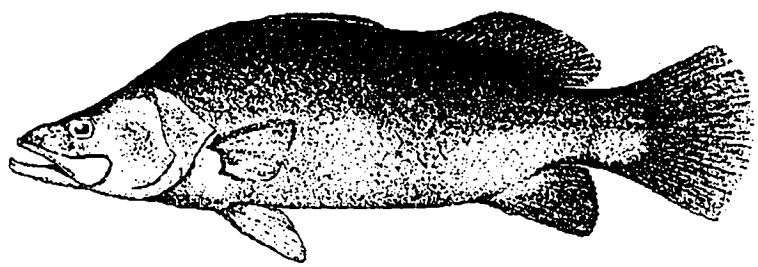
FACTORS AFFECTING SURVIVAL AND GROWTH OF LARVAL BARRAMUNDI *Lates calcarifer* (Bloch) IN AQUACULTURE, WITH PARTICULAR REFERENCE TO FEEDING AND EXTENSIVE REARING



Thesis submitted by

Michael Albert RIMMER

BSc (*Sydney*), MSc (*Macquarie*)

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for the degree of Doctor of Philosophy in

the Department of Zoology

James Cook University of North Queensland

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ABSTRACT

Early attempts to intensively rear barramundi, *Lates calcarifer*, larvae in Australia were periodically beset with problems of high or total mortalities. To determine whether these problems could be ascribed to deficiencies of polyunsaturated fatty acids in the live food organisms used for larval rearing, barramundi larvae were reared in an experimental system comprising replicate 2 litre plastic containers and fed on four diets, representing combinations of supplemented and unsupplemented rotifers (*Brachionus plicatilis*) and supplemented and unsupplemented brine shrimp (*Artemia salina*). Supplementation of rotifers and brine shrimp with a commercially available microencapsulated diet increased the levels of several polyunsaturated fatty acids in the food organisms. Two test diets using freshly hatched brine shrimp produced near total mortality by day 30, while the other two diets, using supplemented brine shrimp, produced negligible mortality over the same period. Supplementation of rotifers with microcapsules resulted in slightly increased growth, but also slightly decreased survival. A second experiment showed that starvation of brine shrimp prior to feeding delayed the onset of the mortality syndrome, but only supplementation with the microencapsulated diet prevented substantial mortalities within the rearing period.

Attempts to replace live prey organisms with microparticulate diets, in order to reduce the costs associated with intensive rearing of barramundi larvae, were unsuccessful. Barramundi larvae fed a microparticulate diet began dying 6

days after initiation of the experiment, presumably from starvation. Mortalities amongst a control group, fed supplemented brine shrimp, were negligible over the same period. Factors which appeared to contribute to the poor acceptance of the microparticulate diet by barramundi were the availability of particles in the water column, and perception of particles by the larvae.

Extensive larval rearing trials with barramundi in a 1350 m^2 brackishwater pond showed that this technique has numerous advantages over traditional intensive rearing methods. Pond productivity was enhanced by adding inorganic and organic fertilisers to promote 'blooms' of phytoplankton, bacteria and protozoans to provide food sources for zooplankton. Barramundi larvae were stocked into ponds the day after hatching (day 2) and grew to 21.5 - 40.8 mm total length in about 3 weeks; representing growth rates of 1.1 to 1.7 mm/day. Survival of barramundi in extensive rearing trials ranged from 0 to 86% (mean 22%), and production (excluding those trials with survival $<1\%$) ranged from 9,215 to 637,037 fish/ha and 0.05 to 7.1 kg/ha/day.

Survival of barramundi in these larval rearing trials was found to be correlated with water temperature and pH during the first week after stocking larvae into the pond. Growth rate was correlated with fish length, fish age, degree days, water temperature, and air temperature.

The diet of barramundi larvae in extensive rearing ponds was examined to determine which types of prey were important as food sources. Barramundi showed a consistent pattern of dietary progression, initially feeding on rotifers and copepod nauplii, then changing to larger zooplankton (copepodites, copepod adults, and cladocerans) and finally to benthic organisms (chironomid larvae).

This pattern was modified by the addition of aeration to the pond, when the larvae commenced feeding on copepod nauplii rather than rotifers.

The results of this research are discussed with a view to developing research strategies to improve the reliability and cost-effectiveness of larval rearing techniques for barramundi. Extensive larval rearing of barramundi has several advantages over traditional intensive rearing techniques, the most important of which is the lower cost of production of fingerlings. Consequently, extensive rearing techniques have been widely adopted by the barramundi aquaculture industry in northern Australia.

TABLE OF CONTENTS

PART TWO

EXTENSIVE LARVAL REARING

LIST OF ILLUSTRATIONS

LIST OF TABLES

STATEMENT ON SOURCES

DECLARATION

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

Michael A. Rimmer 24 August 1996

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CHAPTER 1

General Introduction

Aquaculture is an increasingly important part of Australia's fisheries. In 1989-90, the gross value of aquacultural production in Australia was estimated at $178 million, which represents 23% of the total fisheries production for that year (Treadwell *et al.* 1991). Much of Australia's aquaculture involves the farming of aquatic organisms for utilisation as food by humans, but aquaculture is also becoming increasingly important as a fisheries management tool in Australia. Large numbers of fish, particularly native freshwater species and the introduced salmonids, are produced each year to stock waterways where recruitment of such species is limited as a result of habitat destruction or environmental changes, such as the construction of impoundments. These stocking programs are primarily aimed at providing fish for recreational fishermen (Cadwallader 1983, Rowland *et al.* 1983, Rutledge *et al.* 1990, Rimmer and Russell 1994), or to conserve endangered native fish species by restocking fingerlings into waterways in which the species was formerly abundant (Ingram and Rimmer 1992, Ingram *et al.* 1994).

Barramundi, *Lates calcarifer* (Bloch), is the premier freshwater sportfish of northern Australia. It supports an important commercial wildstock fishery and is a major component of the recreational fisheries of Queensland, the Northern Territory and Western Australia. The recreational fishery for barramundi in Queensland is estimated to be worth approximately $8 million per annum (Rutledge *et al.* 1990). Because barramundi require salt water for spawning and

early larval development, this species cannot support self-maintaining populations in freshwater impoundments, which are important recreational fishing areas throughout eastern Australia. Consequently, there is considerable public demand for barramundi to be stocked into impoundments, as well as into rivers and streams where existing barramundi populations are believed to be in decline (Pearson 1987, Rutledge *et al.* 1990, Rimmer and Russell 1994).

Research into the development of techniques to breed and rear barramundi fingerlings for stocking commenced at the Queensland Department of Primary Industries' (QDPI) Northern Fisheries Centre (NFC), Cairns, in 1984 (Pearson 1987). In addition to the demand for fingerlings for stocking, the relatively high price consistently obtained for barramundi (generally around $11.00-$12.00 per kilogram farm gate price) has led to the development of a rapidly growing aquaculture industry centred around this species. The first commercial barramundi hatchery and farm, 'Sea Hatcheries' (now 'Bluewater Barramundi'), commenced operation at Mourilyan, Queensland, in 1986 (Trendall and Fielder 1991). Techniques used for both research and commercial barramundi production were initially based on those developed in Thailand for the culture of sea bass (*L. calcarifer*) (MacKinnon 1985). Since that time, the techniques used to produce barramundi in Australia have gradually diverged from those used in Asian sea bass culture. There are various reasons for this divergence, but the main influences have been:

1. Differences in the biology of barramundi and Asian sea bass, although both are regarded as the same species (*L. calcarifer*).

2. Different cost structures, particularly with respect to labour, between Australia and Asia, which influences the cost of production.
3. A relatively low level of vertical integration in the Australian barramundi aquaculture industry, with most of the larger operators involved in all facets of barramundi aquaculture, from broodstock maintenance and production of larvae through to grow-out and, in some cases, marketing.

Aquacultural production of barramundi has increased substantially since farming started in 1986 (Table 1.1). At least some of this increased production can be attributed to the research described in this thesis, which has led to the widespread adoption of more cost-effective larval rearing techniques for barramundi; this is discussed further in Chapter 10. Queensland is the major producer of farmed barramundi, with 74% of Australian production in 1993-94. In comparison, production of barramundi from the capture fishery has decreased slightly over recent years (Table 1.2). Because of the higher value of farmed barramundi, the value of aquaculture production is rapidly approaching that of the capture fishery, although production levels are substantially lower (Tables 1.1, 1.2).

Table 1.1 Production and value of production of farmed barramundi in Australia in the financial years 1989-90 to 1993-94 (updated from Barlow and Rimmer 1993). Production figures are tonnes live weight (t).

State	1989-90		1990-91		1991-92		1992-93		1993-94	
	t	$,000	t	$,000	t	$,000	t	$,000	t	$,000
Qld	37	429	103	1100	152	1538	235	2313	260	2860
NT	0	0	0	0	5	51	10	100	20	220
SA/NSW	0	0	2	22	5	55	10	110	70	770
Total	37	429	105	1122	162	1644	255	2523	350	3850

Table 1.2 Production and value of production of barramundi from the capture fishery in Australia in the financial years 1989-90 to 1991-92 (Barlow and Rimmer 1993). Data for 1992-93 and 1993-94 not available at the time of publication. Production figures are tonnes live weight (t).

State	1989-90		1990-91		1991-92	
	t	$,000	t	$,000	t	$,000
Qld	733	5864	783	3913	780	3900
NT	550	2052	459	1819	460	1817
WA	56	346	61	282	46	212
Total	1339	8262	1303	6014	1286	5929

The development of aquaculture technology for finfish species requires reliable and cost-effective techniques for:

1. Broodstock maintenance, spawning and the production of fertilised eggs and larvae.
2. Rearing of larvae from first feeding to a stage suitable for stocking or for weaning onto artificial diets.
3. Grow-out of juvenile fish to market size.

This thesis examines the second of these requirements, larval rearing. The overall aim of the research described in this thesis was to develop reliable and cost-effective techniques for the production of juvenile barramundi for use in stocking or grow-out. This research covers two main areas:

1. The investigation of nutritional influences in the intensive larval rearing of barramundi; and
2. The development of extensive larval rearing techniques for barramundi using earthen ponds.

Accordingly, the thesis has been divided into two parts, each of which deals with one of the above aspects of larval rearing.

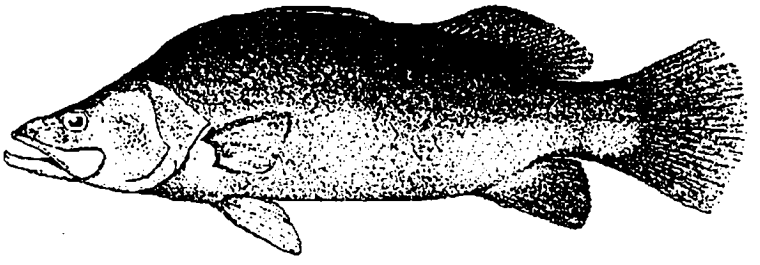
The terms 'intensive' and 'extensive' are loosely used in many aquacultural applications, but have fairly precise meaning when applied to the larval rearing of fishes. Throughout this thesis, 'intensive' is used to indicate the rearing of larval and juvenile fishes in a relatively controlled environment, a hatchery, where factors such as temperature, water quality, light, food quantity, and food quality

can be readily controlled. 'Extensive' rearing involves the use of relatively uncontrolled systems, where fish larvae are introduced into a closed body of water, usually an earthen pond, which is managed to produce adequate quantities of zooplankton for survival and growth of the larvae (Rowland 1983). Both systems of larval rearing have inherent advantages and disadvantages, and these are discussed in Chapter 10. Specific recommendations for aquaculture practitioners arising from this research are listed in Appendix 5.

Two papers were published from the results of research included in this thesis. These papers are presented in Appendix 6.

PART ONE

NUTRITIONAL ASPECTS OF INTENSIVE LARVAL REARING OF BARRAMUNDI



CHAPTER 2

Review: Nutritional Aspects of Intensive Larval Rearing

Introduction

The rearing of marine fish larvae requires a source of food which is suitable for larval survival and growth throughout the period of larval development. The most important criteria which must be met by any foods used are: adequate density to allow larvae to find food without extensive searching; contrast against the background to enable the larvae to sight the food; suitable movement to elicit a feeding ('strike') response; and suitable size for ingestion. In order to meet these criteria, barramundi, and most other marine fish larvae, are fed on live zooplankton reared in the hatchery: rotifers *Brachionus plicatilis* and brine shrimp *Artemia franciscana* (Sorgeloos and Beardmore 1995). Barramundi larvae are initially fed on rotifers (at a density of 10-20 rotifers/ml) from the commencement of feeding (day 2, where day 1 is the day of hatching) until day 14, and on brine shrimp (2-10 brine shrimp/ml) from day 10 until metamorphosis at about day 20 (Fig. 2.1).

Rotifers and brine shrimp are widely used for marine fish larval rearing because of their ease of culture. However, both organisms are now recognised as being deficient in several important nutritional components, particularly highly unsaturated fatty acids (HUFA's). Deficiencies of HUFA's in the live food organisms used for intensive larval rearing have been implicated in the larval mortality syndromes seen in a number of marine fish species, including barramundi (Rodgers and Barlow 1987, Rimmer *et al.* 1988, Dhert *et al.* 1990).

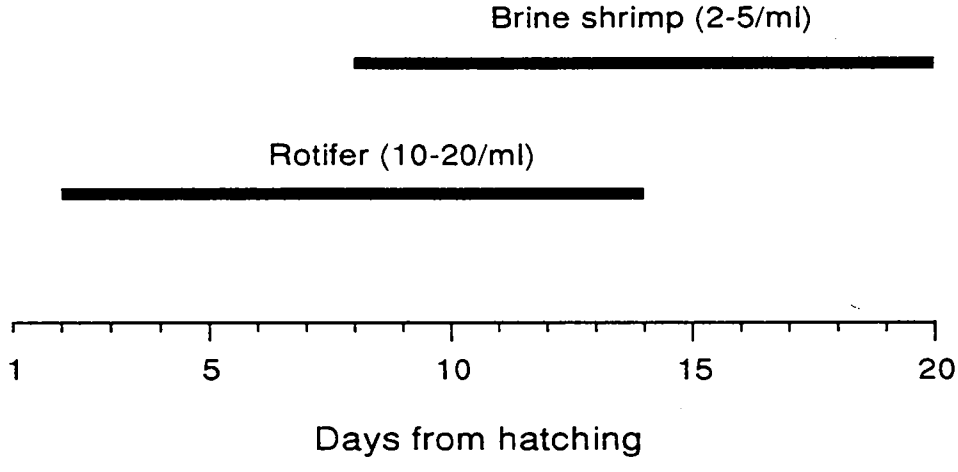


Figure 2.1 Schedule for feeding rotifers and brine shrimp to barramundi larvae reared intensively.

