; Battaglene *et al.* 1994; Soares *et al.* 1994).

During the yolk-sac phase *C. laticeps* larvae were distributed throughout the water column. During the first two days after yolk-sac depletion, they were distributed along the bottom of the tank in the absence of light. Similar sinking behaviour at this stage has been described for sablefish *Anopoloma fimbria* larvae (Alderdice *et al.* 1988) and Atlantic halibut *H. hippoglossus* (Opstad & Bergh 1993). Within an hour of the covers being removed, most of the larvae distributed themselves within the top 15cm of the water column, being attracted to the surface by the light gradient (Barahona-Fernandes 1979; Blaxter 1968; Blaxter 1969; Naas *et al.* 1995). This behaviour was contrary to the well known natural vertical migration of larvae in the wild and could have been indicative of an uninflated swim bladder (Kitajima *et al.* 1985, 1993). On the morning of the fourth DAH, most of the population were distributed within the upper half of the water column, indicating that swim bladder inflation had occurred. Similar changes in diel patterns with swim bladder inflation have been observed by Kitajima *et al.* (1993).

In order to determine the effect of photoperiod on *C. laticeps*, larvae were cultured under three different photoperiods. Larvae from each spawning were stocked into two 50$\ell$ and one 400$\ell$ tank at equivalent stocking densities (see Chapter 3, Table 3.2). As soon as the yolk-sac was completely absorbed, the larvae of each spawning were given 12, 18 and 24 hours of light per day for the remainder of the rearing period. Dark periods were created with the use of plastic lids which covered the tanks completely. Ten larvae from each of the large tanks were removed at 17 DAH, by which time swim bladder inflation should have been completed, then

anaesthetized with 2-phenoxyethanol ($0.5x10^{-3}m\ell.m\ell^{-1}$ seawater) and observed for inflated swim bladders with the use of transmitted light as described by Barrows *et al.* (1993a). Larvae were then placed in fresh seawater where $85\pm3.2\%$ recovered within 15 minutes and were returned to the tank.

Examination of 17-day-old *C. laticeps* larvae for swim bladder inflation showed that 66%, 67% and 72.2% of the larvae from the 24, 12 and 18 hour photoperiod tanks respectively had inflated swim bladders, indicating that photoperiod did not have a significant influence on swim bladder inflation. Unfortunately survival of larvae in the 50$\ell$ tanks was too low to warrant the risk of sacrificing fish and were thus not sampled, prohibiting statistical analysis of the results.

Although many more larvae survived through to metamorphosis in the 400$\ell$ tank receiving an 18-hour photoperiod, there was no observable difference in swim bladder inflation at 17 DAH. All the treatments were maintained under constant dark conditions before first-feeding. Drawing conclusions on the basis of buoyancy changes could have been incorrect and swim bladder inflation could have occurred before first-feeding, obscuring the effects of subsequent photoperiod on swim bladder inflation.

Survival after the initial first-feeding mortalities was lowest under a 24-hour photoperiod, improved under a 12-hour one and was best at 18 hours as many more fish were reared through to metamorphosis under this photoperiod. Little is known about the role that the dark period plays in the physiology of larval fish although Quasim (1959 in Duray & Kohno 1988) found that a dark period was instrumental to survival in *Blenneus pholis,* and reduction in growth, condition factor and survival have been recorded for sea bass *D. labrax* as a result of continuous lighting (Barahona-Fernandes 1979; Johnson & Katavic 1984). It has been postulated that optimum photoperiod would be typical of the spawning season and location of the species in question (Tandler & Helps 1985). The 18 hour photoperiod corresponded well with the photoperiod along the Tsitsikamma coast from November to January (Buxton 1990).

*Settlement*

Settlement was determined by observation. Fish were considered settled when they maintained constant contact with the substratum and no longer swam in the water column. Towards the end of the larval period, both *A. argyrozona* and *C. laticeps* exhibited a distinct change in their behaviour. After spending the majority of their time as larvae in the water column and along the walls of the tank, the largest individuals moved down to the bottom of the tank where they spent all their time touching, or very near to the substrate. Although some larvae fed on the bottom throughout the rearing period, settlement was characterised by the fact that the larvae did not leave the bottom of the tank at all. These were the most advanced individuals in the population and were noticeably paler in colour. It was at this time that larvae were first observed to be aware of movement outside of the tank and would be startled by sudden movements. This behaviour was observed to occur earlier for *A. argyrozona* than *C. laticeps* larvae, although it lasted an equivalent time period (Table 4.3). Settlement also occurred at the same stage of development for both species, being associated with the end of the post flexion stage and beginning of metamorphosis (see Chapter 5). After approximately two weeks of very close association with the tank bottom, the fish (as partially or fully metamorphosed juveniles) loosened their association with the substrate and started to move back into the water column and began to cannibalise their siblings.

Whether the settlement that occurs in the wild and that which is observed in culture conditions is the same phenomenon, still needs to be proven. Fisheries literature describes the phenomenon as "a departure of larvae from the pelagic arena and adoption of a life in the immediate vicinity of the benthos or other physical structure" (Kaufman *et al.* 1992). This closely approximates what was observed in this project and described in other literature (Foscarini 1988; Battaglene & Talbot 1994). Gadomski *et al.* (1992) observed that settlement of the Californian halibut *P. californicus* in the laboratory occurred at the same size (8mm SL) and age (1 month) as in the field. Young sea bass *Atractoscion nobilis* were also observed to move toward structures in laboratory experiments at the same size as their wild counterparts (Allen & Franklin 1992). If the two phenomena are indeed the same, then culturists could gain a great deal of insight by taking note of the occurrence and its governing factors in the wild.

There are three major factors which have been hypothesised to have an effect on settlement in the wild: food, predation and recruitment. Of the three, the first holds the most direct interest for culturists. There appears to be a change in the diet of larvae coinciding with a change of position in the water column. For example, larvae of the red drum *Sciaenops ocellatus* in the wild feed exclusively on zooplankton until they reach approximately 15mm TL when they shift to small shrimp, amphipods and small fish (Henderson-Arzapalo 1992). Similarly, two species of stichaeid fishes exhibit less dependence on water column (calanoid copepods) prey and greater preference for benthic (harpatecoid copepods) prey at settlement indicating a dietary shift to benthic prey (Setran & Behrens 1993). Gut morphology and structure of the eye also changes at settlement to prepare fish for new food in a new habitat (Senoo *et al.* 1994). *C. laticeps* larvae were observed to start preying on copepods inhabiting the bottom of the tank at about the time of settlement. These examples might reflect a transition in predation tactics (Setran & Behrens 1993; Sweatman 1993) in a search for a more nutritionally satisfying diet to fulfil the energetic demands that come with metamorphosis (Pfeiler & Luna 1984; McCormick 1993). A transition to larger prey by growing fishes would ensure maximum energy intake relative to energy expenditure (Setran & Behrens 1993). In fact if newly settled larvae do not find the necessary food organisms in the wild they will return to a pelagic way of life (Noichi *et al.* 1993; Keef & Able 1994) and some species, such as the red sea bream *P. auratus* are guided by abundance gradients of their post-settlement prey species to find their nursery grounds (Tanaka 1985).

Providing the correct food and substrate during this distinct phase may help in reducing the stress of culture conditions, and further study of the phenomenon in the laboratory may shed some light on a process which is vital to maintaining natural stocks.

*Cannibalism*

For a period of approximately two weeks, starting from ±26 DAH, *C. laticeps* larvae exhibited intense cannibalism. Non-cannibalistic (prey) fish restricted their activities to relatively small areas, remaining close to the tank wall and settled, prey fish swam nearer the tank bottom, turning frequently while feeding and ignoring their siblings.

In contrast, cannibals swam vigorously around the tank, ignoring both live and inert food offered and targeting their siblings. Initially attacks took the form of biting, with the target being the eye. Dead fish were siphoned from the bottom of the tanks during periods of cannibalism and examined under a dissecting microscope. The carcasses were measured and larvae were classified according to the number of eyes they possessed and the condition of the gut. During the eye-biting phase of cannibalism, 19.6±12.25 fish were siphoned off the bottom of the tanks per day from each tank. Of these fish, 75.59±9.98% had either one or both eyes missing. The success of attacks was dependent on behaviour, with less developed fish being oblivious of attacks until they occurred. More developed fish seemed aware of their siblings and either took evasive action by swimming away rapidly or challenged their attackers, so avoiding predation.

Approximately a week after the eye-biting type of attacks, cannibals were seen swimming with their victims in their mouths and during peak cannibalistic activity, up to half of the larvae could be seen with prey fish in their jaws at any one time. Although a portion of the fish victims were swallowed head-first, the gut region appeared to be the most common target area for this latter type of attack. Dead larvae siphoned off the bottom revealed that 64.10±8.18% had a partially or completely eaten gut, with the remainder being partially damaged in other areas. Although cannibalistic behaviour was only displayed by fish which had undergone metamorphosis, there was no discernable selection of prey according to size, with cannibals targeting fish smaller, of the same size or even larger than themselves. Similar observations have been made for walleye *S. vitrium* (Li & Mathias 1982). Towards the end of the cannibalistic period the pattern changed, with larger fish targeting and then swallowing smaller fish. Cannibalism ceased abruptly, approximately 14 days after it had begun; once most of the smaller fish had been consumed the juveniles again started to target *Artemia* and inert food.

*Argyrozona argyrozona* larvae displayed several eye-biting type attacks, similar to that displayed by *C. laticeps* for several days. However, no mortalities were recorded as a result of these attacks. Cannibalism amongst *C. laticeps* occurred amongst larvae undergoing or having just completed metamorphosis. Metamorphosis is a critical interval in the early life history of marine fish (Thorisson 1994), with mortality between larval and juvenile stages of cultured fish sometimes exceeding 90% (Fukuhara 1985; Loadman *et al.* 1986).

The factors influencing mortality at metamorphosis are not entirely understood (Foscarini 1988; Battaglene & Talbot 1992). One possibility is that in addition to the energy that is constantly used for maintenance and growth, extra energy is needed during metamorphosis to remodel larval structures into juvenile form (Pfeiler & Luna 1984; Diaz & Pfeiler 1993; McCormick 1993). The growth of new structures not only demands energy but also essential amino acids which are found in abundance in the body tissues of conspecifics (Crump 1986). Hecht and Pienaar (1993) state that the relationship between cannibalism and food availability is an inverse one.

*Chrysoblephus laticeps* larvae which had adopted cannibalism targeted their siblings exclusively. They ignored *Artemia* nauplii (their food source until then), even when offered high concentrations, as well as formulated feeds and minced fish flesh. Identical behaviour has been observed by other workers (Braid 1981; Katavic *et al.* 1989) with cannibalistic larvae ignoring feed items and swimming from fish to fish, selecting other larvae as preferred prey items. Initially, fish may not be specifically targeting siblings but may be searching for a larger prey item than *Artemia* nauplii. A transition to larger prey by growing fishes would ensure maximum energy intake relative to energy expenditure (Setran & Behrens 1993). Gadomski (1992) suggests that mortalities at settlement for cultured halibut was because of inadequate size of brine shrimp at that time. Loadman *et al.* (1986) speculate that cannibalistic attacks may be triggered by predator fish targeting the eye as a large food particle. The eye-biting stage has been noted for other species with many of the attacked larvae attacked not being consumed, but fatally wounded (Barrows *et al.* 1993b; Li & Mathias 1982). However, once larvae seemed to discover that their siblings were an abundant, valuable food source, cannibalism increased and the nature of the attacks changed from eye-biting to attempts to swallow. These two types of cannibalism are described as type I and type II in the literature (Hecht & Appelbaum 1988). The change between the two types signifies a change in how the cannibals perceive their siblings and is a direct result of growth depensation caused by cannibalism which in turn facilitates ingestion of smaller by larger larvae (Li & Mathias 1982; Hecht & Appelbaum 1988; van Damme *et al.* 1989).

There are several possible management strategies which could be used to reduce the effects of cannibalism.

1) It has been suggested that coeval, sibling cannibalism is a tactic to ensure that a certain percentage of the population survive through metamorphosis, and that high fecundity at spawning is a mechanism to facilitate this (Hecht & Appelbaum 1988). A management strategy could

therefore be to produce extra larvae which would be fed to older fish as an alternative prey source. Most mortality in this study occurred before metamorphosis. Therefore, fish which have survived the rigors of early development, particularly first-feeding, have greater value than newly hatched individuals. Paller & Lewis (1987) used fish eggs as a possible alternative food for striped bass *Morone saxatilis* larvae between the time *Artemia* became nutritionally inadequate and the time that formulated feeds were readily accepted. This strategy has also been suggested by Ako *et al.* (1994) for excess milkfish *Chanos chanos* eggs. Fecundity of *C. laticeps* and *A. argyrozona* was high, and incubation was not problematic.