This chapter details the nutritional requirements of marine fish larvae and the nutritional problems associated with the use of live prey organisms used for intensive larval rearing.

Nutritional Requirements of Fish Larvae

Although there have been numerous studies on the nutritional components of live foods for fish larvae, there is little published data on the precise nutritional requirements of marine fish larvae. This is at least partly attributable to the difficulty in experimentally manipulating diets to test each nutritional factor independently of other factors.

The majority of studies on nutritional components of larval fish foods have indicated that fatty acids, particularly long chain fatty acids of the *n*-3 series, are the major limiting factors in larval fish nutrition (Watanabe *et al.* 1983, 1989). However, Dendrinos and Thorpe (1987) found one amino acid (proline) to be present in substantial amounts in the egg yolk of sole (10.89 g / 100 g protein) but present in only trace amounts in the rotifers and brine shrimp used as foods, and suggested that this may indicate a nutritional deficiency.

Fatty acids are important components of the biomembranes in fish (Cowey and Sargent 1972, Rodriguez *et al.* 1993). The predominant fatty acids in fish tissues are in the *n*-3 series such as linolenic (18:3*n*-3), eicosapentaenoic (20:5*n*-3) and docosahexaenoic (22:6*n*-3) (Cowey and Sargent 1972, New 1986). (See Appendix 1 for an explanation of fatty acid nomenclature). In comparison, the

tissues of terrestrial animals are high in *n*-6 fatty acids, such as linoleic (18:2*n*-6) and arachidonic (20:4*n*-6) (New 1986).

The essential fatty acids (EFA's) in marine fish are generally considered to be the C20 and C22 HUFA's, particularly 20:5*n*-3 and 22:6*n*-3 (Watanabe *et al.* 1983, 1989, New 1986, Watanabe 1991). However, survival in some species may also be limited by levels of C18 fatty acids (Dendrinos and Thorpe 1987).

The requirement for *n*-3 HUFA's in marine fish larvae ranges from 0.5% - 2.0% of total fatty acids (Watanabe *et al.* 1989), although these requirements vary between species and may also vary between different growth stages in the same species (Izquierdo *et al.* 1989). Cowey and Sargent (1972) describe the major symptoms of fatty acid deficiency as the occurrence of a 'shock syndrome' in which fish swim in an erratic fashion and then 'faint' to either die or recover; depigmentation of the skin; and erosion of the skin and fins. Low levels of *n*-3 HUFA's have also been associated with increased incidence of disease (Watanabe *et al.* 1989, Corneillie *et al.* 1990) and decreased rates of survival and growth in a range of marine fish species (Witt *et al.* 1984, Dendrinos and Thorpe 1987, Sorgeloos *et al.* 1988, Watanabe *et al.* 1989, Dhert *et al.* 1990, Rodriguez *et al.* 1993).

Although all living organisms can synthesise *de novo* saturated and monounsaturated fatty acids, only photosynthetic organisms are able to synthesise *de novo* HUFA's (Sargent 1976). Fish larvae obtain HUFA's from three main sources:

1. Stored fatty acids in the lipid component of the yolk and oil globule.

2. By elongating and desaturating shorter chain fatty acids to produce HUFA's.
3. From prey animals or plants.

The first of these sources, HUFA's stored in the yolk and oil globule, is the sole source of fatty acids for fish larvae until the commencement of exogenous feeding. In the case of barramundi larvae, the yolk is absorbed rapidly over the first 24 hours after hatching and is almost completely used up by 50 hours after hatching (Kohno *et al.* 1986, Avila and Juario 1987). The oil globule is absorbed more slowly and persists for about 140 hours after hatching (Kohno *et al.* 1986, Avila and Juario 1987).

Some authors have defined the nutritional requirements of fish larvae as being the equivalent of the nutritional composition of the yolk, on the assumption that the yolk provides a complete 'diet' for the yolk-sac larva (Dendrinos and Thorpe 1987, Kanazawa *et al.* 1989, Corneillie *et al.* 1990, Rønnestad *et al.* 1994). While this assumption is probably reasonable for newly hatched larvae, it should be noted that larval development is a dynamic process which includes the development of internal organs and their associated metabolic pathways and thus the nutritional requirements of larvae may change substantially as they develop (Dabrowski *et al.* 1984). For example, Dendrinos and Thorpe (1987) suggested that the requirements of sole larvae for 20:5*n*-3 and 22:6*n*-3 fatty acids during the first few days of larval development could be met by the small quantities in the foods used, and that after this period these requirements could be met by elongation and desaturation of C18 fatty acids. Rønnestad *et al.* (1994) noted that

free amino acids are a significant energy substrate (60-70%) for embryonic *Sparus aurata*, but fatty acids from neutral lipids derived from the oil globule are the main metabolic fuel after hatching (80-90%).

The relative importance of the second source of fatty acids for fish larvae, elongation and desaturation of shorter chain fatty acids, is poorly known. Marine percoid fish are less able to elongate and desaturate fatty acids than freshwater percoids (New 1986). As previously mentioned, the metabolic capabilities of fish larvae change substantially during early development and the ability of fish larvae to metabolise HUFA's may also change. In addition, the process of fatty acid desaturation has been found to be temperature dependent in some fish species and may not take place at relatively low temperatures (Dendrinos and Thorpe 1987).

The third source of HUFA's for fish larvae, their prey, is discussed in detail in the following section.

Nutritional Composition of Live Food Organisms

Microalgae

The different types of microalgae cultured for use in intensive hatchery systems vary significantly in their nutritional composition (Brown *et al.* 1989). To date, much research has centred on the *Chlorella* and *Nannochloropsis* species which are commonly used in intensive aquaculture of marine fishes (Hirayama and Nakamura 1976, Scott and Middleton 1979, Watanabe 1979, Watanabe *et al.* 1983).

Freshwater *Chlorella* species are high in 18:3*n*-3 (an EFA for freshwater fish) but have low levels of 20:5*n*-3 (an EFA for marine fish), while marine *Chlorella* species (at least some of which have recently been found to belong to the eustigmatophyte genus *Nannochloropsis* [Anon 1990]) have low levels of 18:3*n*-3 and high levels of 20:5*n*-3 (Watanabe *et al.* 1983, Brown *et al.* 1989). Neither type contains significant amounts of 22:6*n*-3, but this fatty acid is found in reasonably high levels in golden-brown flagellates such as *Isochrysis* and *Pavlova* (Scott and Middleton 1979, Lubzens *et al.* 1985, Brown *et al.* 1989).

The precise nutritional composition of the microalgae used depends on culture techniques (particularly temperature), the phase of the growth cycle and the genetic strain of microalga used (Sargent 1976, Scott and Middleton 1979, Lubzens *et al.* 1985, Brown *et al.* 1989).

Nutritional deficiencies can be overcome by feeding a range of microalgae rather than a single species (Brown *et al.* 1989). However, large scale culture of many microalgal species is difficult and unreliable. As a result, commercial hatcheries tend to rely on one or two easily cultured species, regardless of their nutritional value.

Yeasts

Several types of yeast have been used as food for rotifers and brine shrimp in intensive culture systems. The main advantage of yeast as a food source is that far less facilities are required compared with microalgal culture. However, yeasts are

deficient in *n*-3 HUFA's, particularly 20:5*n*-3 and 22:6*n*-3 (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987).

These HUFA's can be incorporated in the diet by culturing the yeast on a medium containing high levels of *n*-3 HUFA's, such as fish liver oils; such yeasts have been termed omega-yeasts (Watanabe *et al.* 1983).

Rotifers

The mineral composition of yeast-fed rotifers (*Brachionus plicatilis*) is largely unaffected by the mineral composition of the yeasts used as food (Watanabe *et al.* 1983). In contrast, the proximate composition of the food organisms is reflected in the proximate composition of the rotifers. Water content is greater in rotifers cultured on yeast than those cultured on microalgae, while lipid levels are higher in the latter (Watanabe *et al.* 1983). The lipid levels of yeast-fed rotifers directly reflect the lipid levels of the yeasts used as food (Dendrinos and Thorpe 1987).

The amino acid composition of rotifers fed on yeasts does not differ from those fed on microalgae (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987).

The fatty acid composition of rotifers reflects the fatty acid composition of the foods used to rear them (Watanabe 1979, Scott and Middleton 1979, Watanabe *et al.* 1983, Lubzens *et al.* 1985, Dendrinos and Thorpe 1987). However, the relationship between fatty acid levels of rotifers and their food organisms is not always proportional. In particular, rotifers fed on a diet deficient in C20 and C22 HUFA's have been found to contain significant levels of 22:6*n*-3, suggesting that

Brachionus is capable of elongating and desaturating fatty acids (Lubzens *et al.* 1985, Dendrinos and Thorpe 1987). However, the rate of synthesis of these HUFA's is low and the levels reached are well below the levels regarded as essential for adequate nutrition of marine fish larvae.

The nutritional composition of rotifers can be upgraded by several techniques:

1. Secondary culture of yeast-fed rotifers with microalgae; this technique has been shown to increase the levels of 20:5*n*-3 from trace amounts to a maximum of 27% after 2 days secondary culture with *Chlorella* (Watanabe 1979, Watanabe *et al.* 1983).
2. Indirect method: the levels of *n*-3 HUFA's in the rotifers can be increased by culturing yeasts on media incorporating fish liver oil (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987).
3. Direct method: rotifers can be fed a homogenised mixture of fish oil and egg yolk (Fontaine and Pevera 1980, Watanabe *et al.* 1983), a liquid supplement (Sorgeloos *et al.* 1988) or microcapsules (Walford and Lam 1987). Maximum levels of HUFA incorporation are reached after 8 hours of secondary culture with microcapsules (Walford and Lam 1987).

Brine Shrimp

The nauplii of the brine shrimp, *Artemia franciscana* (Sorgeloos and Beardmore 1995), are widely used as food for larval and juvenile fishes, primarily

because of the convenience with which the resistant cysts can be stored for long periods before use.

Nutritional composition varies between different strains of brine shrimp. Japanese workers have classified *Artemia* strains into two types: a 'freshwater' type which is high in 18:3*n*-3 and a 'marine' type which has high levels of 20:5*n*-3. Neither type has high levels of 22:6*n*-3 (Watanabe 1979, Watanabe *et al.* 1983).

The water and lipid content of brine shrimp is highest in newly hatched nauplii (Dendrinos and Thorpe 1987). Unfed brine shrimp nauplii show a number of changes in their nutritional composition during starvation. Lipid levels initially decrease and protein levels increase concomitantly, followed by a decrease in protein as carbohydrates, lipids and proteins are sequentially utilised as energy sources. In addition, the level of 18:3*n*-3 fatty acid decreases and 20:5*n*-3 and 22:6*n*-3 levels increase, presumably as a result of elongation and desaturation of C18 fatty acids (Watanabe 1979, Dendrinos and Thorpe 1987).

Brine shrimp can be fed on yeasts, microalgae or particulate artificial feed to increase the size of the food organisms or to supplement existing nutritional components (Jones *et al.* 1974, Sakamoto *et al.* 1982, Walford and Lam 1987). The proximate biochemical composition of yeast-fed brine shrimp is largely affected by the biochemical composition of the yeast supplied (Dendrinos and Thorpe 1987).

Similarly, the fatty acid composition of brine shrimp fed on yeasts or microalgae reflects the fatty acid composition of the foods used, as well as

biochemical changes during growth (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987). The proportion of 20:5*n*-3 and 22:6*n*-3 increases in fed brine shrimp even when the food organisms are deficient in these fatty acids, apparently due to elongation and desaturation of shorter chain *n*-3 fatty acids (Dendrinos and Thorpe 1987).

The levels of *n*-3 HUFA's in brine shrimp can be increased by feeding the nauplii on a lipid emulsion which is rich in these fatty acids (Watanabe *et al.* 1983, Ballaer *et al.* 1985, Sorgeloos *et al.* 1988, Fernández-Reiriz *et al.* 1991). The level of *n*-3 HUFA's reaches a maximum level after 12 hours of feeding using this technique (Watanabe *et al.* 1983). However, not all *n*-3 HUFA's are taken up proportionally by this method since brine shrimp incorporate longer fatty acid molecules less readily than shorter chain fatty acids (Dendrinos and Thorpe 1987). The fatty acid composition of brine shrimp can also be enhanced using microencapsulated diets (Sakamoto *et al.* 1982, Walford and Lam 1987). Maximum incorporation of HUFA's by brine shrimp cultured with microcapsules occurs after 8 hours (Walford and Lam 1987).

Other Food Organisms

The freshwater cladocerans *Daphnia* and *Moina* have been used as a supplementary food organism in the intensive rearing of marine fish larvae, including *L. calcarifer*, in South-east Asia (Tattanon and Tiensongrusmee 1984, NICA 1986, Parazo *et al.* 1990, Fermin 1991). *Moina* cultured on yeasts have been found to contain low levels of *n*-3 fatty acids, while those cultured using

poultry manure were high in 20:5*n*-3 (Watanabe 1979, Watanabe *et al.* 1983). A major disadvantage in using freshwater cladocerans is that they rapidly die when introduced to sea water and thus small numbers of cladocerans must be added regularly at short intervals (NICA 1986). Alternatively, euryhaline fish larvae, such as *L. calcarifer*, can be transferred to low salinities (c. 10 ppt) for rearing on *Moina* (Parazo *et al.* 1990, Fermin 1991). However, this strategy restricts use of *Moina* as a supplementary food organism to the period when larvae are tolerant of extremely low salinities, i.e. 15 days or older (Fermin 1991).

Copepods have long been recognised as useful supplementary food organisms for the culture of marine fish larvae. The marine copepods *Tigriopus* and *Acartia* contain high levels of both 20:5*n*-3 and 22:6*n*-3 fatty acids, even when cultured on media deficient in HUFA's (Watanabe 1979, Watanabe *et al.* 1983, Witt *et al.* 1984). Better survival and growth rates for larvae fed cultured copepods compared with larvae fed rotifers and brine shrimp has been demonstrated for mahimahi *Coryphaena hippurus*, mullet *Mugil cephalus* (Kraul 1990, Kraul *et al.* 1991) and sobaity *Acanthopagrus cuvieri* (James and Al-Khars 1986). Dhesprasith *et al.* (1986) examined the effects of copepod density on consumption by 14-24 day old larvae of *L. calcarifer*, but supplementary feeding of *L. calcarifer* larvae with copepods does not seem to have been widely adopted.

There have been numerous studies on the nutritional composition of wild zooplankton, and some studies on the effects that wild zooplankton as a prey source have on growth and survival of intensively reared marine fish larvae. For example, Atlantic halibut (*Hippoglossus hippoglossus*) larvae reared primarily

using brine shrimp are subject to pigmentation problems that can be overcome by feeding wild zooplankton in combination with brine shrimp, but this treatment is only effective if zooplankton is supplied prior to a critical stage (beyond 19 days of feeding) (Næss *et al.* 1995). However, due to the diversity of the composition of 'wild zooplankton', the application of the results of these studies is largely limited to the species and geographic areas where the research was carried out. For this reason, and because wild zooplankton was not used as a treatment in the experimental work described in this section of the thesis, this literature is not reviewed in detail here.

Nutritional Supplements for Marine Fish Larvae

Several nutritional supplements are now available for use in mariculture. There are basically two modes of application of these supplements:

1. Indirect supplementation: this technique involves feeding rotifers and brine shrimp with a supplement which enhances the nutritional composition of the organism prior to feeding to the fish larvae.
2. Direct supplementation: microparticulate or microencapsulated diets with a nutritional composition specifically developed for marine fishes can be fed directly to fish larvae.

There are three main types of supplementary feeds used in intensive larval rearing of marine fishes: liquid, microparticulate and microencapsulated supplements.

A number of liquid enrichment diets have been developed for rotifers and brine shrimp (e.g. 'Selco', produced by Artemia Systems, Ghent, Belgium); the liquid (usually lipid-based) is added to the vessels containing the cultured organisms before they are fed to the fish larvae in order to enhance the nutritional composition of the food organisms. However, these liquid supplements are generally unsuitable for use in tropical aquaculture applications because high water temperatures lead to rapid bacterial decomposition of the supplement with resulting water quality problems in the culture vessels (Rodgers and Barlow 1987).

Microparticulate diets have been developed for use as a substitute for live food organisms in intensive rearing. They have been successfully used to rear a range of temperate marine fish species by wholly or partly replacing rotifers and brine shrimp (Appelbaum 1985, Zitzow and Millard 1988, Jones *et al.* 1993). However, fish fed on microparticulate diets may develop deformities caused by nutritional deficiencies in the composition of the feed (Zitzow and Millard 1988).

Microencapsulated feeds were originally developed as a balanced diet for larval crustaceans. Microcapsules with relatively high levels of HUFA's are now commercially available and the smallest microcapsules (5-20μm) can be used to supplement the nutritional composition of rotifers and brine shrimp fed to fish larvae. Each microcapsule is coated in a protein membrane that prevents leaching of water soluble nutrients but which is digested when the particle is eaten (Jones *et al.* 1974, Walford and Lam 1987). The membrane coating also delays bacterial decomposition of the microcapsule, allowing for extended immersion of the

supplement in the culture vessels without severe degradation of water quality. Frippak Feeds (U.K.) has recently developed a microencapsulated diet specifically for supplementary feeding of live food organisms for marine fish larvae (Frippak 'Booster').

Nutritional Requirements of Barramundi Larvae

The preceding notes indicate that the nutritional deficiencies of live food organisms used in intensive larval rearing of marine fishes are well established. However, the precise nutritional requirements of most marine fish larvae, including barramundi, are not known. In addition, methods of supplementing the nutritional composition of the live food organisms vary considerably. The research described in Part One of this thesis describes experiments which were designed to develop techniques for the nutritional supplementation of barramundi larvae in intensive culture which would increase survival and growth and which could be adopted by commercial hatcheries for minimal cost and with minimal modification to existing procedures and equipment.

Chapter 3 describes a series of experiments which were designed to examine the role of nutritional deficiencies in the live food organisms used for intensive rearing of barramundi and to develop techniques for the indirect supplementation of barramundi larvae. Chapter 4 examines the potential for direct supplementation of barramundi larvae, replacing live food organisms with inert diets. Chapter 5 discusses the application of the experimental results from this

section, with particular emphasis on the cost-benefits of these techniques in commercial aquaculture.

CHAPTER 3

Effects of Nutritional Enhancement of Live Food Organisms on Growth and Survival of Barramundi Larvae

Introduction

Barramundi larvae reared in Australian hatcheries have periodically suffered severe mortalities (up to 90% in some batches) at around 12 to 14 days after hatching. Affected larvae became pale and swam erratically in a corkscrewing motion before dying (MacKinnon 1987). Histological examination of the affected larvae showed extensive vacuolation of the brain and spinal cord and accumulation of excessive fat deposits in the liver (Rodgers and Barlow 1987). The cause of these mortalities has been variously ascribed to nutritional deficiencies in the live food organisms fed to the barramundi larvae (Rodgers and Barlow 1987) and to the action of a picorna-like virus in the larvae (Glazebrook *et al.* 1990, Glazebrook and Heasman 1992, Munday *et al.* 1992). Similar symptoms have been described for a mortality syndrome seen in *L. calcarifer* larvae reared in Tahiti (AQUACOP *et al.* 1990, Renault *et al.* 1991).

This syndrome has not recurred at the barramundi hatchery at NFC since supplementary feeding techniques for rotifers and brine shrimp were adopted in 1987, using a microencapsulated diet (Frippak 'CAR 1' and 'Booster') known to contain high levels of HUFA's (Walford and Lam 1987). This supports the hypothesis that the syndrome may have been caused by nutritional deficiencies in the diet of the larvae (Rimmer *et al.* 1988).

Another mortality syndrome has been described for *L. calcarifer* larvae reared in hatcheries in the Philippines (Dhert *et al.* 1990). Mortalities commenced 23 days after hatching and this syndrome was found to be associated with deficiencies of HUFA's in the brine shrimp component of the larval diet (Dhert *et al.* 1990).

In order to investigate the cause of the observed mortality syndrome in intensively reared barramundi and to develop larval diets which would provide maximal survival and growth, a series of experiments was conducted on the nutritional requirements of barramundi larvae. The experiments described in this chapter were designed to:

1. Determine whether the mortality syndrome observed in larval barramundi 12-14 days after hatching could be ascribed to nutritional deficiencies in the live food organisms used to rear barramundi intensively.
2. Investigate the nutritional influences which affect survival and growth in intensively reared barramundi.
3. Increase the survival of barramundi larvae reared intensively by developing techniques to enhance the nutritional composition of the live food organisms used in intensive rearing.

Materials and Methods

Barramundi larvae used in these experiments were reared from fertilised eggs obtained from wild-spawning barramundi at Weipa in the north-eastern Gulf of Carpentaria in northern Queensland. The fertilised eggs were air-freighted to

Cairns and arrived around the time of hatching (12-17 h after fertilisation at 28-30°C).

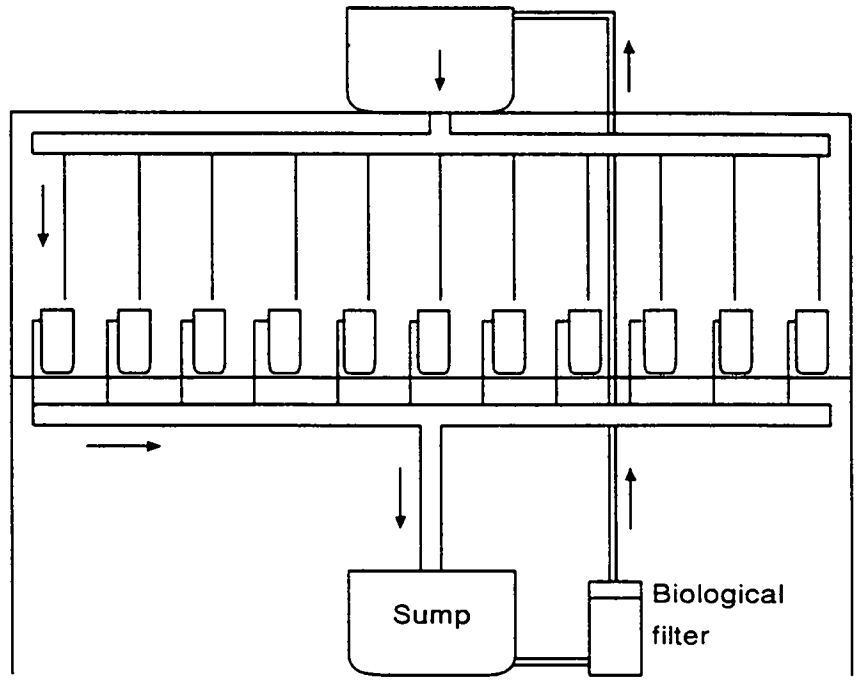
For convenience, the term 'larva' has been used to describe the fish used in these experiments, although the larger fish used in these experiments are properly termed 'juvenile' (Kendall *et al.* 1984). Barramundi larvae metamorphose at 8-12 mm in length (MacKinnon 1987, Russell 1987). Similarly, the term 'nauplius' is used to include the various naupliar stages of brine shrimp.

Experimental Larval Rearing Unit

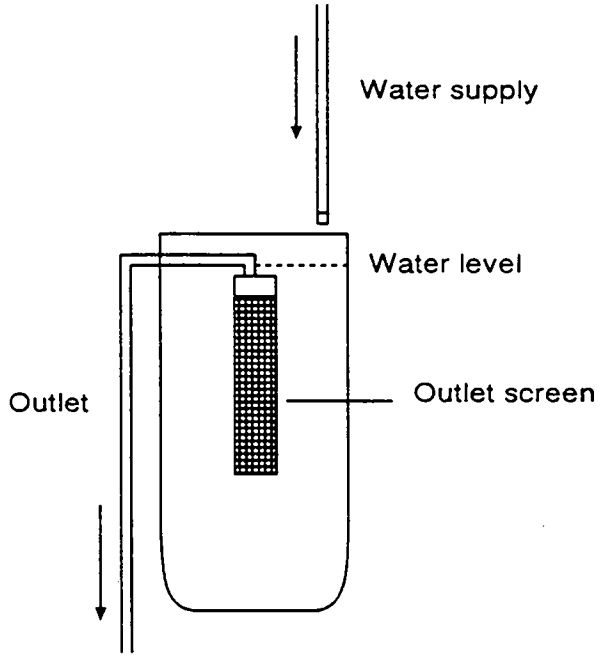
The experimental larval rearing unit used in these experiments comprised 22 individual chambers each of 2 litres capacity (Fig. 3.1a). Water from a header tank was gravity-fed to the rearing chambers, from which it drained via small nylon screens (60, 120 or 200μm aperture) allowing water exchange while retaining the food organisms (Fig. 3.1b). The water from the rearing chambers collected in a sump and was pumped back to the header tank via a biological filter. Temperature was maintained at 29$\pm$1°C by air-conditioning the room and heating water in the header tank. Lighting was provided by fluorescent lamps at an intensity of 400 lux at the water surface. Water quality parameters (temperature, pH, salinity, ammonia, nitrite and nitrate) were monitored daily. Dissolved oxygen was monitored irregularly, but was always in the range 90-100% saturation.

Figure 3.1 Diagram of the experimental larval rearing unit used for the nutrition experiments described in this chapter showing (a) general arrangement of experimental unit, and (b) details of larval rearing chamber. Arrows indicate the direction of water flow within the unit. Note diagrams not to scale.

a


Header tank
Sump
Biological
filter

b


Water supply
Water level
Outlet
Outlet screen

The larvae were fed twice daily to ensure that they had constant access to freshly supplemented food organisms. Before each feed, approximately 90% of the water from each chamber was siphoned through a large surface area screen (200 or 400μm) to remove the remaining food organisms while retaining barramundi larvae.

Larval Feeding Schedule

Rotifers were reared outdoors on the microalga *Nannochloropsis oculata* (CSIRO isolate CS-179) together with small quantities of yeast. Rotifers were harvested daily in the morning and fed to the barramundi larvae that afternoon and the following morning. Rotifers used in the afternoon were supplemented with a microencapsulated diet (Frippak 'Booster') in aerated 3 l bottles for 4 h at 1.0 g microcapsule dry weight (DW) / 4 $\times$ 10^6 rotifers / l. Those used the next morning were supplemented for 20-22 h at 0.2 g microcapsule DW / 2 $\times$ 10^6 rotifers / l. Rotifers not supplemented with microcapsules were retained in aerated 3 l bottles at the same density as supplemented rotifers.

Brine shrimp nauplii ('Aquarium Products' brand; origin Columbia) were harvested daily and fed immediately ('freshly hatched' treatment) or starved for 24 h to ensure that the yolk was absorbed before they were offered to the barramundi larvae ('starved' treatment) or were supplemented. Supplementary feeding of brine shrimp took place in a 50 l glass aquarium or a 70 l hemispherical fibreglass tank. Microcapsules (Frippak 'Booster') were added at 0.3 g microcapsule DW / 0.5-1.0 $\times$ 10^6 nauplii/l.

Barramundi larvae were fed rotifers at 10-20 rotifers/ml from day 2 (where hatching is designated as day 1) to day 14, and brine shrimp at 2 nauplii/ml from day 8, increasing to 5 nauplii/ml by day 12 and continuing at this density until the completion of each experiment.

Reference samples were taken from two sources to allow a comparison of natural dietary regimes with those applied in the hatchery. Dendrinos and Thorpe (1987) suggested that the biochemical composition of the egg yolk reflects the nutritional requirements of the larvae because the egg yolk is the sole source of nutrition prior to the commencement of exogenous feeding. Oocytes were stripped from running-ripe female barramundi caught at Weipa and the chorion removed by filtering the macerated egg material through 20μm mesh. The filtered egg yolk was then used for biochemical analyses.

Juvenile barramundi reared in a brackishwater earthen pond were also used for reference samples. These fish were reared on naturally occurring zooplankton (Rutledge and Rimmer 1991) and can thus be assumed to have a biochemical composition similar to juvenile barramundi in the wild which recruit to similar habitats in estuarine mangrove swamps (Russell and Garrett 1985).

Experimental Design

The experimental larval rearing unit was used to test 4 replicates of 4 diets (experiments N1 and N2) or 3 diets (N3) in a block design. The rotifer and brine shrimp treatments used for the experimental diets in these three experiments are listed in Table 3.1. Experiment N1 was used to determine the effect of the test

diets on survival, whereas N2 was used to examine the effects of the same diets on growth. Experiment N3 was used to further differentiate the effects of different brine shrimp treatments on survival of barramundi larvae. The same procedures and experimental conditions were used in all experiments.