2) If sea bass *D. labrax* are not fed early in the morning there is an increase in cannibalism, and in walleyes *Stizostedion vitreum* it increases as a function of a delay in food availability (Li & Mathias 1982; Katavic *et al.* 1989). Fish often form a search image based on the food item found to be most abundant at the start of foraging. Once the search image is established, other prey are generally ignored in order to optimise foraging (Hart 1993). This adds to the argument in favour of providing a dark period for larvae which could enable foraging patterns to be established daily by the culturist by feeding an alternative food source each morning when (or before) lights are switched on.

3) Very little cannibalism was observed amongst *A. argyrozona* larvae, which were reared at a very low stocking density, despite the piscivorous nature of the adults (Hecht and Pienaar 1993). Cannibalism was worst amongst *C. laticeps* larvae from the third spawning which were reared at the highest stocking density (Table 3.1). Stocking density is a major factor influencing cannibalism, with the rate of cannibalism appearing to be positively density dependent among species with pelagic larvae (Li & Mathias 1982; Katavic *et al.* 1989; van Damme *et al.* 1989; Hecht & Pienaar 1993; Hecht *et al.* 1995). Reducing stocking density just before metamorphosis would be the logical solution as it is a standard practice among certain commercial hatcheries (Foscarini 1988). The timing of this tactic would be critical. High larval densities increase the time larvae spend feeding, which enhances growth (Hecht *et al.* 1995). Type I cannibalism is not influenced by density and reduction of stocking densities should thus coincide with the start of type II cannibalism. *Chrysoblephus laticeps* displayed classic types I and II cannibalism, with the latter beginning approximately a week after the appearance of the former.

## Conclusions

1) The pattern of growth of *C. laticeps* follows that of many commonly cultured larvae such as sea bream *Archosargus rhomboidalis* (Dowd & Houde *et al.* 1980), red sea bream *P. auratus* (Battaglene & Talbot 1992), brown-marbled grouper *Epinephelus fuscoguttatus* (Chao *et al.* 1993); especially growth rate was comparable to other cultured sparid species, implying that *C. laticeps* would be suitable as an aquaculture candidate. Indications that the growth rate of *C. laticeps* was well below its potential, as suggested by comparisons with wild juveniles and studies on species with an established rearing protocol, contributes to this potential.

2) Both *A. argyrozona* and *C. laticeps* exhibit growth and developmental patterns typical to other cultured sparid species.

3) Both species exhibit high mortality after first-feeding, which in *C. laticeps* may have been attributable to the large size of the *Brachionus* food strain used and the disturbing effects of water currents and consequent heterogenous distribution of larvae in the rearing vessels. The gape of *C. laticeps* is however not particularly small and a more suitably sized first-food organism might enhance survival through first-feeding.

4) Swim bladder inflation appears to be concurrent with first-feeding for both species; there was no evidence that for *C. laticeps* swim bladder inflation was influenced by photoperiod. Inflation success was good, which may reflects the efficiency of the skimmers as discussed in Chapter 3.

5) Both species undergo a period of settlement during which the larvae metamorphose into juvenile form. This highlights the need for larvae to be given access to suitable substrate and source of food available to fish foraging on the bottom (in order to ease the physiological stress of metamorphosis).

6) Fish returning to the water column after settlement cannibalised smaller, less developed siblings, by first attacking the eye and then attempting to swallow the prey fish, ie. displaying classical types I and II cannibalism, as described by Hecht & Appelbaum (1988). If grading is to be used as a preventative measure, the natural separation of larger from smaller fish in the water column according to size during the settlement phase would facilitate it.

7) A growth spurt occurs for *C. laticeps* after 26 DAH. This coincides with the onset of cannibalism, which may be the influencing factor. This growth spurt may also, however, be an indication of a change in digestive anatomy, suggesting a suitable point for the introduction of an inert diet. A useful study would be detailed investigation of the development of the digestive organs and anatomy of the gut, as well as an investigation into the enzymology of developing *C. laticeps* larvae.

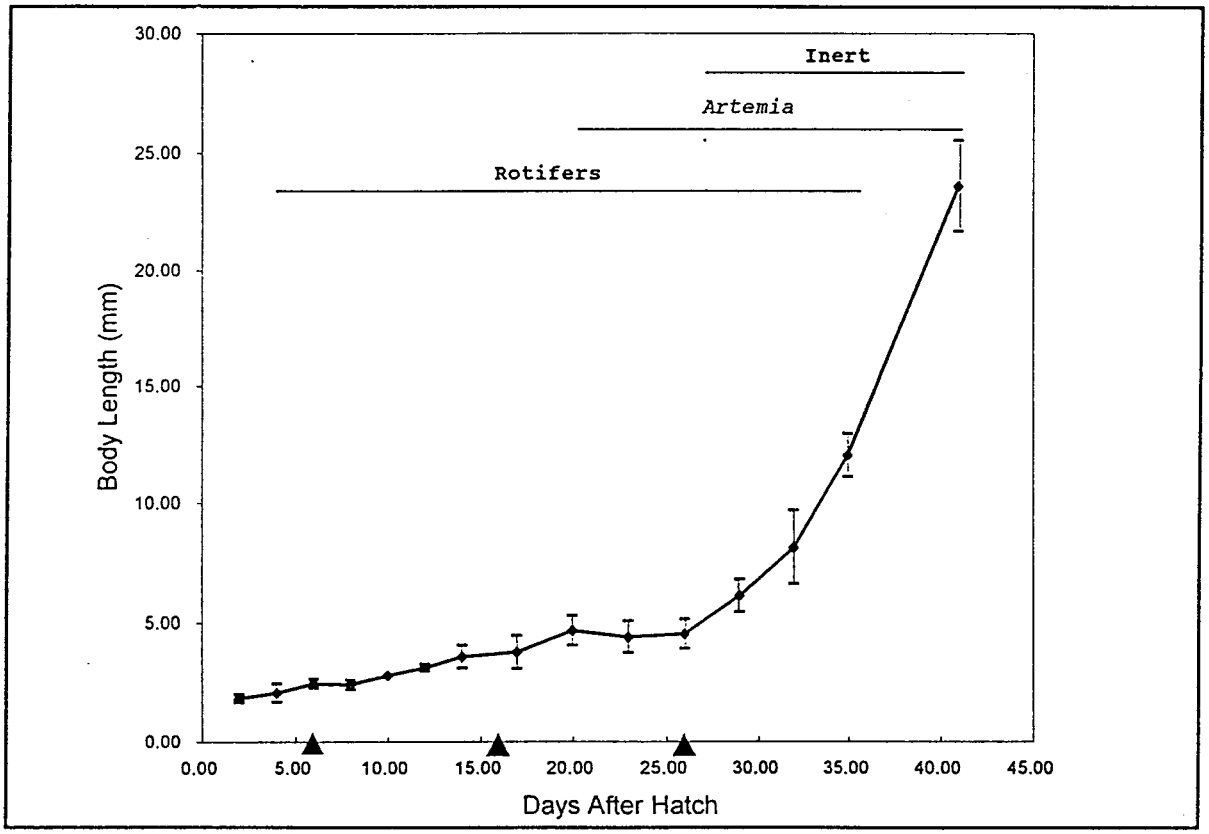



**Figure 4.1** Growth of *C. laticeps* larvae from hatch to metamorphosis. First arrow head=first-feeding; second arrow head=onset of settlement; third arrow head=onset of cannibalism; horizontal lines indicate periods of feeding.

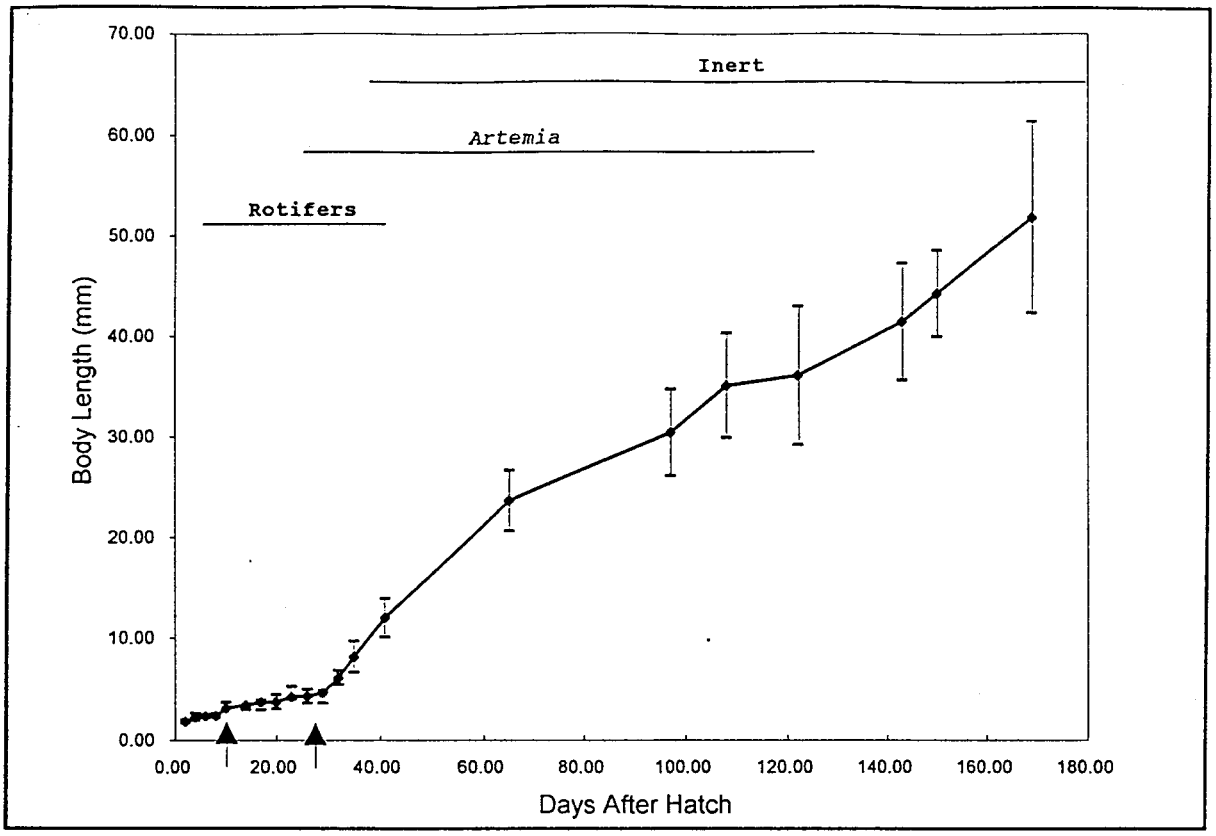



**Figure 4.2** Growth of *C. laticeps* larvae and juveniles. First arrow indicates first-feeding; second arrow indicates onset of cannibalism; horizontal lines indicates period of feeding.