Larvae used in each experiment were taken from a single 1200 l tank containing hatched larvae. Because direct counting of larvae resulted in severe but highly variable mortality, three 50 ml samples from the tank containing hatched larvae were counted to provide an estimate of larval density, and a volume of water estimated to contain approximately 500 larvae was added to each rearing container. Five additional volumes were preserved for enumeration after the larvae had been introduced to the rearing chambers and these indicated that approximately 550 larvae were introduced to each chamber for experiment N1 and 400 larvae for N3. Low numbers of barramundi larvae available for experiment N2 resulted in only about 130 larvae being added to each rearing container for this experiment.

Mortalities could only be accurately estimated after about day 10, when larvae were large enough to leave visible corpses. Experiment N1 was terminated at day 30 when cumulative mortalities in two treatments were at or near 100%. Experiment N2 was terminated at day 22, when all survivors were preserved in 10% formalin for later measurement of total length (TL); this measurement was used to compare growth between treatments. Experiment N3 was terminated at day 40 when cumulative mortalities in two treatments approached 100%.

Key to abbreviations in Tables 3.1 - 3.5.

Rotifer Treatments

CR: rotifers reared on *N. oculata*.

SR: rotifers reared on *N. oculata*, then supplemented with microcapsules.

Brine Shrimp Treatments

FH: freshly hatched brine shrimp.

St: brine shrimp starved for about 24 h after hatching to allow yolk resorption.

Supp: brine shrimp harvested about 24 h after hatching, then supplemented with microcapsules.

Reference

Eggs: egg yolk component of barramundi oocytes stripped from running-ripe female fish at Weipa.

Fish: whole juvenile barramundi reared in an earthen pond containing naturally occurring zooplankton.

Table 3.1 Treatments of rotifers and brine shrimp used for the test diets in experiments N1-N3. See text for key to abbreviations.

Experiment	N1, N2	N3
Diet 1	CR, FH	SR, FH
Diet 2	SR, FH	SR, St
Diet 3	CR, St/Supp*	SR, Supp
Diet 4	SR, St/Supp*	-

* Larvae fed starved brine shrimp (St) from day 8 to day 12, then supplemented brine shrimp (Supp) from day 13.

Statistical Analyses

Analysis of variance (ANOVA) and Tukey's HSD multiple range test were carried out on TL data from experiment N2 using the statistical package Statistix. Patterns in mortality over time were compared by fitting proportional hazards models with the statistical package GLIM. This approach is widely used in medical research and relates the probability of failure for a treatment to some baseline risk (or hazard) by estimating a quantity termed the hazard ratio. Although the baseline hazard may vary with time, the hazard ratio for any treatment is assumed to remain constant. The methods used are similar to those described by Bartlett (1978). Hazard analyses were carried out by A. Lisle (QDPI, Biometry Branch, Mareeba).

Histology

Samples of dead and live moribund larvae were taken for histological examination at irregular intervals. Most of the larvae sampled live at day 22 in experiment N2 (a total of 389 larvae) were used for histological examination. Specimens for histology were preserved in 10% formalin, and forwarded to the QDPI veterinary laboratory at Oonoonba, Townsville, where the specimens were processed using conventional wax embedding techniques, sectioned and stained with haematoxylin and eosin (J. Norton, pers. comm.).

Biochemical Analyses

Four to six replicate samples of each dietary treatment (incorporating organisms from both morning and afternoon feeds), ten samples of barramundi oocytes and two samples of barramundi juveniles were used for the biochemical analyses. Samples of rotifers and brine shrimp were sieved to remove small particles such as algal cells and uneaten microcapsules before preparation for analysis.

Proximate Composition

The samples were weighed, freeze-dried, then forwarded to A. Reed at the QDPI laboratory at Malanda for determination of proximate composition.

Aliquots were taken from a prepared homogenised sample for the following analyses. Moisture was determined in duplicate by drying 2g samples to constant weight at 100°C. Fat was extracted using the method of Folch *et al.* (1957) and determined by evaporating a portion of the chloroform (fat) layer from the extraction and weighing the residue. Protein content was determined using the Kjeldahl method for total nitrogen and applying a factor of 6.25 to calculate crude protein. Ash was determined by drying 4g samples on a steam bath for 2h, igniting at 550°C in a furnace overnight then weighing the cooled residue. Carbohydrate content was calculated by difference (A. Reed, pers. comm.).

Fatty Acid Composition

The samples were extracted with chloroform/methanol (2:1 v/v) using the modified methods of Folch *et al.* (1957) and Bligh and Dyer (1959). The samples

were stored under nitrogen at -25°C until analysed for fatty acid composition by A. Reed at the QDPI laboratory at Malanda.

The excess solvent was removed using a rotary evaporator and the lipid residue taken up in a minimum of hexane. The base-catalysed transesterification procedure of Christopherson and Glass (1969) was used to prepare the fatty acid methyl esters from the lipid solution. The esters were separated by gas-liquid chromatography on a Shimadzu R1-A with a 2.1m x 3mm i.d. glass column packed with 15% OV-275 on 100/120 Chromosorb PAW-DMCS. The column oven was temperature programmed from 190° to 220°C increasing at 2°C/min and the carrier gas (nitrogen) flow rate was 65 ml/min.

The peaks were identified and quantified on a Shimadzu RPR-G1 GC processor calibrated using the methyl esters of authentic triacyloglycerol standards supplied by Sigma (Sigma Chemical Co., St Louis, MO, USA). A comparison was also made with a standard methylated cod liver oil sample supplied by R. Johns of the University of Melbourne (A. Reed, pers. comm.).

Amino Acid Composition

The samples were weighed, freeze dried, then forwarded to M. Levitt at the QDPI Animal Research Institute at Yeerongpilly for determination of amino acid composition.

The samples were further dried in a vacuum desiccator over P_2O_5. The dry samples were fat extracted with hexane, ground in an agate pestle and mortar, and placed in bottles in a vacuum desiccator over P_2O_5. A subsample of 0.5g was

taken for Kjeldahl Nitrogen analysis. These results were corrected for fat content and expressed as g/kg crude protein (N x 6.25) on sample DM basis. A further subsample of 200mg was hydrolysed with 20ml 6N HCl in a sealed tube under nitrogen for 18 hours at 110°C for amino acid analysis. A 100mg subsample was treated with performic acid prior to hydrolysis for the estimation of cystine and methionine. Samples were analysed for amino acids using ion exchange chromatography in a Waters amino acid analyser (M. Levitt, pers. comm.).

Results

Water Quality

Water temperature in the experimental unit ranged from 28-30°C; pH from 7.8-8.0; salinity from 27-32 g/l; total ammonia from 0-0.2 mg/l; nitrite from 0-0.2 mg/l; nitrate was constant at about 20 mg/l.

Survival

Experiments N1 and N2

The four test diets used in experiments N1 and N2 showed dramatically different effects on survival of barramundi larvae. Patterns of mortality were similar in both N1 and N2, despite the different initial densities used in N2. Larvae fed diets 1 and 2 began showing stress symptoms (pale colouration, erratic swimming followed by 'fainting') on day 18. Large-scale mortalities began on day 20 (N1) or day 21 (N2), and continued until most larvae were dead within the next 5 or 6 days (Fig. 3.2a). In comparison, larvae fed diets 3 and 4 had

substantially less mortalities over the same period. Diet 2 (which incorporated supplemented rotifers) showed consistently higher mortality over the duration of the experiment than diet 1 (which incorporated unsupplemented rotifers); and, similarly, diet 4 showed consistently higher mortality than diet 3 (Fig. 3.2a).

Analysis of the mortality data from experiment N1 used the unsupplemented diet 1 as the baseline treatment. Supplementation of brine shrimp substantially reduced the hazard (i.e. increased the probability of survival) for diets 3 and 4 relative to diets 1 and 2 (Table 3.2). In comparison, supplementation of rotifers slightly increased the hazard for diets 2 and 4 relative to diets 1 and 3. No interaction between the effects of supplementing rotifers and brine shrimp was found. The lowest hazard ratio was associated with diet 3 (Table 3.2) indicating that this was the diet which produced maximal survival in barramundi larvae over the duration of the experiment.

Table 3.2 Hazard ratios for test diets using supplemented and unsupplemented rotifers and brine shrimp (diets 1 - 4) from experiment N1.

		Rotifers	
		CR	SR
Brine shrimp	FH	1.000 (Diet 1)	1.785 (Diet 2)
	St/Supp	0.014 (Diet 3)	0.026 (Diet 4)

Figure 3.2 Cumulative mortality of barramundi larvae fed 4 test diets in experiments (a) N1 and (b) N2. Plotted values represent means of four replicates.

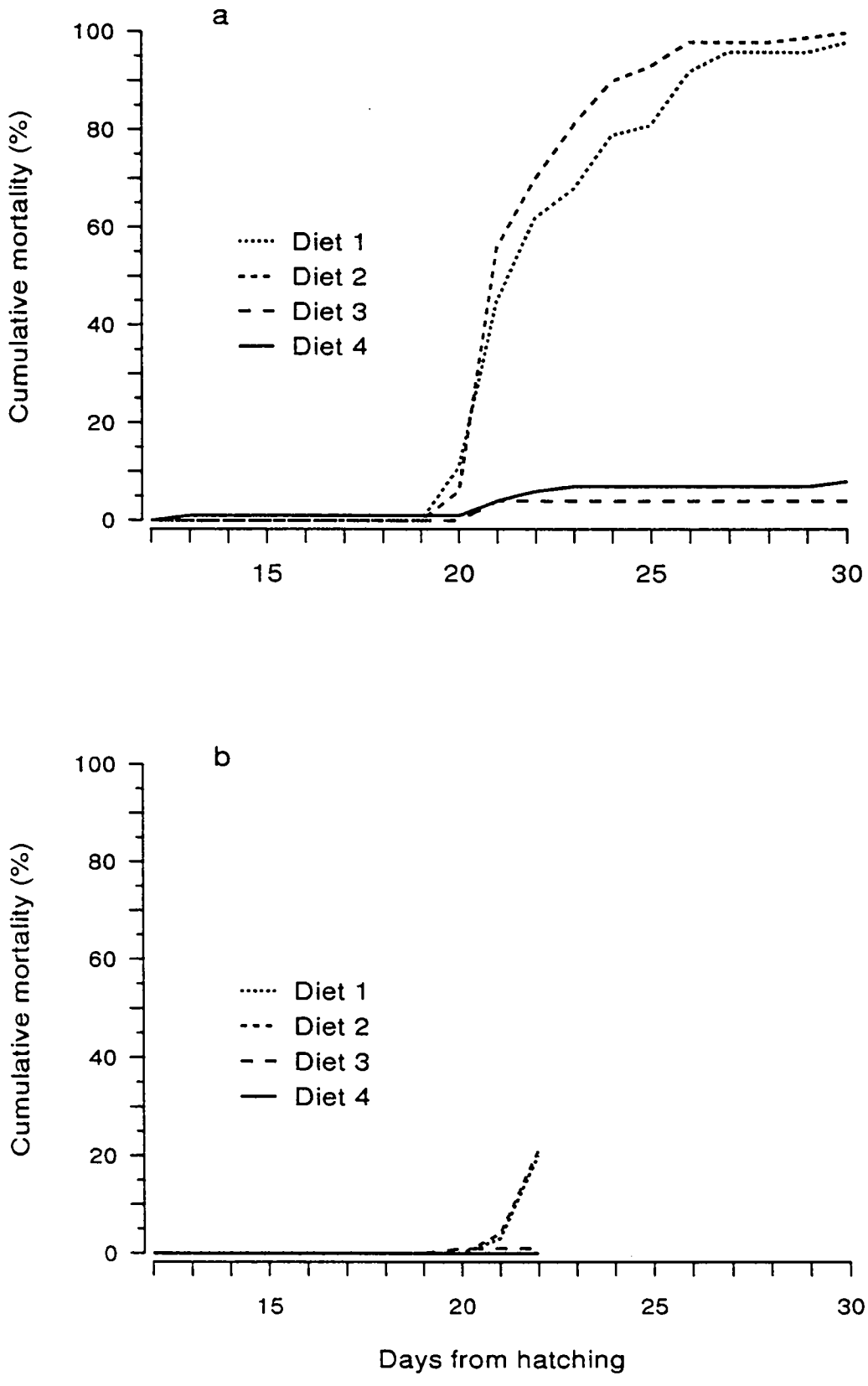
Treatments (see text for details):

Diet 1 CR, FH

Diet 2 SR, FH

Diet 3 CR, St/Supp

Diet 4 SR, St/Supp


a
b
Cumulative mortality (%)
100
80
60
40
20
0
Diet 1
Diet 2
Diet 3
Diet 4
15
20
25
30
Days from hatching

Experiment N3

The three brine shrimp diets used in experiment N3 also showed substantially different effects on survival of barramundi larvae. Extensive mortalities of barramundi larvae began on day 19 (diet 1) and day 27 (diet 2) and continued until most of the fish in these treatments were dead by day 40 (Fig. 3.3). Larvae fed supplemented brine shrimp (diet 3) showed only negligible mortalities over the same period (Fig. 3.3). The hazard ratio for diet 1 relative to diet 2 in experiment N3 was 0.21, indicating that barramundi larvae fed starved brine shrimp exhibited a higher probability of survival than those fed freshly hatched brine shrimp.

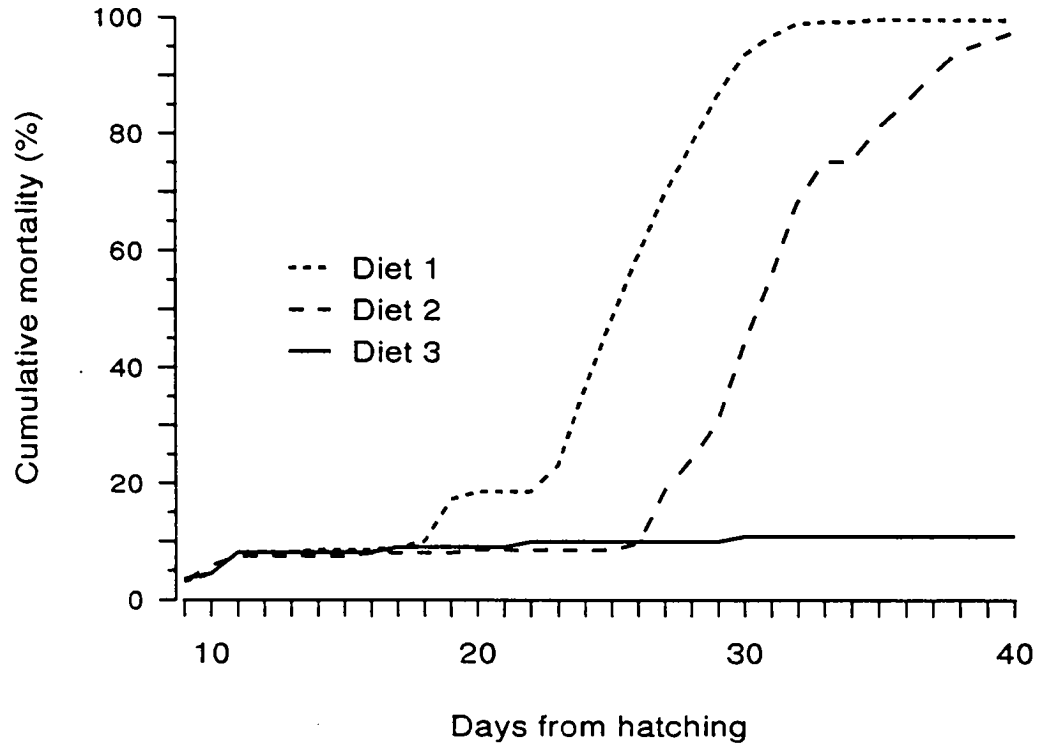


Figure 3.3 Cumulative mortality of barramundi larvae fed 3 test diets in experiment N3. Plotted values represent means of four replicates.

Treatments (see text for details):

Diet 1 SR, FH

Diet 2 SR, St

Diet 3 SR, Supp

Growth

The four test diets used in experiments N1 and N2 produced significantly different growth rates in barramundi larvae by day 22 after hatching (ANOVA, $F=22.6$, $P<0.01$). Larvae fed on diets 3 and 4 were significantly larger at day 22 than those fed on diets 1 and 2 (Tukey's HSD, $P<0.05$). Larvae fed on diets 1 and 2 averaged 8.3 mm TL and 8.7 mm TL respectively at day 22, while larvae fed on diets 3 and 4 averaged 10.0 mm TL and 10.5 mm TL respectively at day 22 (Fig. 3.4).

The effects of rotifer and brine shrimp supplementation on growth were further analysed using TL data from experiment N2 and rotifer supplementation, brine shrimp supplementation and experimental block as factors. The results of this analysis indicated that barramundi larvae fed on supplemented rotifers (in diets 2 and 4) were significantly larger at day 22 than those fed on unsupplemented rotifers (diets 1 and 3) (ANOVA, $F=8.1$, $P<0.05$). Larvae fed on unsupplemented rotifers averaged 9.1 mm TL while those fed on supplemented rotifers averaged 9.6 mm TL at day 22 (Fig. 3.5a).

Similarly, barramundi larvae fed on starved and supplemented brine shrimp (diets 3 and 4) were significantly larger at day 22 than those fed on newly hatched brine shrimp (diets 1 and 2) (ANOVA, $F=59.7$, $P<0.01$). Larvae fed on newly hatched brine shrimp averaged 8.5 mm TL while those fed on starved and supplemented brine shrimp averaged 10.2 mm TL at day 22 (Fig. 3.5b).

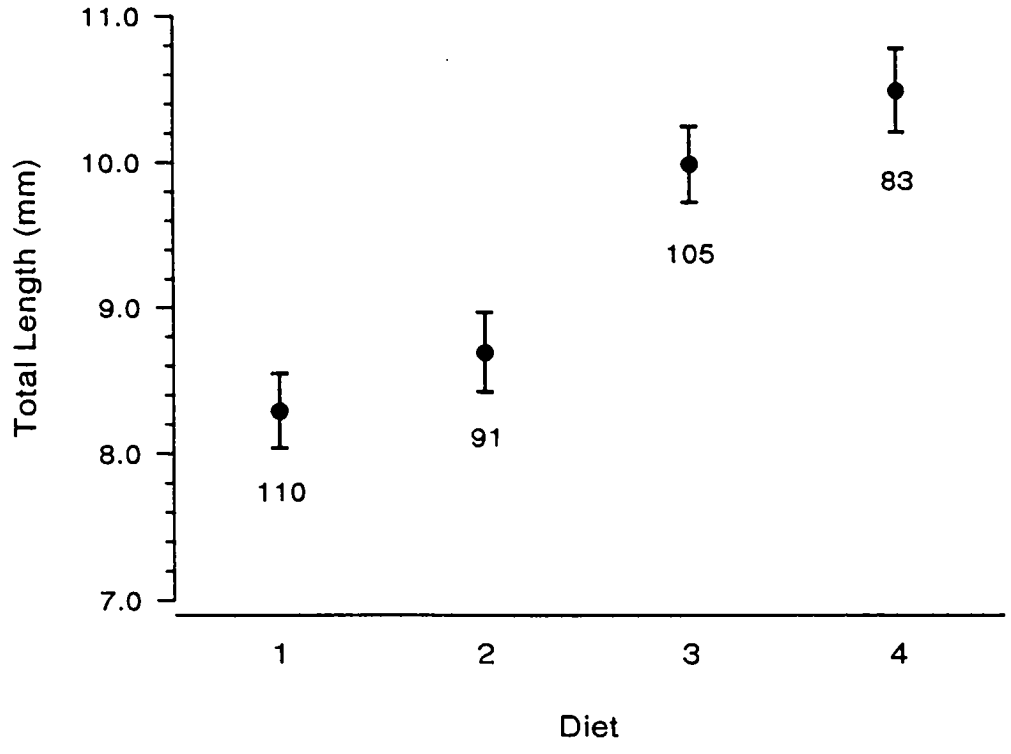


Figure 3.4 Total length of barramundi larvae fed on four test diets in experiment N2 and measured at day 22. Means and 95% confidence limits shown; numbers below bars represent sample sizes.

Treatments (see text for details):

Diet 1 CR, FH

Diet 2 SR, FH

Diet 3 CR, St/Supp

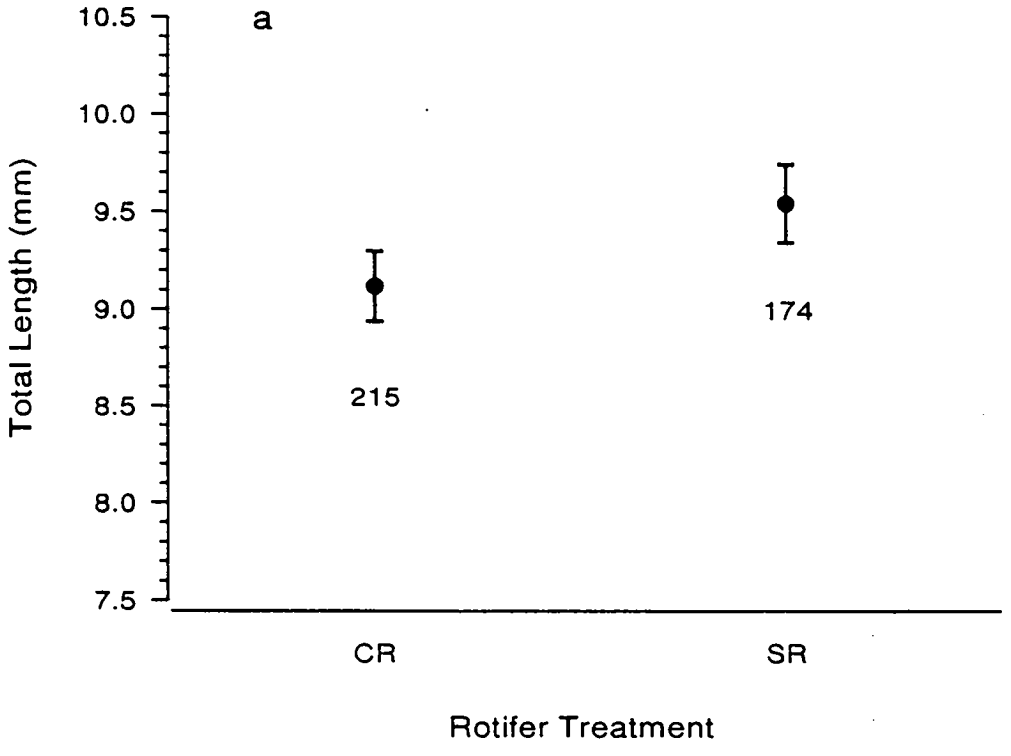
Diet 4 SR, St/Supp

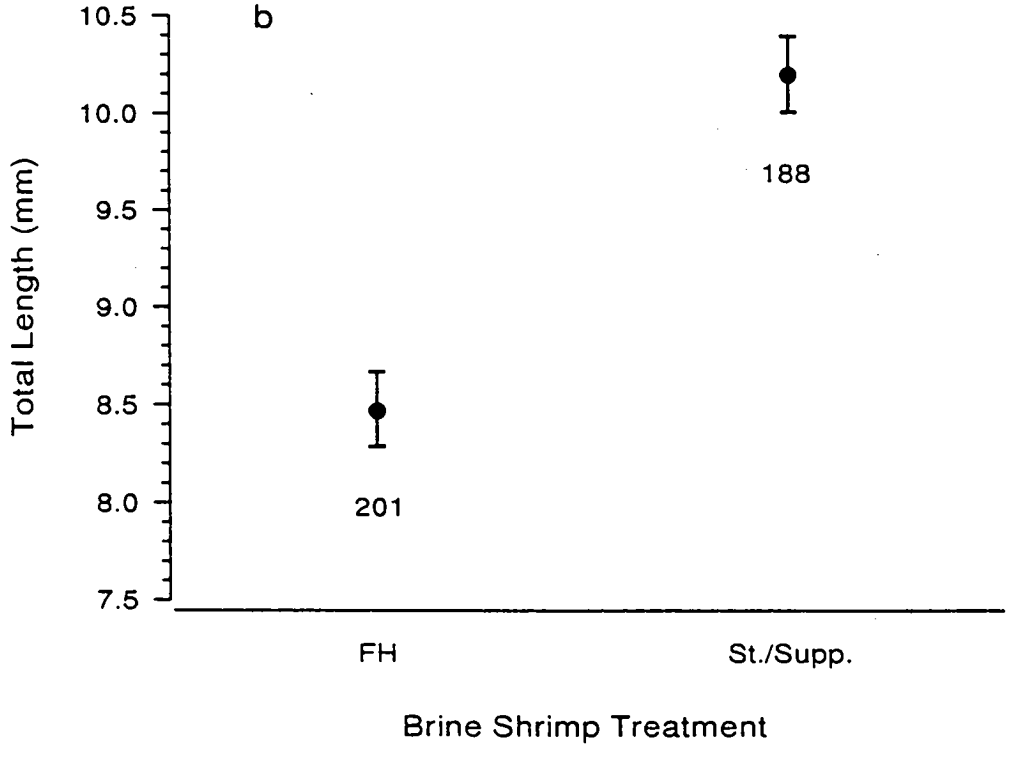
Figure 3.5 Total length of barramundi larvae fed on four test diets in experiment N2 and measured at day 22.

(a) Larvae fed on unsupplemented rotifers (diets 1 and 3) and supplemented rotifers (diets 2 and 4).

(b) Larvae fed on freshly hatched brine shrimp (diets 1 and 2) and starved and supplemented brine shrimp (diets 3 and 4).

Means and 95% confidence limits shown; numbers below bars represent sample sizes.


a
10.5
10.0
9.5
9.0
8.5
8.0
7.5
Total Length (mm)
215
174
CR
SR
Rotifer Treatment


b
10.5
10.0
9.5
9.0
8.5
8.0
7.5
Total Length (mm)
201
188
FH
St./Supp.
Brine Shrimp Treatment

Histology

Larvae fed on diets incorporating starved or supplemented brine shrimp showed no abnormal pathology. Several larvae fed on diets incorporating freshly hatched brine shrimp showed some minor vacuolation of the spinal cord at day 22. The extensive vacuolation of the brain and spinal cord seen in larvae reared in previous seasons was not observed in any larvae reared in these experiments.

Biochemical Composition

Since only minor differences were found in the biochemical composition between replicate samples of the dietary treatments, barramundi oocytes, and pond-reared barramundi juveniles, data from replicate samples for each treatment and reference were averaged.

Proximate Composition

There were differences between the proximate composition of the live food organisms used in the test diets and the reference samples (Table 3.3). Eggs and juvenile barramundi had higher levels of fat and moisture, and lower levels of carbohydrate and ash, compared with the live food organisms fed to the barramundi larvae. All brine shrimp treatments were sustantially higher in fat than rotifers, but had lower levels of fat than barramundi egg yolk or juveniles. Supplementation of brine shrimp resulted in a decrease in the relative amount of carbohydrate and concomitant increases in ash.

Fatty Acid Composition

The fatty acid composition of the rotifers more closely matched the fatty acid composition of juvenile barramundi than that of the barramundi egg yolk (Table 3.4). Unsupplemented rotifers were lacking 4 fatty acids found in barramundi egg yolk: 18:4, 22:4*n*-6, 22:5*n*-6, and 22:6*n*-3. Both supplemented and unsupplemented rotifers contained high levels of 20:5*n*-3. Supplementation of the rotifers incorporated 22:6*n*-3 into the diet and resulted in minor changes in the proportions of other unsaturated fatty acids. Both unsupplemented and supplemented rotifers were lacking only in 18:4 in comparison with barramundi juveniles.

Freshly hatched and starved brine shrimp lacked 6 fatty acids found in barramundi egg yolk: 15:0, 20:4*n*-6, 22:4*n*-6, 22:5*n*-3, 22:5*n*-6, and 22:6*n*-3. Supplemented brine shrimp lacked only 3 fatty acids in comparison with barramundi egg yolk: 20:4*n*-6, 22:4*n*-6, and 22:5*n*-6. Supplementation of brine shrimp resulted in levels of 20:5*n*-3 increasing from about 1% to 8%, and incorporation of 15:0, 22:5*n*-3, and 22:6*n*-3 into the diet.

Freshly hatched and starved brine shrimp were lacking 3 fatty acids found in barramundi juveniles: 15:0, 20:4*n*-6, and 22:5*n*-3. Supplemented brine shrimp were lacking only in 20:4*n*-6 in comparison with barramundi juveniles.

There were substantial differences in the fatty acid composition of barramundi egg yolk and barramundi juveniles. Juvenile barramundi lacked 3 fatty acids found in the egg yolk: 22:4*n*-6, 22:5*n*-6, and 22:6*n*-3. Barramundi egg yolk

contained much higher levels of 22:6*n*-3 than was found in any of the dietary treatments.

Amino Acid Composition

No substantial differences were found in the amino acid composition of the live food organisms and the reference samples used in these experiments (Table 3.5). Supplementation of rotifers and brine shrimp resulted in only minor changes in the amino acid composition of these organisms.

Table 3.3 Proximate composition of live food organisms used in experiments N1-N3, barramundi egg yolk, and pond-reared juvenile barramundi. See text for key to abbreviations.