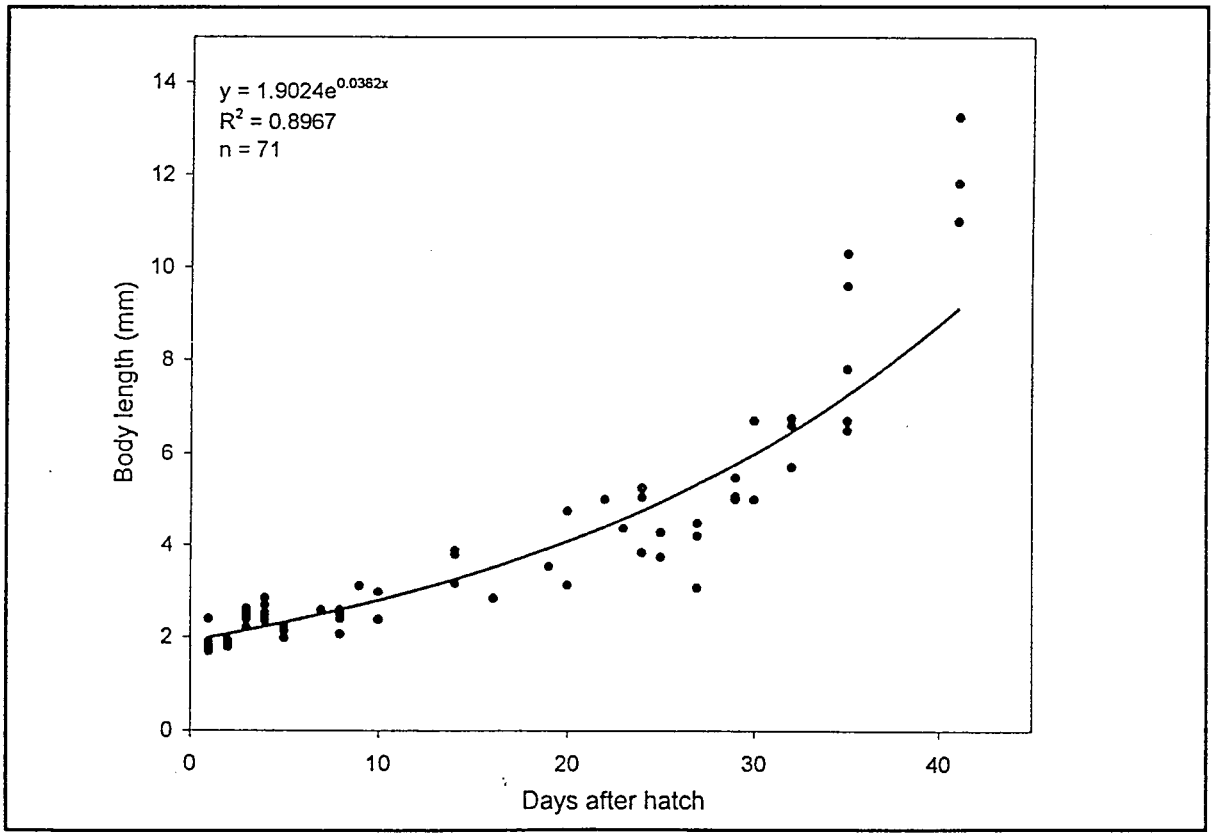



**Figure 4.3** Fitted growth curve for *C. laticeps* larvae up to metamorphosis.

# CHAPTER 5. DESCRIPTIONS OF THE EARLY LIFE HISTORY OF *ARGYROZONA ARGYROZONA* AND *CHRYSOBLEPHUS LATICEPS*

## Larval development of laboratory reared *Argyrozona argyrozona*

The larval development of the sparid *Argyrozona argyrozona* is described and illustrated from sixteen individuals, representative of a batch reared in the laboratory from artificially spawned eggs. A general account of development is given as well as detailed descriptions of pigmentation, fin development, head spination, myomere counts and morphometrics. The general developmental pattern is similar to other sparids but is unique in regard to preopercular spination, premaxillary and medio-lateral pigmentation and morphometrics.

## Introduction

The carpenter *Argyrozona argyrozona* (Valenciennes, 1830) is endemic to South Africa, occurring from Saldanha Bay to Port St Johns (van der Elst 1988). This carnivorous, schooling sparid spawns during the summer months between September and March (Nepgen 1977) and is of considerable importance to inshore fisheries in the region (Smith & Heemstra 1991; Hecht & Tilney 1989). Previous descriptions of early life history have been limited to short accounts of the yolk-sac and early preflexion stages (Gilchrist 1904, 1916; Nepgen 1977). In this study the early life history stages of *A. argyrozona* are described and illustrated from a batch spawned and reared in captivity.

## Materials and methods

Adult carpenter were caught on hand-line at the peak of the natural spawning season in the Tsitsikamma National Park situated on the Southern Cape coast between Natures Valley (34°59'S, 23°34;E) and Oubos Strand (34°59'S, 24°12'E) in February 1994. After being transported to shore in 80$\ell$ plastic bins the fish were placed in a 5000$\ell$ circular holding tank and given a 0.1m$\ell$.kg$^{-1}$ body weight injection of pituitary homogenate. After approximately 12 hours the fish were stripped and the eggs fertilised by mixing them in bowl with sperm and a small amount of seawater, following the methods of Battaglene and Talbot (1992) for red sea bream *P. auratus*. Eggs were transported 300km within 12 hours of fertilisation to a recirculating seawater rearing facility at the Department of Ichthyology and Fisheries Science (DIFS), Rhodes University, Grahamstown, where they were reared through metamorphosis. Samples were removed at irregular intervals from the rearing tanks, anaesthetized using 2-phenoxyethanol (0.1m$\ell$.100m$\ell^{-1}$) and preserved in 4% buffered formalin (Markle 1984). Preserved specimens were drawn using a camera lucida attachment on a binocular microscope. Drawing techniques followed those recommended by Faber and Gadd (1983) and Leis and Trnski (1989). Unless internal melanophores were obvious in uncleared specimens, they were not illustrated. Specimens were cleared and stained for cartilage and bone following the methods of Taylor & van Dyke (1985). Xanthophores were not illustrated.

Sixteen specimens ranging in size from 2.48-34mm were examined. Morphometric features were measured using a micrometer eyepiece for specimens under 30mm body length (BL) and larger specimens were measured with callipers. Terminology used in the written description follows that used by Leis and Trnski (1989). Body length (BL) corresponds to notochord length in preflexion and to standard length in post-flexion larvae and juveniles. All specimens have been lodged in the JLB Smith Institute fish collection in Grahamstown, South Africa (RUSI catalogue numbers 48839-48853).

# Results

*Description of eggs and hatching*

The water hardened egg was spherical, buoyant, and transparent. The egg envelope was unsculptured and the yolk unsegmented. Average egg diameter was 0.82mm (±0.015). The average diameter of the single oil globule was 2.2% of the egg diameter. Eggs hatched within 26-30 hours at 19°C, which is similar to other sparid species (Griswold & McKennedy 1984; Fukuhara 1987). Hatched larvae measured approximately 2.4mm BL and hung head downwards in the water column. Yolk was resorbed within two days after hatch (DAH), although the oil globule persisted until first-feeding. Opening of the mouth and anus with concurrent first-feeding on rotifers *Brachionus plicatilis* occurred 3-4 DAH (±2.5mm BL).

*Pigmentation*

Pigmentation is described in the order in which the melanophores appeared on the fish. First pigmentation of the embryo was evident after 20 hours in the form of two rows of light brown melanophores running dorsally along the length of the body. Slight pigmentation of the oil droplet was also evident under strongly transmitted light (Figure 5.1A).

A row of melanophores developed along the ventral midline of the tail region in larvae larger than 2.5mm BL, one being located at the base of each post-anal myoseptum. Myomeres eight to sixteen possessed an additional melanophore in the middle of the myomere's ventral edge in pre-flexion larvae. This pigmentation became more diffuse as the fish developed but remained until metamorphosis, eventually becoming part of juvenile coloration.

The melanophore on the fin fold immediately anterior to the anus first appeared 2 DAH (2.5mm BL), increasing in size as the fish grew. It persisted as a remnant in fish of about 5.0mm BL, finally disappearing along with the fin fold.

A melanophore on the ventral surface of the gut and just posterior to the cleithral symphysis appeared at 3.2mm BL (Figure 5.1B). This melanophore persisted through notochord flexion (Figures 5.1C and 5.1D(i)) and was still well established in fish of 5mm BL (Figure 5.1E(i)). Eventually this pigmentation disappeared in post-flexion fish of 7.5mm BL and over.

An isolated melanophore appeared on the dorsal surface of the hindgut in fishes larger than 3mm BL. This pigmentation expanded anteriorly and became pronounced in fishes of about 3.5mm BL (Figure 5.1B, C and D). Gut pigmentation started to become obscured by body musculature in late-flexion larvae (>5mm BL). The pigment was partially obscured in fish undergoing metamorphosis and was finally visible only as a subtle band of subsurface pigmentation in early juveniles. A melanophore dorsal to the swim bladder was visible in larvae larger than 3mm BL. This pigmentation remained visible until being obscured by body musculature in fish larger than 5mm BL.

An isolated melanophore ventral to the notochord tip appeared just prior to the emergence of the caudal anlage (3.7mm BL) and persisted through flexion. It disappeared by the commencement of post-flexion.

The edge of the hypural plates in post-flexion fish was unpigmented.

Pre-flexion larvae lacked cranial pigmentation. A bilateral pair of melanophores appeared over the hindbrain and three smaller dorso-lateral melanophores were visible on the hind, mid and forebrain of flexion animals (Figure 5.1D(i)). They were supplemented by several smaller melanophores as the fish developed (Figure 5.1D(i)). The cranium of juvenile fish was darkly pigmented dorsally.

Pigmentation of the snout first appeared on fish of 4mm BL. The pigmentation spread and darkened as the fish developed and was prominent in juveniles (Figures 5.1D-5.1G).

Pigmentation of the lateral body surfaces was first visible on the tail as a single melanophore, positioned midway between the dorsal and anal anlagen, in fish larger than 4.5mm BL (Figure 5.1D). Pigmentation spread in a patch around the original melanophore, remaining in an area bound anteriorly by the gut and posteriorly by the caudal peduncle. Dorso-lateral pigmentation spread anteriorly to the nape region (Figures 1D-1E). The lateral body surface remained heavily pigmented throughout development. The distinctive feature of the lateral pigmentation was its exclusion from the caudal peduncle until after metamorphosis.

Pigmentation of the dorsal fin was associated with the distal margin of the fin membrane. Melanophores started to develop between the spinous fin elements at approximately 8.6mm BL (Figure 5.1F). This pigmentation was conspicuous in larger fish appearing as a dark band

fringing the distal edge of the dorsal fin in juveniles. In large juveniles (>33mm BL) dorsal fin pigmentation spread to the fin rays where it ran along the length of the rays.

*Morphology*

**General**

Nares differentiated from the olfactory pit at 5mm BL. Fine teeth were visible on both jaws at about 7.6mm BL. Scales first appeared at 7.6mm BL and were fully developed by 8.6mm BL. Large canines developed by 12mm BL. The lower jaw (dentary) projected anteriorly beyond the upper jaw (premaxilla) in larvae measuring 3mm BL and above. The protrusion of the lower jaw became a prominent feature in juveniles over 30mm BL.

**Myomere counts**

The myomere count for all larvae two days and older was 25. In one-day-old larvae, either the most posterior myomere was still undeveloped or was too small to be detected. As the gut lengthened, the ratio of pre-anal to postanal myomeres increased from 7+18 in early preflexion larvae to 9+16 in larvae just prior to flexion. Flexion larvae had myomere counts of 10+15 while early juveniles possessed 12+13 myomeres.

**Fin development**

Fin development is described in the order in which the fins developed in the fish. Fin counts are summarised in Table 5.1. Pectoral fin buds were present in newly hatched larvae. Fin rays started differentiating in fish larger than 5mm BL. A final count of 16 pectoral rays occurred in fish over 32mm BL.

The caudal fin anlage appeared posterio-ventrally on the tail just prior to commencement of flexion in fish larger than 4mm BL. Fin elements increased in number through flexion and the caudal fin was fully differentiated in 8.6mm BL fish.

Dorsal fin anlage appeared in fish of 4.7mm BL (Figure 5.1D). The adult complement of fin bases was present by 5mm BL. The first incipient rays appeared on the middle elements in fish of this size (Figure 5.1E). The eleventh and twelfth (most posterior) spines formed first as rays. The twelfth was changed in fish of 8.6mm BL (Figure 5.1G) and the eleventh in fish over 33mm BL. Dorsal spines ossified before the rays.

Anal fin anlage appeared in fish of 4.7mm BL (Figure 5.1D). The adult complement of fin-ray bases was present by 5mm BL. The first incipient rays appeared on the middle elements in fish of this size (Figure 5.1D). The third (most posterior) spine formed first as a ray and the change was complete by 32mm BL). Anal spines ossified before rays. Anal fin elements ossified prior to dorsal.

Pelvic fin buds were visible at 5mm BL and the fin elements were fully formed (the first being a hard spine and the rest soft rays) in fish larger than 7mm BL.

Full ossification of the fin elements was complete at 33mm BL. The smallest juvenile to posses full adult fin counts (D XII,10; A III,8) was 33mm BL.

**Head spination**

Head spination is described in the order of development. Spination of the outer preopercle first appeared in fish larger than 3.5mm BL as two short, blunt spines (Figure 5.1C). These spines increased in number to a total of eleven in juvenile fish (Figure 5.1D). The spines reached their greatest relative length in larvae of 5mm BL. The longest of these spines was at the preopercular angle and reached the posterior edge of the branchiostegal rays (Figure 5.1D). The relative size of the spines decreased with age until the spines were reduced to a serrated edge on the outer preoperculum of juvenile fish.