Composition (%)	Rotifer		Brine shrimp			Reference	
	CR	SR	FH	St	Supp	Eggs	Fish
Protein	51.3	52.2	49.4	50.2	50.5	50.1	54.2
Fat	7.7	9.4	16.4	14.3	14.4	20.0	18.1
Carbohydrate	15.2	14.2	16.6	12.9	9.3	8.0	7.7
Ash	18.2	16.9	9.6	13.1	16.9	5.3	8.7
Moisture	7.6	7.3	8.0	9.5	8.9	16.6	11.3

Table 3.4 Fatty acid composition of live food organisms used in experiments N1-N3, barramundi egg yolk, and pond-reared juvenile barramundi. See text for key to abbreviations.

% Total Fatty Acids	Rotifer		Brine shrimp			Reference	
	CR	SR	FH	St	Supp	Eggs	Fish
14:0	2.7	3.6	0.5	0.5	0.7	1.3	8.3
15:0	1.0	1.0	-	-	0.2	1.1	1.1
16:0	10.4	12.3	10.8	10.8	9.4	26.5	18.3
16:1	22.2	19.0	5.9	5.6	6.1	8.6	22.1
18:0	3.2	5.8	6.1	6.3	6.7	8.3	8.3
18:1	27.8	22.3	22.0	22.1	20.9	17.6	15.5
18:2	6.3	8.9	10.8	9.9	12.4	4.6	5.4
18:3/20:1	6.6	5.0	23.1	23.0	15.8	1.5	2.0
18:4	-	-	13.0	11.6	9.6	2.6	2.8
20:4*n*-3	0.5	1.0	2.5	2.3	2.1	-	-
20:4*n*-6	2.7	2.3	-	-	-	3.9	1.5
20:5*n*-3	11.5	13.0	0.9	2.1	7.7	4.4	7.4
22:1	2.2	1.1	6.2	6.2	6.8	-	-
22:4*n*-6	-	-	-	-	-	1.3	-
22:5*n*-3	3.3	2.5	-	-	0.7	3.4	1.8
22:5*n*-6	-	-	-	-	-	1.2	-
22:6*n*-3	-	2.5	-	-	1.4	17.2	-

Table 3.5 Amino acid composition of live food organisms used in experiments N1-N3, barramundi egg yolk, and pond-reared juvenile barramundi. See text for key to abbreviations.

Amino Acids (g/16gN DM)	Rotifer		Brine shrimp			Reference	
	CR	SR	FH	St	Supp	Eggs	Fish
Lysine	5.9	6.1	6.3	6.2	7.0	7.8	7.7
Histidine	1.3	1.4	1.5	1.6	1.6	2.6	1.9
Arginine	5.3	5.6	7.0	7.0	6.4	6.8	7.1
Aspartic acid	7.8	8.0	7.6	7.9	8.1	7.7	11.1
Threonine	3.4	3.5	4.0	4.1	4.1	4.8	4.1
Serine	4.2	4.4	5.0	4.8	4.9	5.9	3.9
Glutamic acid	10.1	10.7	11.0	11.1	10.9	12.7	13.3
Proline	4.1	4.0	4.4	4.1	4.3	4.6	3.6
Glycine	3.2	3.3	4.1	4.2	4.2	3.3	6.3
Alanine	3.5	3.8	4.6	4.9	5.5	8.4	6.1
Valine	4.5	4.6	4.8	4.8	4.9	6.9	4.6
Methionine	1.2	1.3	1.9	1.7	1.7	2.2	2.7
Isoleucine	4.3	4.3	4.5	4.5	4.6	6.5	4.0
Leucine	6.8	7.0	6.6	6.7	7.1	9.7	7.2
Tyrosine	3.0	3.2	3.3	3.4	3.8	4.4	3.0
Phenylalanine	3.8	4.0	3.7	3.8	3.8	4.6	3.7
Cystine	1.6	1.6	1.3	1.4	1.3	1.1	1.0

Discussion

The mortality syndrome previously observed in larval barramundi at day 12-14 was not seen during these experiments and could not be induced using rearing techniques identical to those used in previous seasons (experiment N1, Diet 1) when this mortality syndrome caused substantial mortalities in some batches of larvae (MacKinnon 1987, Rodgers and Barlow 1987). Thus the results of these experiments do not support the hypothesis that this syndrome is primarily related to nutritional deficiencies in the live food organisms used for intensive rearing of barramundi larvae (MacKinnon 1987, Rodgers and Barlow 1987, Rimmer *et al.* 1988).

A picorna-like virus has been found in *L. calcarifer* larvae affected by this syndrome in hatcheries in Australia (Glazebrook *et al.* 1990, Glazebrook and Heasman 1992, Munday *et al.* 1992) and in Tahiti (Renault *et al.* 1991), although a causal link has not yet been established which would indicate that this virus is a primary pathogen of fish larvae (Glazebrook and Heasman 1992).

The mortality syndrome observed in the barramundi larvae used in these experiments appears to be identical to that described by Dhert *et al.* (1990) in *L. calcarifer* larvae reared in the Philippines and ascribed to a deficiency in the levels of *n*-3 HUFA's in the brine shrimp fed to the fish larvae. The results of the experiments presented in this paper support this conclusion, as most of the variation in the nutritional composition of the live food organisms used for intensive larval rearing of barramundi was found in the fatty acid composition,

with relatively little variation in the proximate and amino acid composition of the test diets and reference samples.

A comparison of the test diets using supplemented rotifers (diets 2 and 4) in experiment N1 indicated that barramundi larvae fed these diets exhibited slightly lower survival but slightly higher growth rates than those fed diets containing unsupplemented rotifers (diets 1 and 3). Since unsupplemented rotifers lacked 22:6*n*-3 (Table 3.4) and supplementation resulted in the incorporation of this fatty acid in the diet, these results indicate that the presence of 22:6*n*-3 in the diet of barramundi larvae during the rotifer feeding stage had no positive effect on survival, although it may have contributed to growth.

In most marine fishes, including barramundi, the egg yolk is high in 22:6*n*-3 which is quickly reduced during larval development, suggesting that the larval requirement for 22:6*n*-3 is initially met from this source and an exogenous source is not required during this stage (Watanabe *et al.* 1989, Ostrowski and Divakaran 1990, Webster and Lovell 1990). Marine fish larvae generally are incapable of elongating and desaturating shorter-chain fatty acids to produce 22:6*n*-3 (Witt *et al.* 1984, Watanabe *et al.* 1989, Ostrowski and Divakaran 1990, Webster and Lovell 1990) and are thus dependent on endogenous stores or dietary sources of 22:6*n*-3. The larvae of dolphin fish (*Coryphaena hippurus*) are subject to substantial mortality following the exhaustion of endogenous levels of 22:6*n*-3 if an alternative source is not provided (Ostrowski and Divakaran 1990). Similarly, barramundi larvae may have their initial requirement for 22:6*n*-3 met by the high levels of this fatty acid in the yolk (Table 3.4) and may not require an exogenous

source of 22:6*n*-3 until this is exhausted. However, the absence of 22:6*n*-3 in pond-reared juveniles (Table 3.4) suggests that this fatty acid is not conserved by barramundi larvae. Thus the importance of 22:6*n*-3 in the diet of barramundi larvae remains uncertain.

The results of experiment N1 also indicate that the effects of supplementation of the rotifer component of the diet are relatively small, whereas supplementation of brine shrimp has marked effects on growth and survival of barramundi larvae. Experiment N3 provided further information on the effect of different brine shrimp treatments on survival of barramundi larvae.

Diets 1 and 2 in experiment N3 both caused extensive mortality of barramundi larvae, but barramundi larvae fed diet 2 began dying 8 days after larvae fed diet 1 and continued to die at about the same rate, while diet 3 was the only diet adequate for long-term larval survival (Fig. 3.3). This pattern suggests that some nutritional factor was present at a very low level in diet 1 and at a higher, but still inadequate, level in diet 2 and was only present at an adequate level in diet 3. This is further supported by the proportional hazards analysis of these results which indicated that the probability of failure associated with diet 2 was significantly lower than that of diet 1 (hazard ratio 0.21).

The concentration of 20:5*n*-3 in the three brine shrimp treatments is the only measured biochemical component of the diets that reflects this pattern. Starvation of brine shrimp for about 24 h after hatching increased the levels of 20:5*n*-3 from about 1% to 2%, while supplementation of brine shrimp increased the levels of this fatty acid to about 8%. Supplementation of brine shrimp with microcapsules

resulted in the incorporation of 15:0, 22:5*n*-3, and 22:6*n*-3 into the diet (Table 3.4), but the addition of these fatty acids in diet 3 does not readily explain the observed differences in mortality between diets 1 and 2. The levels of 20:5*n*-3 in supplemented brine shrimp are similar to those found in pond-reared barramundi (Table 3.4).

The confounding effects of the different levels of various fatty acids found in the treatments used in these experiments prevent a detailed analysis of the precise roles of individual fatty acids in promoting growth and survival of barramundi larvae. Further research is required to investigate the role of fatty acids from endogenous and exogenous sources to determine precisely the biochemical requirements of barramundi larvae during and after absorption of the yolk and oil globule.

CHAPTER 4

Direct Nutritional Enhancement of Barramundi Larvae

Introduction

The production of live prey organisms is one of the most expensive aspects of intensive larval rearing because of the facilities and staff required for production of algae, rotifers, and brine shrimp. In order to reduce production costs, there have been attempts to replace live food organisms, partly or completely, with inert diets. In addition to their economic advantages, inert diets can be prepared to the precise nutritional requirements of the species being cultured and hence overcome many of the nutritional deficiencies associated with live prey organisms (Person-Le Ruyet 1990). Additional components, such as feeding attractants, can be also incorporated into inert diets (Person-Le Ruyet 1990).

Although several freshwater fish species have been reared exclusively on microparticulate or microencapsulated diets, notably carp, *Cyprinus* spp., and whitefish, *Coregonus* spp. (Dabrowski *et al.* 1984, Rösch and Appelbaum 1985, Zitzow and Millard 1988, Jones *et al.* 1993), attempts to rear marine fish larvae using such diets exclusively have generally resulted in poor survival (Adron *et al.* 1974, Person-Le Ruyet 1990, Walford *et al.* 1991, Jones *et al.* 1993, Southgate and Lee 1993). However, partial replacement of live prey organisms by microparticulate or microencapsulated diets has generally been more successful than complete replacement for marine fish larvae, although larvae reared on a combined diet of live prey and inert diet generally show inferior growth and survival compared with those fed only on live prey (Kanazawa *et al.* 1982, 1989,

Appelbaum 1985, Person-Le Ruyet 1990, Walford *et al.* 1991, Holt 1993, Jones *et al.* 1993, Barnabé and Guissi 1994).

The experiment described in this chapter was designed to investigate the feasibility of using a commercially available microparticulate diet to partly or completely replace brine shrimp during intensive larval rearing of barramundi.

Materials and Methods

Barramundi larvae used in this experiment (designated experiment M1) were reared from fertilised eggs obtained from broodstock held at NFC and spawned using hormone induction techniques (Garrett and Connell 1991). The larvae were fed on supplemented rotifers and supplemented brine shrimp according to the schedule described in Chapter 3. Following the completion of the rotifer feeding stage (day 15), approximately 50 barramundi larvae were transferred to each of 12 containers in the experimental unit (Fig. 3.1).

This experiment tested 4 replicates of 3 diets in a block design. The test diets consisted of brine shrimp and microparticles introduced to the containers at a total density of 5/ml. This density was based on the density of brine shrimp used for intensive larval rearing of barramundi. The diets tested were:

Diet 1: brine shrimp at 5 nauplii/ml;

Diet 2: brine shrimp at 2.5 nauplii/ml and microparticulate diet at 2.5 microparticles/ml;

Diet 3: microparticulate diet at 5 microparticles/ml.

Brine shrimp and microparticles were introduced to the experimental containers twice daily. Brine shrimp densities were determined volumetrically, as described in Chapter 3. Brine shrimp were supplemented with a microencapsulted diet as described for the 'supplemented' treatment in Chapter 3. The microparticulate diet used in this experiment was Zeigler 350 AP (i.e. Artificial Plankton with a nominal particle diameter of 350μm). This is a microparticulate diet, rather than a microencapsulated diet as was used to boost HUFA levels in live food organisms (Chapter 3). The Ziegler microparticulate diet was chosen for these experiments because it was developed to be used as a direct food source for fish larvae, whereas the microencapsulated diets were developed as crustacean larval feeds or as zooplankton supplementary feeds.

To determine the number of microparticles per unit weight, 20 samples of Zeigler 350 AP were weighed and the number of microparticles counted, giving an estimated 83,000 microparticles per gram. At each feed, 0.06 g and 0.12 g of Zeigler 350 AP was added to the containers with barramundi larvae fed diet 2 and diet 3 respectively, to provide the required numbers of microparticles to achieve the densities listed above.

Mortalities were monitored daily by counting dead larvae. Experiment M1 was terminated at day 24 and all surviving larvae were preserved in 10% formalin. TL data from these larvae were used to compare growth between treatments with ANOVA and Tukey's HSD test using the statistical package Statistix.

Results

This experiment revealed various problems with the use of microparticulate diets in a standard intensive larval rearing system. Circulation in the containers was inadequate to suspend the microparticles in the water column and the microparticles sank rapidly to the bottom of the containers. The barramundi did not appear to feed on microparticles which had settled to the bottom of the tank.

Survival

The three test diets used in this experiment resulted in markedly different survival during the experiment. Barramundi larvae fed a diet of microparticles alone (diet 3) began dying in large numbers on day 21 and substantial mortalities continued to occur up until the end of the experiment at day 24 (Fig. 4.1a). The combination of microparticles and brine shrimp (diet 2) resulted in a lower mortality during the experiment, and the diet comprising brine shrimp alone (diet 1) resulted in only negligible mortalities over the same period (Fig. 4.1a).

Growth

Surviving barramundi larvae fed the three diets used in experiment M1 were significantly different in length at day 24 (ANOVA, $F=125.1$, $P<0.01$; Tukey's HSD, $P<0.01$). Larvae fed diets 1, 2 and 3 averaged 9.4, 8.2 and 6.6 mm TL respectively at day 24 (Fig. 4.1b).

Figure 4.1 Survival and growth of barramundi larvae in experiment M1:

(a) Cumulative mortality of barramundi larvae fed three test diets in experiment M1. Plotted values represent means of four replicates.