Spination of the inner preopercle first appeared in fish of 4.75mm BL (Figure 5.1D) as two short spines. The longest at the inner preopercular angle extended past the edge of the outer preopercular plate. The relative size of the spines decreased with larval development. Juvenile fish developed a third small spine anterior to the original two (Figure 5.1G). These spines were reduced in larger juveniles.

Spination of the opercle developed in post-flexion fish. It consisted of one interopercular, one subopercular and one opercular spine. Juvenile fish lost the interopercular spine and developed a second subopercular spine (Figure 5.1G). The opercular spine was conspicuous in juvenile fish.

Three small supracleithral spines were visible in cleared and stained specimens from 7mm BL. These spines were reduced in juvenile fish, disappearing in fish larger than 9mm BL.

Spination of the posttemporal was visible as a single small spine in cleared and stainedspecimens from 7mm BL. A second posttemporal spine was visible in cleared and stained juveniles of 8.6mm BL (Figure 5.1G). These spines were invisible in larger juveniles.

## Discussion

The larvae and juveniles of *A. argyrozona* exhibited typical sparid developmental patterns, as described by Leis & Trnski (1989), including general body shape, myomere count, sequence and nature of fin development, head spination and general pattern of pigmentation. A comparison between the larvae of *A. argyrozona* and those sympatric sparid larvae which have been described revealed some important differences.

*Diplodus cervinus* (Brownell 1979a).

The preflexion larvae had heavy pigmentation of the otic capsule, nape, base of the brain and along the entire length of the symphysis and both ventral and lateral surfaces of the gut. Post-flexion larvae had no pigmentation on the snout and displayed heavy pigmentation of the rest of the head, nape and gut. The pelvic fin was pigmented as was the edge of the hypural plates. Pigmentation of the tail was restricted to the posterior ventral edge. There were four inner and six outer preopercular spines and no interopercular spine.

*Diplodus sargus* (Brownell 1979a; Divanach *et al.* 1982).

Preflexion larvae lacked pigmentation on the fin fold anterior to the anus and possessed conspicuous pigmentation along the dorsal midline of the tail. Flexion larvae lacked pigment on the tail and the outer preopercular spines did not reach the opercular margin. Post flexion larvae lacked posttemporal spines, possessed only a single subopercular and six preopercular spines. Pigmentation of the tail was restricted to the ventral surface. Juveniles had pigmentation on the edge of the hypural plates and caudal peduncle, and tail pigmentation was restricted to a band along the midline. There were no inner preopercular and six outer preopercular spines. The dorsal fin possessed 26 elements and the anal 16.

*Lithognathus mormyrus* (Brownell 1979a).

Preflexion larvae under 4mm BL were virtually indistinguishable, however, larvae over 4mm BL had a minimum of 18 melanophores on the ventral midline, more than five on the ventral gut surface and lacked pigment on the cleithral symphysis. The lateral tail pigmentation of post-flexion larvae was restricted to a thin line and the ventral hypural plates were pigmented. The inner preopercle was smooth and the outer possessed two blunt spines. There were no supracleithral, posttemporal or opercular spines. Juveniles had widespread pigmentation on the opercular region and pigment of the spinous region of the dorsal fin extended down the membrane. Tail pigment extended into the caudal peduncle. The fin count at this stage was D XI,12; A III,11.

*Pachymetapon blochi* (Brownell 1979a).

Preflexion larvae showed pigmentation of the posterio-ventral surface of the otic capsule and hindbrain. Groups of ventral midline melanophores were widely separated in post yolk-sac larvae. Post-flexion larvae had pigment on the tail which was restricted to the ventral surface. They seemed to lack a supracleithral spine, had three inner opercular, eight outer opercular and a single subopercular spine.

*Cheimerius nufar* (Connell & Garratt, in prep).

Preflexion larvae had pigmentation of the otic capsule, snout operculum, hindbrain and lateral gut surface. Post-flexion larvae lacked tail pigmentation but possessed a melanophore on the angle of the lower jaw. There were three preopercular, nine outer preopercular and two supracleithral spines. Both the opercular and posttemporal were smooth. The juveniles lacked lateral and dorsal pigmentation and the pigmentation on the ventral surface of the tail extended onto the caudal peduncle. Both the lower jaw and base of the pectoral fin were pigmented. There were nine preopercular, four supracleithral, two subopercular and a single posttemporal spine at this stage. The first soft ray of the pelvic fin was elongate and the dorsal fin was unpigmented.

*Spondyliosoma emarginatum* (Beckley 1989).

Preflexion larvae possessed three outer preopercular spines, but were otherwise indistinguishable. Flexion larvae lacked pigment on the snout, cleithral symphysis and otic capsule. There were three inner and five outer preopercular spines, of which the longest was dorsal to the preopercular angle. Post-flexion larvae lacked pigmentation of the snout or otic capsule. There were five tiny spines on the outer preoperculum and no opercular, supracleithral or posttemporal spines. Juveniles had a fin count of D X,13; A III,10. Pigmentation of the dorsal was restricted to the base of the fin elements.

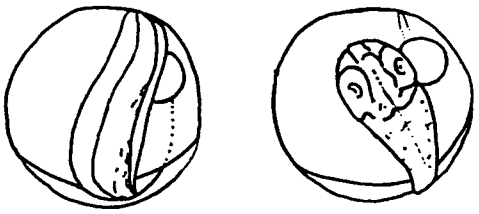
Species "5" (Brownell 1979a).

Preflexion larvae had melanophores on the nape, dorsal edge of the tail and pigmentation spread over the entire ventral gut surface. Descriptions of subsequent stages were not available. Brownell (1979a) could have been wrong in describing Species "5" as *A. argyrozona*. However, his description was limited to two specimens in the preflexion stage and the possibility exists that the preflexion stage of *A. argyrozona* may be subject to some intraspecific variation.

**Table 5.1** Morphometrics and meristics of *A. argyrozona* larvae and juveniles reared in captivity. Measurements are in mm and are absolute. BL=Body Length; HL=Head Length; SnL=Snout Length; ED=Eye Diameter; PAL=Pre-anal Length; BD=Body Depth; C=Caudal; D=Dorsal; A=Anal; P1=pectoral; P2=pelvic.

| Age | Stage | Figure | Morphometrics | | | | | | Fin counts | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | BL | HL | SnL | ED | PAL | BD | C | D | A | P1 | P2 |
| 1 DAH | Preflexion | - | 2.48 | 0.23 | 0.050 | 0.17 | 1.033 | 0.17 | - | - | - | - | - |
| 2 DAH | | - | 2.73 | 0.23 | 0.067 | 0.18 | 1.067 | 0.17 | - | - | - | - | - |
| 3 DAH | | - | 2.96 | 0.65 | 0.24 | 0.23 | 1.08 | 0.20 | - | - | - | - | - |
| 5 DAH | | - | 3.17 | 0.68 | 0.22 | 0.22 | 1.17 | 0.18 | - | - | - | - | - |
| 7 DAH | | 1B | 3.17 | 0.67 | 0.17 | 0.23 | 1.20 | 0.27 | - | - | - | - | - |
| 16 DAH | | 1C | 3.72 | 0.97 | 0.33 | 0.36 | 1.73 | 0.46 | - | - | - | - | - |
| 20 DAH | Flexion | 1D | 4.75 | 1.58 | 0.35 | 0.51 | 2.40 | 0.80 | 0+7+8+0 | - | - | - | - |
| 22 DAH | | 1E | 5.05 | 1.95 | 0.50 | 0.70 | 3.20 | 1.00 | 0+8+8+0 | 0,7 | 0,3 | - | - |
| 24 DAH | Post-flexion | 1F | 7.58 | 2.95 | 0.92 | 1.00 | 4.90 | 2.00 | 3+9+8+4 | X,12 | II,9 | 13 | I,5 |
| 29 DAH | Juvenile | 1G | 8.60 | 3.20 | 1.06 | 0.90 | 5.10 | 1.85 | 9+9+9+7 | XI,11 | II,9 | 15 | I,5 |
| 36 DAH | | - | 10.60 | 4.42 | 0.83 | 1.09 | 6.21 | 2.88 | 9+9+9+7 | XI,11 | III,8 | 15 | I,5 |
| 38 DAH | | - | 11.97 | 4.39 | 1.74 | 1.36 | 7.88 | 3.94 | 9+9+9+8 | XI,11 | III,8 | 16 | I,5 |
| 48 DAH | | - | 16.20 | 6.82 | 1.36 | 2.35 | 10.30 | 3.86 | 9+9+9+9 | XI,11 | III,8 | 16 | I,5 |
| 64 DAH | | - | 32.00 | 11.50 | 3.00 | 4.50 | 21.50 | 9.00 | 10+9+9+9 | XI,11 | III,8 | 16 | I,5 |
| 71 DAH | | - | 33.00 | 12.00 | 2.50 | 4.00 | 20.00 | 9.02 | 10+9+9+9 | XII,10 | III,8 | 16 | I,5 |
| 95 DAH | | - | 34.00 | 13.51 | 3.00 | 4.00 | 20.50 | 10.00 | 10+9+9+9 | XII,10 | III,8 | 16 | I,5 |

A



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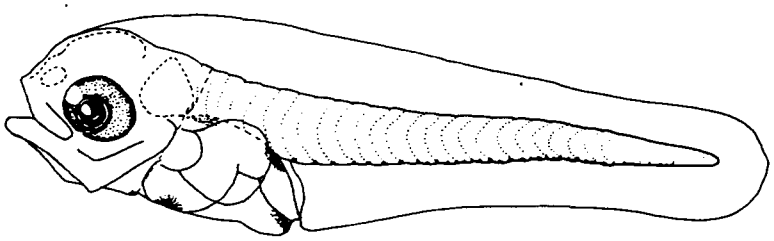

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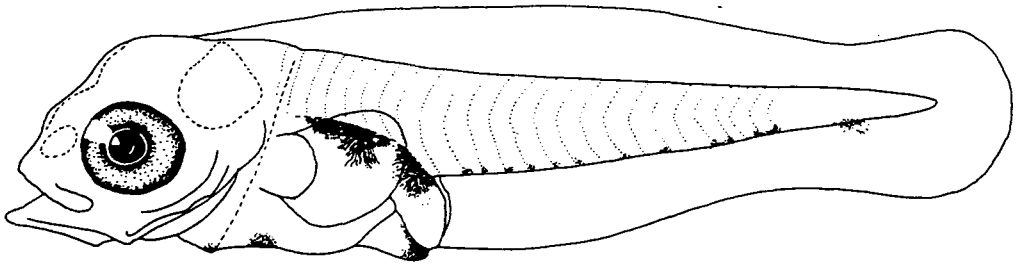

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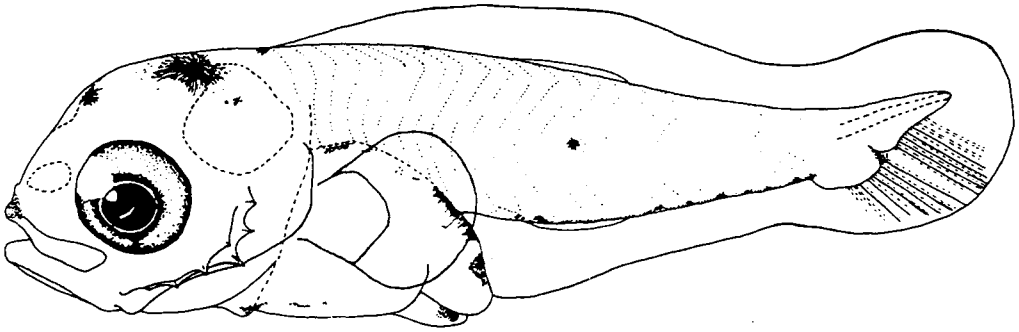
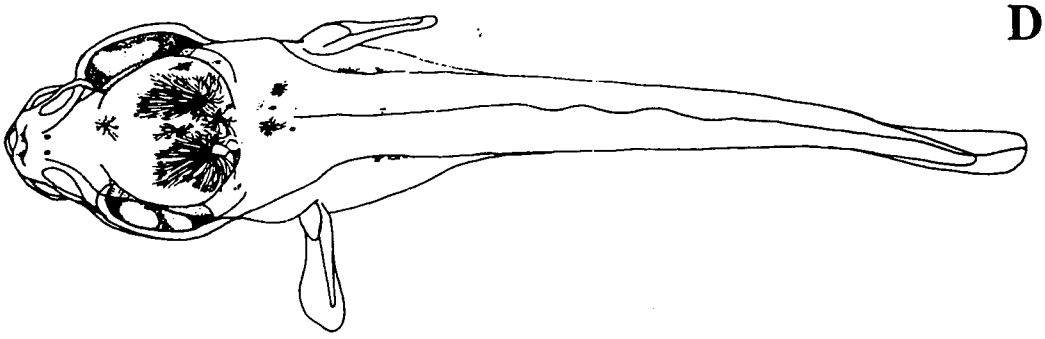


D(i)



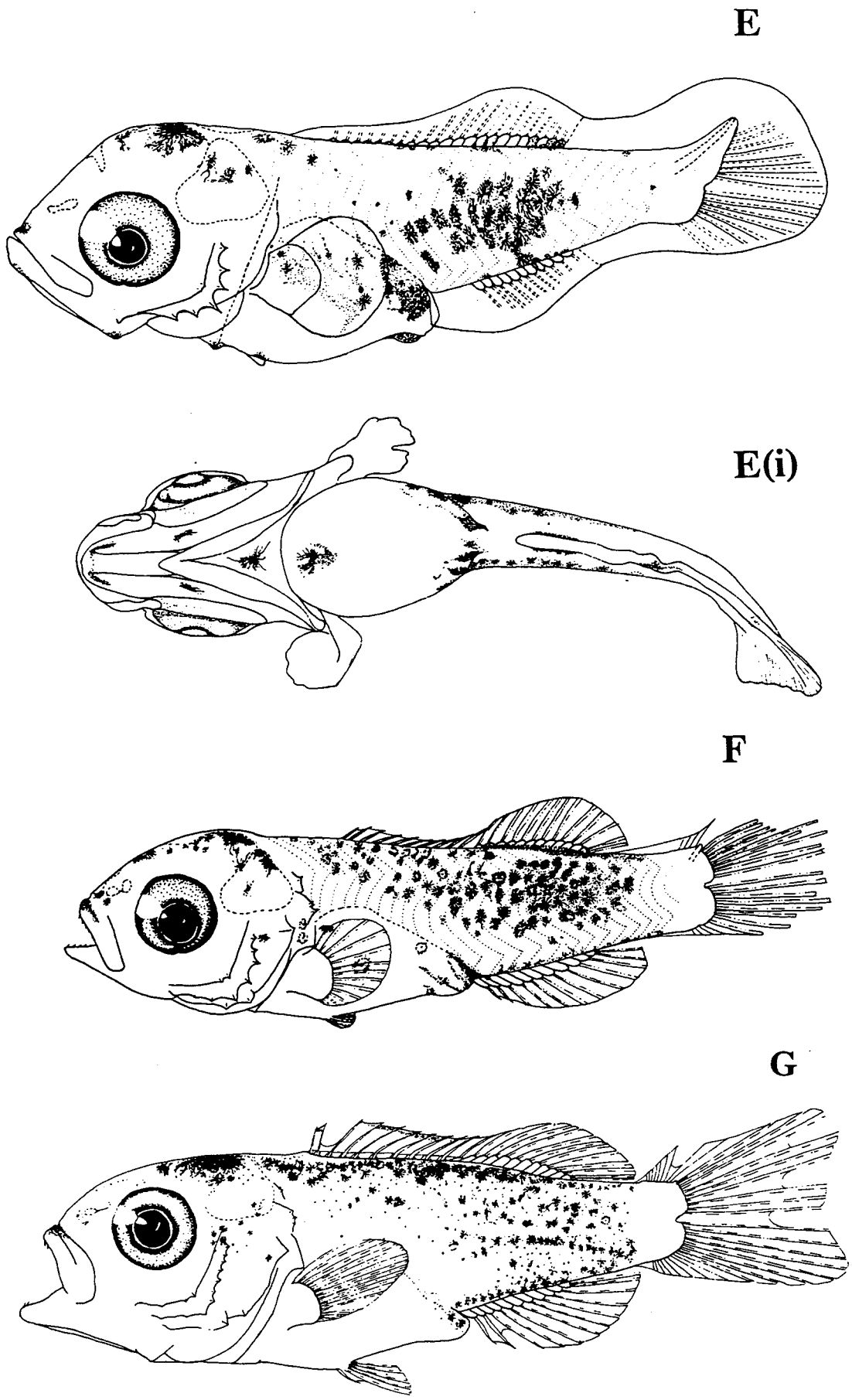



**Figure 5.1** Larval development of laboratory reared *Argyrozona argyrozona*. A=0.82mm diameter; B=3.17mm BL; C=3.72mm BL; D=4.75mm BL; E=5.05mm BL; F=7.58mm BL; G=8.6mm BL.

## Larval development of laboratory reared *Chrysoblephus laticeps*

The sparid *Chrysoblephus laticeps* was reared in the laboratory from artificially spawned eggs. A general account of larval development is given as well as detailed descriptions of pigmentation, fin development, head spination, myomere counts and morphometrics. Developmental patterns in this species are similar to those described for other sparids but are unique with respect to head spination, gut and tail pigmentation and morphometrics.

## Introduction

The roman *Chrysoblephus laticeps* (Valenciennes, 1830) is a sparid endemic to South Africa occurring from Cape Town to Port St Johns (van der Elst 1988). *Chrysoblephus laticeps* are reef dwelling, benthic carnivores (Buxton 1984) and are of considerable importance to inshore recreational and commercial fisheries in the region (Smale & Buxton 1985; Hecht & Tilney 1989; Penney 1990). They are protogynous hermaphrodites (Buxton 1989) spawning during the summer months between October and February (Buxton 1990). The early life history is unknown although Brownell (1979a) has described the post metamorphosis juvenile. Here, the early life history stages of *C. laticeps* are described and illustrated from a group spawned and reared in captivity.

## Materials and methods

Adult *C. laticeps* were caught in December 1994 in the same manner as *A. argyrozona*. After being transported to shore in 80$\ell$ plastic bins, the fish were placed in a 5000$\ell$ circular, flow-through holding tank and given a 0.5m$\ell$.kg$^{-1}$ body weight injection of Aquaspawn$^{R}$ containing 10$\mu$g of LHRHa and 50$\mu$g domperidone. After approximately 48 hours, the fish were stripped and the eggs fertilised in a bowl, following the method of Battaglene and Talbot (1992) for red sea bream *Pagrus auratus*. Eggs were transported to the DIFS, where they were reared through the larval stages.

Samples were collected in the same manner as *A. argyrozona*, and drawn according to the same criteria. Seventy five preserved specimens ranging in size from 1.76-49.80mm were examined. A micrometer eyepiece was used to measure specimens under 30mm body length (BL) and callipers for larger specimens. Terminology used in the written description follows that used by Leis and Trnski (1989). Body length (BL) corresponds to notochord length in preflexion and to standard length (SL) in post-flexion larvae and juveniles. All specimens have been lodged in the JLB Smith Institute fish collection in Grahamstown, South Africa (RUSI catalogue numbers 52500-52561).

## Results

### *Description of the egg and hatching*

The egg was spherical, buoyant, and transparent. Live eggs had an orange tinge. The egg envelope was unsculptured and the yolk was whole. Average egg diameter was 0.76±0.018mm. The average diameter of the single oil globule was 23.33±1.40% of the egg diameter. Eggs hatched in 26-30 hours at 19°C, which was similar to other sparid species (Griswold & McKennedy 1984; Fukuhara 1987). Hatched larvae measured approximately 1.76±0.035mm BL, the yolk-sac was an average of 44.59±1.14% BL and the oil globule was an average of 8.04±0.95% BL. Yolk was resorbed by two DAH, although the oil globule persisted until first-feeding. First-feeding on rotifers (*Brachionus plicatilis*) occurred at ±2.6mm BL or 3-4 DAH.

### *Pigmentation*

Pigmentation is described in the order in which the melanophores appeared on the fish. Pigmentation in the embryo just prior to hatch consisted of small melanophores scattered along the dorsal surface of the body. The rest of the egg was completely unpigmented (Figure 5.2A).

A row of melanophores developed along the ventral midline of the tail region in larvae larger than 1.7mm BL. The most anterior pigment spot was associated with the first post-anal myomere.

The remaining melanophores were relatively evenly distributed along the remainder of the tail growing smaller the more posterior their position. Initially these melanophores were joined by a feint ventral line of light pigment which gradually disappeared as the larvae developed. The pattern remained relatively unchanged until fish reached approximately 3mm BL. As larvae approached 4.0mm BL the two posterior-most melanophores joined. This melanophore became associated with the posterior edge of the ventral hypural plate in flexion larvae and migrated dorsally with flexion, remaining visible until the juvenile stage. The remaining melanophores became larger, spreading away from the ventral midline, either forming the beginning of the ventro-lateral pigmentation of the tail or the elements of the anal fin bases.

The melanophore on the fin fold immediately anterior to the anus first appeared in larvae of approximately 1.8-1.9mm BL, where it was prominent. It persisted as a remnant in fish of about 5.4mm BL, finally disappearing along with the fin fold.

A melanophore on the ventral surface of the gut, just posterior to the cleithral symphysis appeared at approximately 1.8mm BL. Although steadily decreasing in size in relation to the fish, this melanophore persisted through metamorphosis. It migrated posteriorly away from the cleithral symphysis as the fish developed, becoming closely associated with the base of the pelvic fin in fish larger than 5mm BL.

Pigmentation at the tip of the cleithral symphysis appeared simultaneously with the ventral gut melanophore. Similarly, this pigmentation persisted through to metamorphosis, becoming relatively smaller and less distinctive as pigmentation appeared around it.

An isolated melanophore appeared on the dorsal surface of the hindgut in fishes larger than 2mm BL. This melanophore was joined by a second melanophore in fish larger than 4mm BL, eventually joining up with the pigment of the swim bladder to form a continuous line from the colon to the anterior edge of the swim bladder in fish larger than 5.2mm BL. This pigmentation became less distinct as the larvae developed due to the obscuring effect of the developing body musculature (Figures 5.2E-5.2F). It was completely obscured in fish over 7mm BL.

A distinctive melanophore capping the dorsal surface of the swim bladder was visible in larvae larger than 2mm BL. This pigmentation spread as the swim bladder extended posteriorly, linking up with the dorsal gut pigmentation described above.

Pigmentation of the otic capsule appeared in fish larger than 3mm BL. It was visible as a subsurface, horizontal strip until becoming obscured in fish larger than 5.4mm BL.

A distinctive melanophore appeared at the point joining the colon with the ventral surface of the gut coil in fish approaching 4mm BL (Figure 5.2C). This pigment persisted through metamorphosis, becoming less noticeable as pigment spread over the gut in fish larger than 9mm BL.

Preflexion larvae lacked cranial pigmentation. A bilateral pair of melanophores appeared over the hindbrain of larvae in the early stages of flexion. By the late stages of flexion, large melanophores had appeared on the midbrain and by the completion of flexion, the forebrain was also pigmented (Figures 5.2C-5.2F(i)). The cranium was heavily pigmented in juveniles.

A large, prominent melanophore appeared on the dorsal surface of the tail, just posterior to the base of the dorsal fin in the majority of larvae larger than 6mm BL. Not all of the larvae possessed this pigmentation and it must therefore be considered an unreliable diagnostic feature. In those larvae where it did appear, it was associated with an isolated melanophore on the nape and was highly distinctive (Figures 5.2F & 1F(i)).

Pigmentation of the snout, tip of lower jaw and lower jaw angle appeared in larvae over 6mm BL and became part of juvenile pigmentation.

Pigmentation of the lateral body surfaces spread from the dorsal and ventral edges towards the midline, dorsal pigmentation spreading relatively more quickly. The caudal peduncle was the last area of the tail to become pigmented, producing a triangular pattern pointing towards the caudal fin in juveniles. Juveniles sported a distinctive, medio-lateral band of dark pigmentation along the tail, posterior to the anus, as well as a darkened area along the dorsal surface, spreading in a patch below the posterior half of the dorsal fin (Figure 5.2H).

The pelvic fin was pigmented from its emergence. Pigmentation spread distally, until in juveniles it was distinctively darkened with heavy pigmentation.