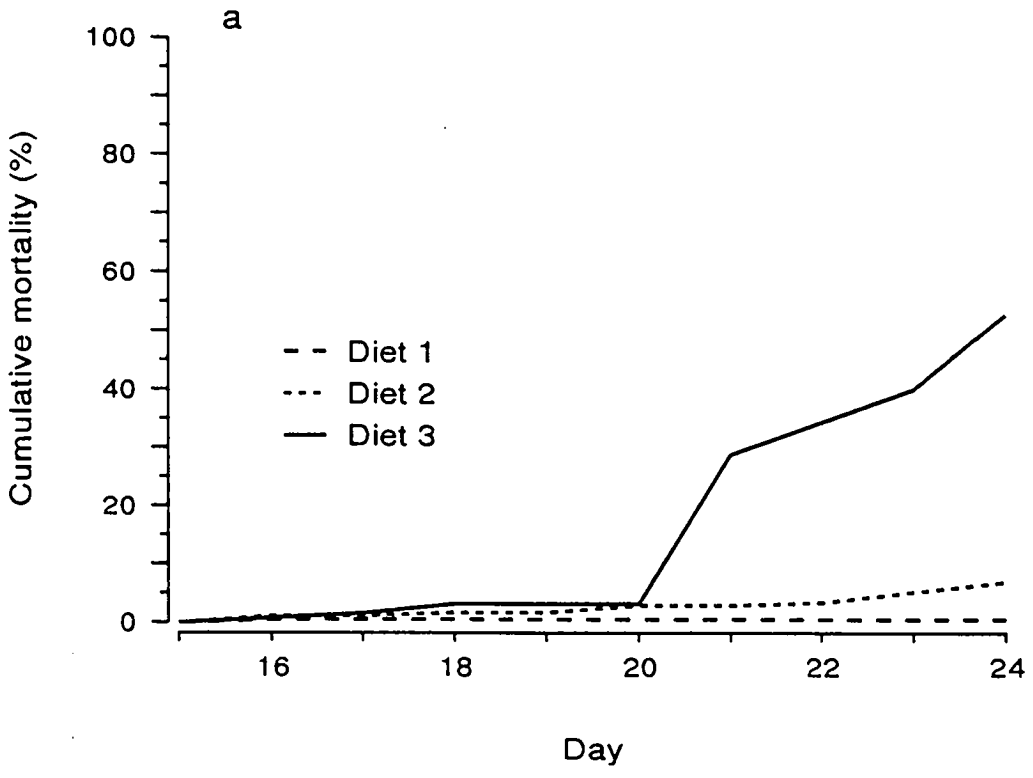
(b) Total length of barramundi larvae fed three test diets in experiment M1 and measured at day 24. Means and 95% confidence limits shown; numbers below bars represent sample sizes.

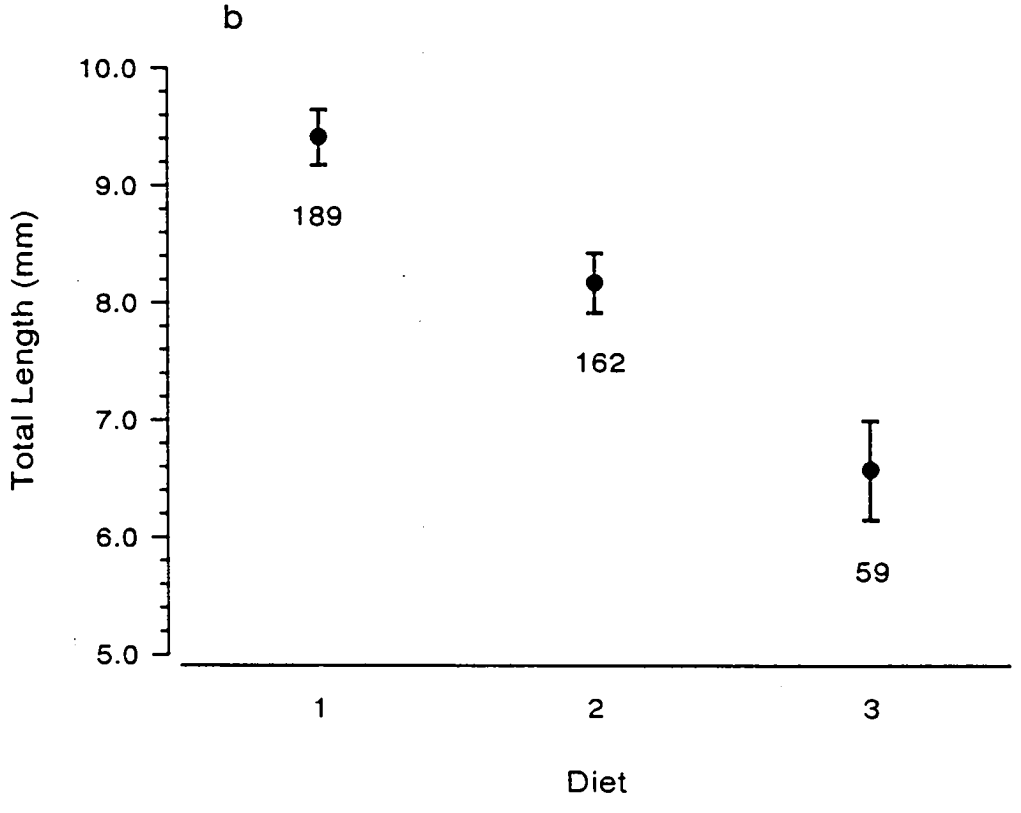
Treatments (see text for details):

Diet 1 Brine shrimp only;

Diet 2 Brine shrimp and microparticulate diet;

Diet 3 Microparticulate diet only.


a
Cumulative mortality (%)
100
80
60
40
20
0
Diet 1
Diet 2
Diet 3
16
18
20
22
24
Day


b
Total Length (mm)
10.0
9.0
8.0
7.0
6.0
5.0
189
162
59
1
2
3
Diet

Discussion

This experiment was unsuccessful in substituting an inert diet for brine shrimp in the intensive larval rearing of barramundi. Although the use of brine shrimp and the microparticulate diet in combination produced better survival and growth than the microparticulate diet alone, this appeared to be due to the brine shrimp component of the diet rather than any effect of the microparticulate diet. Although survival of the barramundi larvae fed brine shrimp only in this experiment was higher than in experiments N1-N3, this is within the normal range of variation observed in the intensive larval rearing of barramundi. Observation of the experimental animals during the course of this experiment indicated that acceptance of the inert diet was very poor. Some of the factors which may have contributed to this poor acceptance are: poor availability of the inert diet, and lack of perception of the inert diet by the larvae.

Availability of the diet was effectively low because the particles sank rapidly to the bottom of the container. This condition did not simulate the behaviour of brine shrimp which remained in suspension due to their active swimming. The reluctance of many marine fish larvae to ingest small particles may be an adaptation to prevent the ingestion of non-food particles in environments where these particles are found in high densities, such as coastal bays and estuaries (Buskey *et al.* 1993). Since suspension of particles depends on the degree of aeration and the size and shape of the tank (Backhurst *et al.* 1989), a major redesign of the tanks used for larval rearing of barramundi would be necessary to accommodate the use of microparticulate diets.

Perception of the microparticles may have been limited by factors such as their colour (and hence their contrast against the background of the containers). Growth and survival of barramundi larvae are strongly influenced by the visibility of prey items (Pearce 1991) and the microparticles may be of insufficient contrast with the tank background to be perceived by the larvae.

Many marine fish larvae require live prey in combination with inert diets to achieve satisfactory growth and survival (Kanazawa *et al.* 1982, 1989, Lauff and Hofer 1984, Walford *et al.* 1991, Walford and Lam 1993, Holt 1993, Jones *et al.* 1993, Barnabé and Guissi 1994). Walford *et al.* (1991) found that *L. calcarifer* larvae fed a microencapsulated diet alone died by day 10 after hatching, whereas those fed a combination of rotifers and microcapsules for 5 days, then microcapsules alone had a survival rate of 2.4%. Southgate and Lee (1993) also found that barramundi larvae fed microbound diets alone readily ingested the particles, but died at the same time as the control (unfed) larvae, suggesting that the microbound diet was not digested by the larvae. Walford and Lam (1993) confirmed the importance of exogenous proteases from live prey to proteolytic activity in larvae of *L.calcarifer*. These enzymes act not only to digest prey, but also to activate the activities of endogenous enzyme (DeSilva and Anderson 1995).

In many fish species, the movement of live prey may be necessary to stimulate feeding by the larvae (Appelbaum 1985, Person-Le Ruyet 1990, Jones *et al.* 1993), although Southgate and Lee (1993) found that barramundi larvae would ingest microbound diet particles in the absence of live prey. Barnabé and Guissi (1994) used rotifers immobilised by 'cold shocking', in combination with

microparticles, to overcome the attraction that sea bass (*Dicentrarchus labrax*) larvae have for live prey in preference for inert particles. This strategy appears to hold considerable promise for future research into the use of microparticulate diets for the larval rearing of marine finfish.

The use of live prey in combination with microcapsules may also be necessary to aid digestion in fish larvae, since the live prey may also serve as an important source of digestive enzymes and bacteria in larvae whose digestive systems have not yet fully developed (Appelbaum 1985, Walford *et al.* 1991). The experimental evidence regarding the contribution of exogenous enzymes to digestion of microparticulate diets is ambiguous. Several studies with gilthead sea bream (*Sparus aurata*) have shown that the addition of pancreatin to microparticulate diets results in improved growth and enhanced protein assimilation (Jones *et al.* 1993). However, Fernández-Díaz and Yúfera (1995) found that the addition of enzymes to microcapsules fed to larval *S. aurata* did not result in any significant change in the degree of capsule breakdown. In the present study, the presence of live prey did not improve the utilisation of the microparticulate diet, although more detailed studies are required to further examine the digestion of microparticles by barramundi larvae.

The age at which larvae are introduced to inert diets also affects the successful use of such diets. Several species have been successfully reared from first feed on inert diets (Adron *et al.* 1974, Dabrowski *et al.* 1984, Rösch and Appelbaum 1985, Zitzow and Millard 1988, Jones *et al.* 1993), while in other species inert diets could only be used to replace brine shrimp (Dabrowski *et al.* 1984,

Appelbaum 1985, Holt 1993, Jones *et al.* 1993). Microparticulate diets were not used in this study to replace rotifers in the early larval rearing of barramundi because of the poor quality of smaller particles. A high proportion of such particles were made up from individual components of the original preparation, and thus could not be presented as a homogeneous diet. However, the poor survival recorded by Walford *et al.* (1991), i.e. 2.4%, using rotifers and a microencapsulated diet to rear *L. calcarifer* larvae, suggests that substitution of inert diets during the rotifer feeding stage of barramundi larval rearing has similar problems to those encountered in this experiment.

Based on these results, the use of inert diets to partly or completely replace live food organisms in the intensive larval rearing of barramundi requires further research. Among the factors which need to be addressed are: redesign of tanks to allow prolonged suspension of microparticles, the development of microparticles of lower specific gravity which remain suspended in the water column for longer, research into the factors affecting perception of the microparticles by barramundi larvae, and the nutritional composition of the inert diet.

CHAPTER 5

Discussion: Larval Nutrition

The results of the experiments undertaken in this study indicate that barramundi larvae, like the larvae of other marine fishes, have specific nutritional requirements which are not met using 'standard' live food culture techniques. Specifically, barramundi require enhanced levels of HUFA's in the brine shrimp component of the diet.

This research examined two approaches to supplying HUFA's from exogeneous sources: direct and indirect supplementation.

Attempts to directly supplement barramundi larvae using a microparticulate diet were unsuccessful. Although the present study was limited in scope because only one inert diet was tested, other workers have reported similar constraints in attempting to replace live food organisms with artificial feeds for the larval rearing of barramundi (Walford *et al.* 1991, Southgate and Lee 1993). Some of the technical difficulties encountered during this study might be overcome by redesigning the rearing tanks and associated equipment used for intensive larval rearing of barramundi. Partial replacement of live prey with inert diets shows greater short-term potential than complete replacement, but is still subject to a number of problems. In particular, barramundi larvae appear to prefer live prey organisms, which may explain why acceptance of the inert diet used in this study was extremely poor.

Indirect supplementation of the live prey organisms fed to barramundi larvae can be readily and relatively cheaply accomplished using microencapsulated diets.

Supplementation with microcapsules enhanced the HUFA composition of both rotifers and brine shrimp. However, supplementation of rotifers resulted in slightly decreased survival and only a marginal increase in growth (0.4 mm at day 22). This minor increase in growth rate does not justify the additional costs associated with the supplementation of rotifers.

In comparison, the supplementation of brine shrimp dramatically improved both survival and growth of intensively reared barramundi larvae. Larvae fed supplemented brine shrimp were 1.7 mm longer (TL) at day 22 than those fed unsupplemented brine shrimp; an increase in size of 20%. Brine shrimp used in barramundi hatcheries should be routinely supplemented to enhance their HUFA composition in order to achieve maximal survival and growth of the larvae.

Although there are other techniques for supplementation of live prey organisms, microcapsules present a number of advantages over other techniques:

1. Ease of use. Microencapsulated diets developed specifically for nutritional supplementation of live prey organisms are now commercially available. Unlike some other nutritional enhancement techniques, such as culture of various algal species, few additional resources are required for supplemetation of rotifers and brine shrimp. The use of microcapsules requires no specialised equipment beyond some small containers for supplementary feeding of the live prey organisms.
2. Maintenance of water quality. The protein membrane surrounding the microcapsule prevents the leaching of water soluble nutrients and provides a relatively poor substrate for bacterial activity. As a result, microcapsules do

not cause the severe and rapid decline in water quality which is associated with the use of some nutritional supplements, particularly those which are lipid-based (Rodgers and Barlow 1987).

3. Low cost. The current cost of microencapsulated diet (Frippak 'Booster') is approximately A$60 for 400 g, or about 15 ¢/g. Supplementation of brine shrimp using microcapsules at the rates used for this research requires the use of approximately 3.4 g DW of microcapsules per 1,000 larvae. This adds only 0.05 cents in direct costs to the production cost for each fish (reared to approximately 1 cm TL).

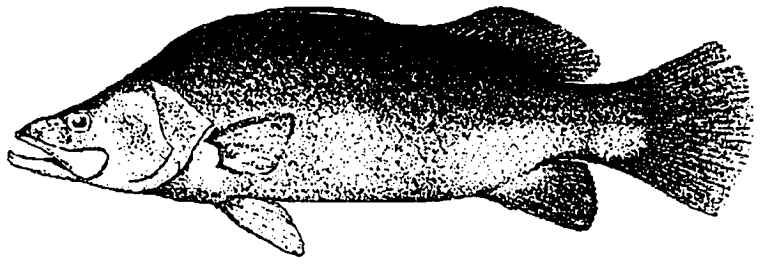
In addition to the direct benefits of increased growth and survival, there are indirect benefits of nutritional enhancement which are also important. Nutritional deficiencies may act as stressors which impair the immune mechanisms of fish, thus reducing their disease resistance (Langdon 1988). Optimal, or at least improved, nutrition will improve the fish's disease resistance and hence indirectly benefit survival and growth.