Pigmentation of the dorsal fin began to spread up the dorsal fin membranes associated with the spinous fin elements. The pattern of spreading was from the distal to the proximal edges and anterior to posterior spines. The pattern of pigmentation was distinctive in its exclusive association with the spinous membrane, being excluded from all but the proximal edge of the soft ray membranes in juvenile fish (Figure 5.2H).

Pigmentation of the anal fin membranes followed a similar pattern of development (Figure 5.2H), despite some of the fin bases being pigmented much earlier on in development (Figure 5.2F).

Pigmentation of the 14th, 15th & 16th elements of the pectoral fin was evident in juveniles.

*Morphology*

**General**

Contrary to most sparids sacrificed under light conditions (Leis & Trnski 1989), *C. laticeps* displayed a prominent swim bladder from just after absorbtion of the yolk sac until it was obscured by the body wall musculature in fish approaching metamorphosis (Figures 5.2B-5.2F). Nares began to differentiate from the olfactory pit at approximately 5.4mm BL and were completely separate by 11mm BL. Fine teeth were visible at the tips of both jaws in fish of approximately 4.8mm BL and spread to the entire jaw in fish of approximately 5.2mm BL. Enlarged incisors appeared in fish larger than 9mm BL. Scales first appeared at approximately 9.5mm BL and were fully developed by 11mm BL.

**Myomere counts**

The total myomere count for all larvae was 24. As the gut lengthened, the ratio of pre-anal to postanal myomeres increased from 7+17 in early preflexion larvae to 9+15 in larvae just prior to flexion. Flexion larvae had myomere counts of 11+13 while larvae just prior to the juvenile stage possessed 12+12 myomeres.

**Fin development**

Fin development is described in the order in which the fins developed in the fish. Fin counts are summarised in Table 5.2. Pectoral fin buds were present in newly hatched larvae. Fin rays started differentiating in fish larger than 4.5mm BL. A final count of 16 pectoral rays occurred in fish over 9.6mm BL.

The caudal fin anlage appeared posterio-ventrally on the tail just prior to commencement of flexion in fish larger than 4.5mm BL. Fin elements increased in number through flexion and the caudal fin was fully differentiated at 9.6mm BL.

The dorsal fin anlage appeared in fish of 4.95mm BL (Figure 5.2D). There were 18 dorsal fin elements at this stage. The number of elements increased to 20 toward the end of flexion and post-flexion fish of 6.6mm BL possessed the full complement of 22 fin bases. The eleventh and twelfth (most posterior) spines formed first as rays. The twelfth was changed in fish of 9.6mm BL (Figure 5.2G) and the eleventh in fish over 11.0mm BL. Dorsal spines ossified before the rays.

The anal fin anlage appeared in fish of 4.95mm BL (Figure 5.2D). The adult complement of fin bases was present at this stage. The first incipient rays appeared in fish of 5.47mm (Figure 5.2E). The third (most posterior) spine formed first as a ray and the change was complete by 13mm BL. Anal spines ossified before the rays. Anal fin elements ossified prior to dorsal.

Pelvic fin buds were visible at 5.5mm BL and the full compliment of elements (the first being a hard spine and the rest soft rays) in fish larger than 6.6mm BL. In fish larger than 9.6mm BL, the first soft ray became elongate, a feature which was noticeable in juvenile fish (<11mm BL).

Full ossification of the fin elements was complete at 13mm BL. The smallest juvenile to posses full adult fin counts (D XI-XII,10-11; A III,8-9) was 23mm BL.

**Head spination**

Head spination is described in the order of development. Spination of the outer preopercle first appeared as a single, small spine in fish of 3.88mm BL. The number increased to six in fish of 4.95mm BL. The longest of these spines were those at, and just ventral to, thepreopercular angle where they extended to the opercular margin. The spines increased in number to a total of eleven in juvenile fish (Figure 5.2H). The spines reached their greatest relative length in larvae of 6.6mm BL (Figure 5.2F). The relative size of the spines decreased as the juveniles developed, finally appearing as a serrated edge on the outer preopercle of juvenile fish larger than 23mm BL.

Spination of the inner preopercle first appeared in fish of 3.9mm BL (Figure 5.2C) as a single short spine. Spines increased to three in larvae of 5.5mm BL, five in 6.6mm BL and six, grouped in three pairs, in fish of 9.6mm BL (Figures 5.2C-5.2G). The margin of the inner preopercle was smooth in juvenile fish (Figure 5.2H).

Spination of the opercle developed towards the end of flexion. It consisted of a single subopercular and an opercular spine in fish of 5.5mm BL. The opercular spine was a prominent feature in cleared and stained individuals until the juvenile stage, where it became inconspicuous. The original subopercular spine continued to be visible in juvenile fish (Figure 5.2H). The number of subopercular spines increased to a maximum of five at 9.6mm BL. The posterior-most spines were much reduced in juvenile fish, with the most posterior being almost invisible. The anterior three subopercular spines were more prominent (Figure 5.2H). Two small supracleithral spines were visible in cleared and stained specimens from 5.5mm BL. These spines increased size and number to a maximum of three in larvae of 6.6mm BL. They were inconspicuous in juvenile fish.

Spination of the posttemporal was visible as a single small spine in specimens of approximately 9.6mm BL. This spine disappeared in juvenile fish.

**Morphometrics and meristics**

Morphometrics and meristics of larvae are summarised in Table 5.2 as a percentage of body length. It must be noted that BL effectively decreased after flexion when it was measured as SL, as opposed to notochord length in preflexion larvae. There is thus a sudden increase in the ratios of the various morphometric characters, which is not reflective of the actual body measurements. For this reason, the relative measurements of post-flexion larvae were often larger than those of more developed juvenile fish.

Head length (HL) increased as fish approached the juvenile stage. Thereafter, fish started to increase in SL and HL therefore decreased slightly. Snout length increased from yolk-sac to post-flexion-stage larvae. As fish became juveniles the snout became more blunt as is typical of sparid development (Leis & Trnski 1989), and was reflected by a decrease in snout length. Eye diameter increased substantially after flexion, probably as a result of measuring techniques described above. As juveniles developed it decreased to preflexion size, and continued to decline as the fish developed. Pre-anal length was relatively large due to the yolk sac in fish 1-2 DAH. It increased as fish approached the juvenile stage. Larger juveniles had smaller relative pre-anal lengths as the tail grew longer. Body depth was relatively large in 1-2 DAH larvae due to the yolk-sac. Body depth increased through flexion, stabilised during the early juvenile stages, but decreased slightly as larvae began to increase in SL.

# Discussion

The larvae and juveniles of *C. laticeps* exhibited typical sparid developmental patterns, as described by Leis & Trnski (1989), including general body shape, myomere count, sequence and nature of fin development, head spination and general pattern of pigmentation. A comparison between the larvae of *C. laticeps* and those sympatric sparid larvae which have been described revealed some important differences.

*Argyrozona argyrozona* (Davis & Buxton, in press).

Preflexion larvae had 25 myomeres and lacked pigmentation at the joining of the colon and gut, and the otic capsule. Otherwise the two species were virtually indistinguishable, especially in the earlier stages. Flexion larvae possessed pigmentation of the snout, nape and medio-lateral surfaces of the tail. Pigmentation of the ventral midline of the tail was less prominent. Early-flexion (4.7mm BL) larvae had two inner and five outer preopercular spines. Late-flexion larvae (5mm BL) possessed two inner preopercular spines but lacked spines on the operculum. Post flexion larvae lacked a dorsal melanophore posterior to the dorsal fin and along the edge of the ventral hypural plate. They possessed widespread pigmentation of the medio-lateral surfaces of the tail and only two inner preopercular spines. Juveniles lacked pigment on the anal, pectoral and pelvic fins, and pigmentation of the dorsal fin membranes was restricted to a fringe on the distal edge. They possessed three inner preopercular, two subopercular and two posttemporal spines. The first soft ray of the pelvic fin was not elongate.

*Cheimerius nufar* (Connell & Garratt, in prep).

Preflexion larvae had pigmentation on the snout, operculum, hindbrain and lateral gut surface. The dorsal and anal anlagen were visible. Pigmentation of the dorsal gut surface was much heavier and they lacked the melanophore at the joining of the gut and colon. Flexion larvae possessed widespread pigmentation on the head and some pigment on the base of the pectoral fin bud. There were nine outer preopercular, four supracleithral, a single posttemporal and no opercular spines. The unpaired fins were much less developed at this

stage (5.8mm BL) having fewer elements. The pectoral fins had not developed rays and the pelvic fins were positioned more posteriorly (the middle of the base of the pelvic fin bud was in line with the posterior edge of the pectoral fin base). Post-flexion larvae lacked pigment posterior to the dorsal fin, had pigmentation on the opercular plate and pectoral fin base. Juvenile fish lacked the lateral, posterior band of pigment on the tail and dorsal area, instead there were broad vertical bands. The posttemporal and supracleithral spines were still evident. There was no pigmentation of the dorsal, anal or pelvic fins.

*Diplodus cervinus* (Brownell 1979a).

The preflexion larvae had heavy pigmentation of the otic capsule, nape, base of the brain, and along the entire length of the symphysis and both ventral and lateral surfaces of the gut. The melanophores of the ventral midline were continuous with the dorsal surface of the gut. Myomere counts were 25. Post-flexion larvae had no pigmentation on the snout but displayed heavy pigmentation of the rest of the head (including the opercular plate), nape and gut. They lacked the pigment posterior to the dorsal and the edges of both the dorsal and ventral hypural plates were pigmented. There were only four inner preopercular and six outer preopercular spines. They lacked the opercular and possessed only a single subopercular spine. The pelvic fin did not display the characteristically lengthened first soft ray.

*Diplodus sargus* (Brownell 1979a; Divanach *et al.* 1982).

Preflexion larvae lacked pigmentation on the fin fold anterior to the anus and possessed pigmentation along the entire ventral surface of the gut. Pigmentation of the otic capsule was heavier and there were a maximum of 14 melanophores along the ventral midline of the tail. Post-flexion larvae lacked pigment posterior to the dorsal, however, preflexion larvae had a prominent melanophore in this position. This feature was therefore even less reliable for this species considering its inconsistency in post-flexion *C. laticeps*. There were only three visible inner preopercular and six outer preopercular spines. The larvae possessed only two posttemporal spines and were lacking an opercular spine. Juveniles lacked pigmentation of the pectoral fin base, anal and pelvic fins, while melanophores on the dorsal fin membranes were sparsely distributed and restricted to the first-four elements.

Tail pigmentation, which included the characteristic black spot on the caudal peduncle in fish over 9mm BL, was restricted to the lateral midline and the edges of both hypural plates were pigmented. The opercular plate was unpigmented as was the lower jaw. Unpaired fin counts were different. The dorsal fin possessed 26 elements, the caudal 6+7 and the anal 16.

*Gymnocrotaphus curvidens* (Brownell 1979a).

The single flexion larva described possessed pigmentation of the nape, lateral gut surface and the edges of both hypural plates. They possessed six prominent inner preopercular spines, one subopercular and no opercular spine. Anal fin count was 12.

*Lithognathus mormyrus* (Brownell 1979a).

Preflexion larvae possessed fewer (14) melanophores on the ventral midline of the tail. They lacked pigmentation on the tip of the cleithral symphysis. There was no spination of the preoperculum. Post-flexion larvae were generally more elongate. Pigmentation of the tail was in three distinct bands on the dorsal and ventral surfaces, and a thin band just dorsal to the midline. Head spination was weak. The inner preopercular margin spination of the outer preopercular consisted of two rounded knobs. There were no posttemporal or opercular spines. Juveniles had a similar pigmentation pattern on the body; however, tail pigment extended into the caudal peduncle and neither the anal nor the pelvic fin was pigmented. Melanophores on the dorsal fin membrane were more disperse. Head spination was weak with only five outer preopercular and two subopercular spines. There was no opercular spine. The first soft ray of the pelvic fin was not elongate. The fin count at this stage was D XI,12; A III,11.

*Pachymetapon blochi* (Brownell 1979a).

Preflexion larvae showed pigmentation of the hindbrain. Pigmentation of the medio-ventral surface of the tail consisted of seven groups of melanophores assembled towards the posterior end of the tail. They did not posses the melanophore at the joint of the gut and colon. Post-flexion larvae had a heavily pigmented gut, inner preopercular plate and nape region, but lacked the pigment spot posterior to the dorsal fin bases. There were three inner preopercular

and eight outer preopercular spines. The operculum possessed only one subopercular and did not have an opercular spine. Juveniles lacked the dark patches on the tail surfaces. Pigmentation of the membrane of the spinous portion of the dorsal fin was similar to *C. laticeps*, however both the dorsal and anal fins possessed dark patches at the base of the most posterior soft fin ray elements. The anal fin otherwise lacked pigmentation and the first (dorsal) elements of the pectoral fin possessed pigment. Head spination had disappeared in the juvenile (20.7mm BL) described. The first soft ray of the pelvic was not elongate and fin counts were DX,12; AIII,10.

*Sparodon durbanensis* (Brownell 1979a).

The single juvenile described lacked the midline band of darker pigmentation and pigment on the pelvic and anal fins. Outer preopercular spination was restricted to three small spines and the edge of the subopercular plate was smooth. There was no opercular spine. The first soft ray of the pelvic fin was not elongate and the fin count was D XI,12; A III,10; P1 15.

*Spondyliosoma emarginatum* (Beckley 1989).

Preflexion larvae possessed three melanophores ventral to the gut, a dorsal melanophore above the midbrain and at the anterior edge of the gut coil. The outer preoperculum possessed three small spines. Post-flexion fish had a characteristic medio-lateral band of pigment and lacked the dorsal tail melanophore. They possessed three inner and five outer preopercular spines. Opercular spines were not described. Juveniles lacked pigmentation of the dorsal and anal fin membranes and had distinctive pigmentation of the tail region in fish smaller than 14.5mm BL. Juveniles had a fin count of D X,13; A III,10. Pigmentation of the dorsal was restricted to the base of the fin elements.

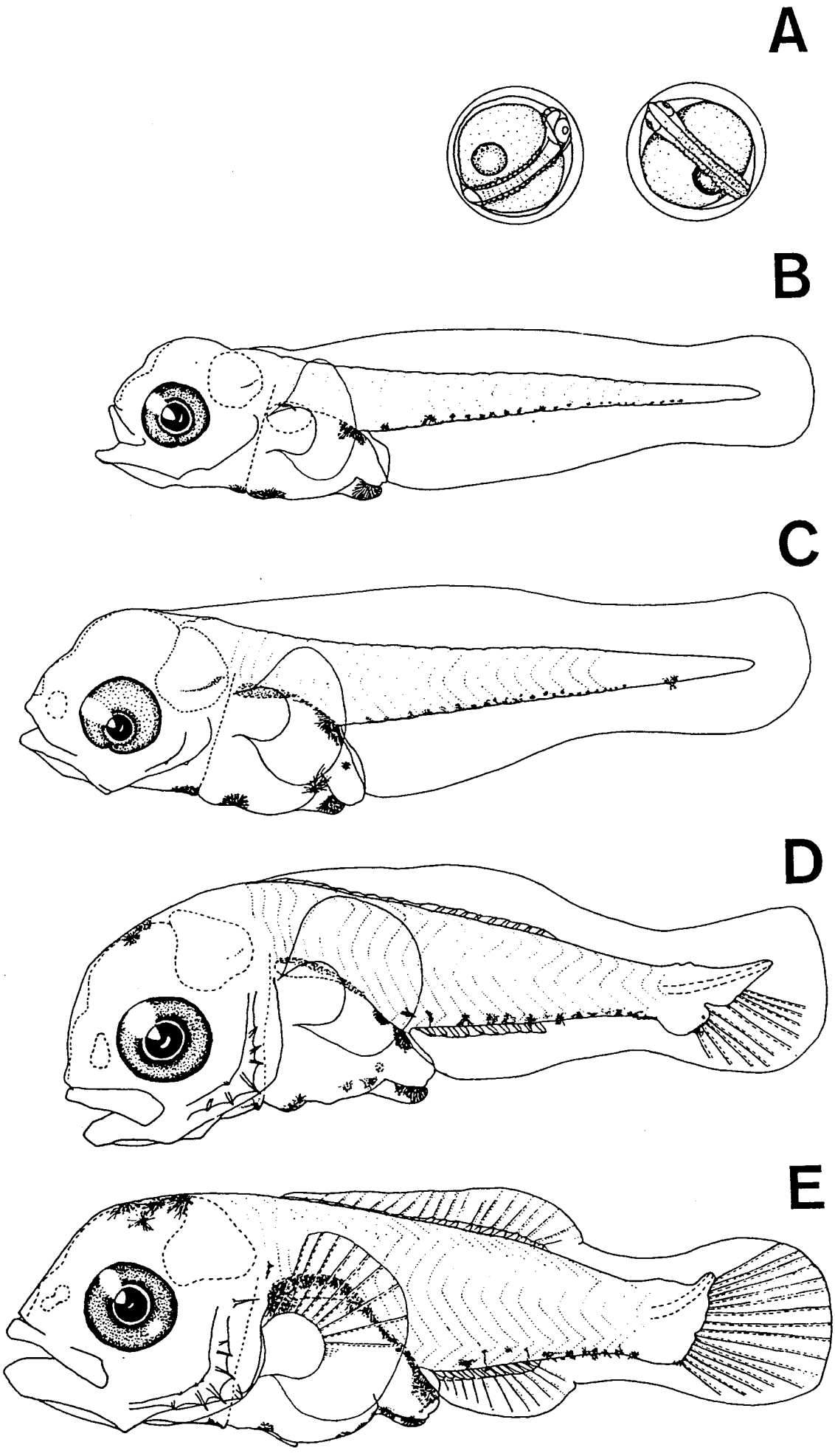
*Rhabdosargus (Sparus) sarba* (Kinoshita 1986).

The fish was elongate and the snout noticeably rounded. Post-flexion fish lacked pigmentation posterior to the dorsal fin, had pigment along the edges of both the dorsal and ventral hypural plates and a melanophore medio-laterally on the caudal peduncle. There were three inner preopercular and four outer preopercular spines. There were no subopercular spines

and what appeared to be a single supracleithral spine. Juveniles had relatively light pigmentation on the head region and the tail pigmentation was restricted to the dorsal and ventral midlines. There were a total of seven outer preopercular spines, and no opercular spines were described. The fin count was D XI,13; A III,11.

**Table 5.2** Morphometrics and meristics of *Chrysoblephus laticeps* larvae and juveniles reared in captivity. Body length is in mm, other measurements are expressed as % body length. BL=Body Length; *n*=No. of Specimens; HL=Head Length; SnL=Snout Length; ED=Eye Diameter; PAL=Pre-anal length; BD=Body Depth; C=Caudal; D=Dorsal; A=Anal; P1=Pectoral; P2=Pelvic.

| Age (DAH) | Stage | Fig | Morphometrics | | | | | | | Fin counts | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | BL | *n* | HL | SnL | ED | PAL | BD | C | D | A | P1 | P2 |
| 1-2 | Preflexion | - | 1.5-1.9 | 10 | 18.36 | 4.39 | 8.81 | 46.33 | 23.51 | - | - | - | - | - |
| 3-8 | | - | 2.0-2.4 | 12 | 20.18 | 5.54 | 9.11 | 38.90 | 19.23 | - | - | - | - | - |
| 3-16 | | - | 2.5-2.9 | 12 | 20.73 | 5.07 | 9.05 | 37.11 | 18.19 | - | - | - | - | - |
| 9-27 | | 1B | 3.0-3.4 | 5 | 22.17 | 6.29 | 10.31 | 40.25 | 19.57 | - | - | - | - | - |
| 14-25 | | 1C | 3.5-3.9 | 5 | 26.64 | 7.05 | 9.5 | 41.94 | 23.97 | - | - | - | - | - |
| 23-27 | | - | 4.0-4.4 | 3 | 27.53 | 6.87 | 9.97 | 48.84 | 29.57 | - | - | - | - | - |
| 20-27 | Flexion | 1D | 4.5-4.9 | 2 | 27.37 | 3.16 | 7.73 | 50.53 | 30.53 | 0+5+5+0 | VIII,10 | 0,11 | - | - |
| 24-30 | | 1E | 5.0-5.9 | 7 | 33.04 | 9.03 | 11.44 | 54.44 | 30.95 | 0+9+7+0 | VIII,12 | II,9 | 9 | - |
| 30-35 | Post-flexion | 1F | 6.0-6.9 | 5 | 37.14 | 10.30 | 13.01 | 63.45 | 37.14 | 6+9+9+4 | X,12 | II,9 | 12 | I,4 |
| 35 | | - | 7.0-7.9 | 1 | 41.03 | 10.26 | 12.97 | 66.67 | 38.1 | 6+9+9+5 | X,12 | II,9 | 12 | I,4 |
| 35 | | 1G | 9.0-9.9 | 1 | 41.67 | 13.57 | 13.02 | 65.63 | 36.46 | 6+9+9+7 | X,12 | II,9 | 14 | I,5 |
| 35 | | - | 10.0-10.9 | 1 | 34.95 | 8.74 | 12.62 | 62.14 | 36.41 | 7+9+9+8 | X,12 | II,9 | 15 | I,5 |
| 41 | Juvenile | 1H | 11.0-11.9 | 2 | 36.36 | 9.94 | 12.77 | 66.43 | 38.60 | 7+9+9+9 | XI,11 | II,9 | 16 | I,5 |
| 41 | | - | 13.0-13.9 | 1 | 39.43 | 7.94 | 13.71 | 65.14 | 37.71 | 7+9+9+9 | XI,11 | II,9 | 16 | I,5 |
| 41 | | - | 23.0-23.9 | 2 | 34.10 | 3.40 | 9.75 | 57.87 | 34.31 | 8+9+9+9 | XI,11 | II,9 | 16 | I,5 |
| 78 | | - | 24.0-24.9 | 1 | 33.97 | 5.73 | 11.65 | 63.01 | 34.77 | 8+9+9+9 | XI-XII,10-11 | III,8 | 16 | I,5 |
| 97-108 | | - | 25.0-25.9 | 2 | 28.48 | 7.91 | 7.91 | 56.33 | 31.01 | 9+9+9+9 | XI-XII,10-11 | III,8 | 16 | I,5 |
| 97-108 | | - | 49.8 | 1 | 32.15 | 7.91 | 9.43 | 52.52 | 34.78 | 9+9+9+9 | XI-XII,10-11 | III,8 | 16 | I,5 |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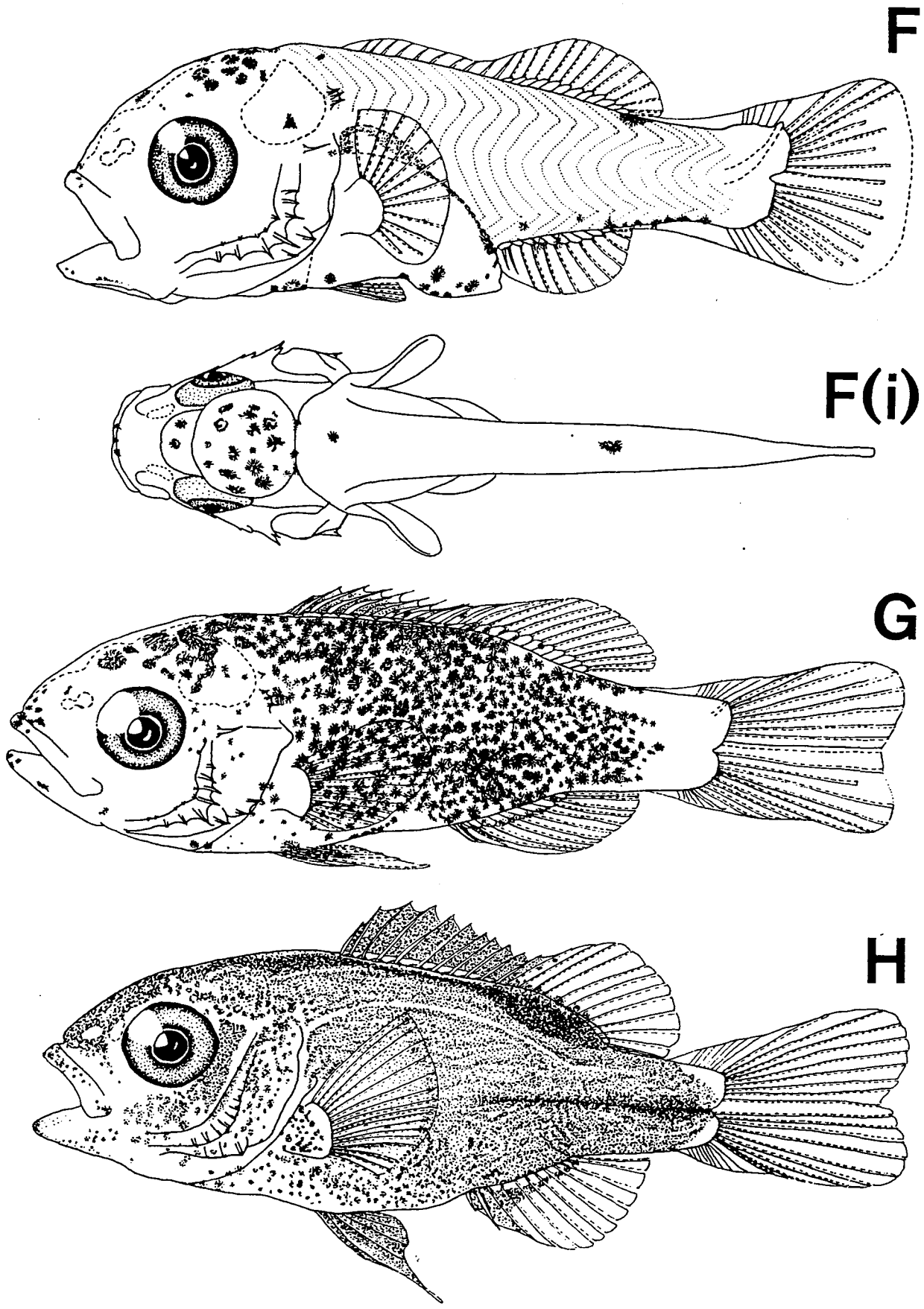


**Figure 5.2** Larval development of laboratory reared *C. laticeps*. A=0.76mm diameter; B=3.23mm BL; C=3.88mm BL; D=5mm BL; E=5.47mm BL; F=6.6mm BL; G=9.6mm BL; H=11mm BL.

# CHAPTER 6. CONCLUSION

The choice of *Argyrozona argyrozona* and *Chrysoblephus laticeps* as model species was justified. Despite the limited opportunities for spawning the fish, sufficient eggs were procured to apply larviculture techniques. *Chrysoblephus laticeps* emerged as a possible candidate aquaculture species due to several factors. Adult *C. laticeps* were easy to catch in sufficient numbers for successful spawning, were not particularly susceptible to barotrauma and the swim bladder was easy to deflate. Because they did not reach sizes above 400mm (Buxton 1990), the largest individuals were relatively easy to handle. They were monandric protogynous hermaphrodites and adults could therefore be sexed by virtue of size (Buxton 1989). Observations by Buxton (1990) of *C. laticeps* in the wild suggest that they are polygynous with one large male spawning with a number of smaller females; this has positive implications for broodstock stocking densities. They have also spawned spontaneously in captivity (Buxton 1990; this study). The adults accepted an inert diet soon after capture and fed even after being handled and stripped. It is one of the few local sparids which has been well researched and long-term studies have been conducted into its reproductive biology, life history characteristics and age and growth (Buxton 1989, 1990, 1993; Lang & Buxton 1993).

Several individuals have been reared from eggs in the past (Brownell 1979a, Brownell & Horstman 1987; A. Connell, Council for Scientific and Industrial Research, Durban, pers. comm.; C. van der Lingen, Sea Fisheries Research Institute, Cape Town, pers. comm.). This combined with the success achieved in this study indicate that the larvae are possible to rear and have the potential for improved survival. Growth, survival and general pattern of development of the larvae are comparable with other cultured sparids, and, according to the evidence presented in Chapter 4, there is a possibility that growth could be significantly improved. The juveniles are gregarious (van der Elst 1988; pers. obs.) and due to protogyny the juveniles were not subject to gender-specific growth rates - one of the major causes of growth depensation (Kristinsson *et al.* 1985). Growth depensation contributes to cannibalism and causes difficulties in marketing and handling (Paller & Lewis 1987; Ottera & Folkvord 1993).

There is a desperate need for a comprehensive market survey to be conducted determining the demand for cultured South African species. Without this, we can only guess

at the market potential of our endemic species. Notwithstanding, *C. laticeps* is a well known local species with a traditional market. It is attractive due to its bright red colour and may have good marketing potential overseas. Although *A. argyrozona* was easily spawned and some larvae reared in captivity, its potential as a candidate species for aquaculture is limited due to the low price it commands in the market place. The adults were also easily stressed when handled - as soon as the fish were caught, they developed a characteristically blotchy stress pattern. The tissue surrounding hook wounds and the area where the deflating needle was inserted was bruised easily. They were skittish when confined in plastic bins used for transporting them to shore, bruising themselves against the sides and requiring the use of anaesthetic. *Argyrozona argyrozona* did not respond well to stripping and their scales came off readily. They possessed no secondary sexual characteristics and were therefore difficult to sex. The adults did not respond to food in captivity and the juveniles were difficult to wean onto an inert diet. Little has been published about the biology of the fish, being limited to a somewhat superficial study by Nepgen (1977) on age and growth and reproduction.

The application of spawning techniques in the study proved effective. Despite limited spawning opportunities, sufficient eggs were procured to apply larval rearing techniques. The number of eggs and spawnings were limited by the logistical problems of depending on wild-caught adults during the spawning season. Although the injection and stripping of wild-caught, ripe adults has been used for supplying eggs for other larval rearing investigations, a dependence on wild-caught adults has generally been found to be impractical (Stickney and Wu-Liu 1991; Battaglene & Talbot 1992, 1994). According to Halls (1992), "The principle difficulty standing in the way of successful cultivation of halibut *H. hippoglossus* in the U.K. have been the establishment and maintenance of a reliable broodstock". In order for future larviculture research to be successful in South Africa, it is essential to establish a captive broodstock. The pre-vittilogenic stages of ovarian and oocyte development manipulation through diet, photoperiod and/or temperature so that the spawning season can be controlled; natural spawning in captivity needs to be stimulated through long-term hormone manipulation with slow-release hormone implants.

In the long term, dependence on artificial hormones might be effectually phased out. Viability, fertility, number, fatty acid content and total protein content of eggs spawned from individuals treated with hormones have been shown to be significantly below that of non-manipulated fish (Caddell *et al.* 1990; Ako *et al.* 1994). The progress of greasy grouper *E. fuscoguttatus* breeding research, for instance, has been hampered primarily because eggs were obtained by artificial spawning methods (Chao *et al.* 1993). Although hormone manipulation is still used to a large extent, the search for environmental cues that are natural triggers of maturation, ovulation and spawning of local species will hopefully produce results which can contribute to more successful manipulation and induction of these processes (Zohar 1990b).

A cooperative Earth Marine and Atmospheric Technology (EMATEK)/Department of Ichthyology and Fisheries Science (DIFS) project to investigate these and other factors influencing seed production in local linefish species was initiated in 1995. It is hoped that techniques will be developed to supply seed to future larviculture research and development.

The fact that survival of *C. laticeps* larvae reared in the relatively small, recirculating system constructed at the DIFS, was comparable to the survival of larvae reared in state-of-the-art hatcheries overseas (Table 6.1), testifies to the effectiveness of the designs described in Chapter 3. According to the available methods of water quality analysis, static flow during first-feeding, coupled with high food densities and high larval mortality caused high ammonia levels. This combined with a drop in dissolved oxygen content and slightly lower temperatures increased the toxicity of un-ionised ammonia. According to the test kit ammonia levels changed with the introduction of intermittent flow following first-feeding, dropping significantly. The trade-off between a static water environment and ammonia buildup at first-feeding should be investigated. Facilitating a turbulence-free increase in flow by means of better tank design and larger tank volumes could go some way to improving survival.

Six *Argyrozona argyrozona* and 212 *Chrysoblephus laticeps* were reared, with survival varying from 0.1-0.5%, through yolk-sac to post metamorphosis. Table 6.1 compares survival rates of *C. laticeps* with several other species reared in other preliminary investigations, using techniques similar to this study.

**Table 6.1** Comparison of survival between *C. laticeps* and other species reared in recent preliminary investigations using similar techniques.

| Species | % Survival of juveniles | Day after hatch fish counted | Reference |
|---|---|---|---|
| Roman *Chrysoblephus laticeps* | 0.125 - 0.51 | 134 | This study |
| Mulloway *Argyrosomus hololepidotus* | 0.0002 | 180 | Battaglene & Talbot 1994 |
| Brown-marbled grouper *Epinephalus fuscoguttatus* | 0.026 | 17 | Supriatna & Khono 1990 (in Chao *et al.* 1993) |
| Southern mullet *Liza richardsonii** | 0.2 | 50 | Bok 1989 |
| Santer sea bream *Cheimerius nufar** | 0.1-0.5 | 150 | Garratt *et al.* 1989 |
| Yellowfin bream *Acanthopagrus australis* | 2.5 | 24-30 | Cowden, unpublished data |
| Red sea bream *Pagrus auratus* | 22 | 50 | Battaglene & Talbot 1992 |
| Yellowfin porgy *Acanthopagrus latus* | 0.7-27.5 | 55-56 | Leu & Chou in press |

*=studies done in South Africa.

Survival of *C. laticeps* larvae was not significantly below that achieved for larvae in similar studies. The studies noted in Table 6.1 refer to the first artificial rearing of a species and do not necessarily refer to the first time larviculture has been attempted by the institution or individuals presenting the publication (both of which may significantly contribute to the success of rearing).

The exact causes for the high mortalities witnessed in this project were difficult to determine. A limited number of eggs obtained at irregular intervals prohibited significant experimentation or statistical confirmation of results. This also prevented sufficient mortality estimates and a reluctance to sacrifice fish for sampling.

Several observations were made concerning larval rearing which are considered to be major factors contributing to low rates of larval survival.

As mentioned in Chapter 4, the highest mortalities were observed in relation to first-feeding for both species. The rotifer strain used as first-food for *C. laticeps* larvae was found to be too large for efficient first-feeding. Three solutions to this problem are suggested:

1) A smaller (S-strain) *Brachionus plicatilis* than the one used here can be imported.

2) Several recent investigations into establishing rearing technologies for lutjanids in South East Asia have found that *B. plicatilis* is not small enough for first-feeding. There has thus been research into local calanoid copepod species (Stottrup *et al.* 1986; Singhagraiwan & Doi 1993; Doi *et al.* 1994). The search for live food species alternative to traditional *B. plicatilis* and *Artemia* spp. is gaining popularity worldwide (Sorgeloos & Leger 1992). Besides the role played by *A. longipatella* in the project as an intermediate food between rotifers and *Artemia* during settlement, their nauplii and the nauplii of other endemic copepods may have good potential as a first-food for future larviculture endeavours in South Africa.

3) Trochophores of the Pacific oyster *Crassostrea gigas* have been used as a successful food organism in several studies (Fukuhara 1984; Garratt *et al.* 1989; Leu & Chou in press). Cryopreserved trochophores are commercially available through overseas companies, but the costs are high. There are, however, several local breeders of *C. gigas* which could be approached.

After first-feeding, larger prey are desirable since larvae select larger prey as they grow (Dabrowski & Bardega 1984). The rotifer and copepod used in this project could be used to bridge the gap between first-feeding and weaning onto *Artemia.*

Algal production was unreliable and the larvae in this study were reared in clear water. The greenwater technique describes the practice of adding algae to the water in which marine pelagic larvae are being cultured. The technique is applied worldwide by commercial hatcheries as well as experimental laboratories culturing a wide variety of species (Fukuhara 1984; Foscarini 1988; Vasquez-Yeomans *et al.* 1990; Mourente *et al.* 1993; Tamaru *et al.* 1994; Reitan *et al.* 1995). There is a recent move towards the elimination of algae in western hatcheries (De Wolf *et al.* 1995), although many of the authors mentioned above claim that the greenwater technique is essential to successful larval rearing. Until larval production technology in South Africa progresses to approximate that prevalent in Europe, the benefits of the greenwater technique cannot be ignored. An irregular supply of microalgae also

hampers efficient live food production, the establishment of which is essential to successful larviculture.

From the larvae surviving first-feeding, a large percentage died as a result of cannibalism. Recommendations for combatting this mortality have been presented in chapter 4. Future areas of research include the effects of grading, feeding frequency, nutrition, photoperiod and stocking density on cannibalism.

Overall, the project demonstrated that established techniques could be applied to local species. It is believed that based on the experience gained during this project, techniques could be refined to the extent that larviculture of marine finfish could become commercially viable in South Africa.

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