Increased survival obviously increases the efficiency of the hatchery by producing more fish with the same amount of effort. Increased growth also increases hatchery efficiency by reducing the time that the fish are in the hatchery, since fish are generally reared to a particular size, rather than over a set time period. Because of the high cost of live food production, even a saving of a few days in the hatchery can result in substantial decreases in the costs associated

with intensive rearing and thus improve the cost-efficiency of the hatchery operation.

PART TWO

EXTENSIVE LARVAL REARING OF BARRAMUNDI



CHAPTER 6

Review: Extensive Rearing of Marine Fish Larvae

Extensive Larval Rearing

Extensive larval rearing involves the production of juvenile fish (termed 'fingerlings' or 'fry') from newly hatched larvae in managed enclosures, usually earthen ponds (Rowland 1983). The essential requirements for efficient fish production are the provision of a stable pond environment and the provision of prey organisms of optimal size which are available in sufficient quantity to provide for survival and growth of the fish throughout the rearing period (Geiger 1983a,b, Sturmer 1990). Since most marine fish larvae are largely zooplanktivorous (Hunter 1981), extensive larval rearing requires the production of zooplankton to provide a source of prey to satisfy the latter requirement (Geiger 1983a,b), while simultaneously maintaining water quality within the tolerances of the species cultured.

Extensive rearing ponds are managed to produce a 'food web' which supports the continual development of a zooplankton community which in turn provides prey for the fish larvae (Fig. 6.1). Phytoplankton and zooplankton communities which develop in larval rearing ponds are influenced by a complex series of interactions between environmental variables such as temperature, photoperiod, water quality and nutrient availability, and biological variables such as feeding habits, the reproductive capabilities of the zooplankton, competition between zooplankton, and the effects of fish predation (Geiger 1983b, Rutledge 1988, Sturmer 1990).

Because of this complexity, no single management strategy can be expected to work effectively for all conditions, and management procedures must be modified for different areas, species, climatic conditions, etc. (Boyd 1990, Sturmer 1990, Colura *et al.* 1991, Rutledge and Rimmer 1991). However, the fundamental principles underlying extensive rearing techniques are common to different situations and these are discussed in the following review. Although I have concentrated on reviewing aspects of extensive larval rearing of marine fishes in this chapter, these techniques are derived from those originally developed to rear freshwater fishes (Geiger 1983a,b). Consequently, I have included some details of extensive larval rearing in freshwater systems, particularly where such information is also applicable to marine or brackishwater ponds.

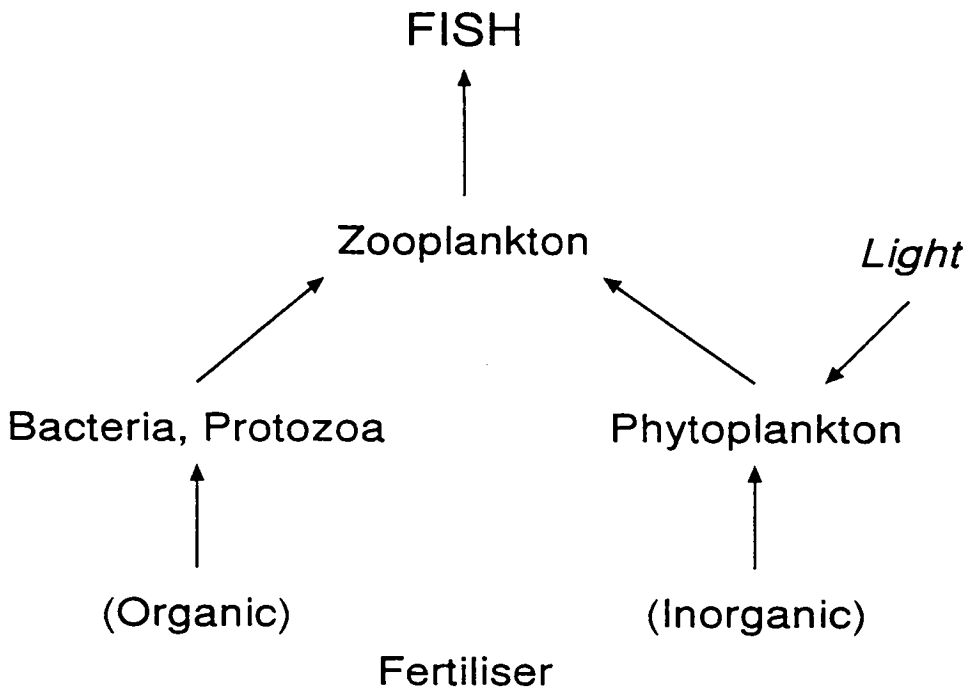


Figure 6.1 Diagrammatic representation of the biological systems underlying extensive larval rearing.

Water Quality

The successful rearing of marine fish larvae requires an environment suitable for their survival and growth. To fulfil this criterion, the physicochemical parameters of the pond must remain within the tolerances of the larvae, and preferably conform to the optima for the species under culture. The following notes summarise the information available on the tolerances of marine fish larvae to the major physicochemical parameters in ponds with particular regard to barramundi larvae. As discussed throughout this chapter, the precise physicochemical tolerances of barramundi are poorly known.

The tolerances of fish larvae to various water quality parameters are generally least in newly hatched larvae, particularly during the period of transition from endogenous to exogenous food sources (commonly referred to as 'first feed') (Brownell 1980a). In the pond environment, physicochemical factors can be expected to exert most influence on larval survival and growth immediately after the larvae have been stocked into the pond.

Brownell (1980a,b) investigated the effects of water quality parameters on the larvae of eight species of marine fishes and concluded that the results for different species were similar enough to use as general water quality criteria for marine fish larvae under artificial rearing conditions. Brownell (1980a,b) also found that several physicochemical parameters indirectly affected larval mortality, through reduced success in first feeding, at levels well below the LC_{50} values for the same parameters. Of the water quality parameters examined, only high pH, low dissolved oxygen and high unionised ammonia were found to significantly inhibit

first feeding at levels likely to be encountered in the rearing of marine fish larvae (Brownell 1980a,b).

Temperature

Temperature may directly or indirectly affect survival and growth of fish. Temperatures beyond the temperature tolerance range of the species concerned may result in death. Low temperature appears to induce mortality through osmoregulatory failure (Langdon 1988). High temperatures within the tolerance range may result in the rapid multiplication of pathogenic organisms, particularly bacteria; reduced dissolved oxygen concentrations; and increased metabolic rate, including increased oxygen consumption (Langdon 1988). All these factors act as stressors and may caused decreased growth rates and increased mortality in fish (Rowland 1983, Langdon 1988, Boyd 1990).

NICA (1986) recommends water temperatures between 28 and 30°C for larval rearing of *L. calcarifer*. Unpublished experimental work undertaken at NFC has indicated that intensively reared barramundi larvae have a similar optimum temperature range (J. Russell, pers. comm.). Although the upper and lower thermal limits of larval barramundi are not known, larval barramundi in hatcheries grow extremely slowly at water temperatures below 25°C. Control of temperature is virtually impossible in an earthen pond, although very high temperatures may be controlled by pumping cooler water into the pond.

Salinity

Salinity levels beyond the survivable tolerance range for a given fish species result in overhydration and hyponatraemia at one extreme or dehydration and hypernatraemia at the other (Langdon 1988). The salinity tolerance range often varies greatly with age and different stages of the life cycle, particularly in catadromous fish such as barramundi (Langdon 1988).

Barramundi larvae appear to have wide salinity tolerances, as larvae as small as 3.3 mm TL have been found in the wild at salinities ranging from 6 to 38 ppt (Davis 1985a, Russell and Garrett 1985). Recommended salinities for the larval rearing of *L. calcarifer* are 28-31 ppt (NICA 1986, Ruangpanit 1987) and unpublished research undertaken at NFC indicated that the optimal salinity for barramundi larvae reared in the hatchery is approximately 28 ppt (J. Russell, pers. comm.). The salinity tolerance of barramundi larvae increases rapidly as the larvae develop and 15 day old *L. calcarifer* larvae (3.58 $\pm$ 0.17 mm standard length) have been successfully transferred to 10 ppt salinity (Fermin 1991).

High salinity may also act as a stressor. Red drum (*Sciaenops ocellatus*) harvested from ponds with high salinities (45 ppt and above) suffered handling stress which resulted in higher post-harvest mortalities than amongst red drum harvested from ponds at lower salinities (Rutledge 1988).

Dissolved Oxygen

The major source of dissolved oxygen in larval rearing ponds is the oxygen produced by phytoplankton during photosynthesis. Because of the high densities

of phytoplankton maintained in larval rearing ponds, dissolved oxygen is usually produced in excess of the respiration requirements of the pond fauna and concentrations commonly reach supersaturation during the day. Supersaturated levels of dissolved oxygen in larval rearing ponds appear to have no adverse affect on barramundi (Rutledge and Rimmer 1991).

Low dissolved oxygen concentration is a potential problem in larval rearing ponds. Minimal dissolved oxygen requirements vary greatly between fish species and vary with the health status of the fish (Langdon 1988). Impairment of the respiratory capability of the fish, such as gill lamellar epithelial hyperplasia caused by infestations of ectoparasitic protozoans, may leave the fish susceptible to normally tolerable falls in dissolved oxygen (Langdon 1988). Saltwater ponds may commonly have dissolved oxygen levels lower than those regarded as optimal in freshwater ponds, e.g. larval rearing ponds for red drum consistently average 4-5 mg/l dissolved oxygen (60-85% saturation) with no apparent adverse effects (Rutledge 1988). Brownell (1980b) found that the LC_{50} values for six species of marine fish larvae ranged from 2.3 to 3.6 mg/l dissolved oxygen, and that dissolved oxygen levels below about 5.0 mg/l reduced first feeding success. Rowland (1983) noted that no obvious losses of golden perch (*Macquaria ambigua*), silver perch (*Bidyanus bidyanus*) or Murray cod (*Maccullochella peeli*) larvae occurred when dissolved oxygen levels remained at 3 mg/l for several days, although complete mortality of golden perch larvae occurred when dissolved oxygen levels fell below 1.5 mg/l.

Because all organisms in the pond (including the phytoplankton) respire at night, minimal dissolved oxygen concentrations occur early in the morning, just before light levels increase enough for phytoplankton to begin photosynthesis (Rowland 1983, Boyd 1990). *L. calcarifer* is relatively tolerant of low oxygen concentrations (Wu 1990), but juvenile barramundi show signs of stress ('gasping' at the water surface) when dissolved oxygen levels fall below about 2 mg/l. Low dissolved oxygen levels are common during harvesting, particularly if ponds are harvested during the day when the warm water draining from the pond is usually deficient in dissolved oxygen. Some form of aeration is commonly used during harvesting to maintain adequate dissolved oxygen levels (Rowland 1983).

Larval rearing ponds which become supersaturated with dissolved oxygen during the day will usually maintain adequate dissolved oxygen levels overnight. As a result, aeration is not widely used in larval rearing ponds except in the event of critically low dissolved oxygen levels, i.e. below 2-3 mg/l (Rowland 1983, Hogan and Bull 1986). However, routine aeration of extensive rearing ponds provides several advantages:

1. Aeration moderates diel water quality variation and increases the dissolved oxygen content of the water when respiration demand is high, or when low densities of phytoplankton severely reduce the rate of photosynthetic production of oxygen (Geiger 1983b).
2. Aeration also increases the production of phytoplankton and heterotrophs by increasing oxidation and degradation of organic fertilisers and increasing primary production, presumably by improving nutrient circulation within the

pond. These increases in productivity result in increased zooplankton production (Geiger 1983b).

pH

The pH of salt water is usually about 8.0. Although salt water is better buffered against pH changes than freshwater (Spotte 1979, Sturmer 1990), pH in extensive larval rearing ponds can undergo significant changes. Photosynthesis consumes carbon dioxide, resulting in higher pH and decreased solubility of calcium carbonate (Spotte 1970, Boyd 1990). Consequently, high densities of phytoplankton in ponds usually cause an increase in pH, and ponds with heavy phytoplankton blooms may show a diel pH pattern caused by photosynthetic consumption of carbon dioxide during the day and release of respired carbon dioxide at night (Boyd 1990).

Extreme high or low pH is lethal to fish (Rowland 1983, Langdon 1988, Boyd 1990). Small changes in pH *per se* probably have little adverse effect on fish but are generally symptomatic of other changes in water quality (Spotte 1970).

Brownell (1980b) found a 10% reduction in first feeding success for the larvae of three marine fish species at pH 8.4-8.5 and concluded that a 'negligible effect pH' is not likely to exceed pH 8.4. 24-h LC_{50} values for the three species tested fell between pH 9.0 and 9.2 (Brownell 1980b). Rowland (1983) recommended that water exchange should commence when pH reached about 9.0 in freshwater fish larval rearing ponds.

Nitrogenous Compounds

Ammonia is the main nitrogenous compound excreted by aquatic organisms and is constantly being produced by fish and other organisms in the pond. In addition, the nitrogenous component of many inorganic fertilisers (e.g diammonium phosphate) is an ammonia compound (Rutledge 1988). Unionised ammonia (NH_3 or NH_3-N) is directly toxic to fish, causing acute intoxication and death at high levels, or inducing severe and eventually fatal gill lamellar epithelial hyperplasia at lower levels (Langdon 1988).

Brownell (1980a) found the 24-h LC_{50} values for five species of marine fish larvae to range from 0.36 to 0.46 mg/l NH_3-N, and the maximum concentration of unionised ammonia-nitrogen which did not affect first feeding following a 24 h exposure to be between 0.02 and 0.05 mg/l NH_3-N. The precise ammonia tolerances of barramundi larvae are unknown, but barramundi reared at NFC were on one occasion exposed to total ammonia-nitrogen concentrations in excess of 0.6 mg/l (equivalent to 0.03 mg NH_3-N per litre) for several days due to equipment failure. No noticeable mortality occurred during this time, although growth effectively ceased while ammonia concentrations were high.

In order to reduce ammonia concentrations in the pond, the use of inorganic fertilisers is usually restricted to frequent applications of small quantities in preference to the application of large quantities.

Nitrite and nitrate are products of the nitrification process which is undertaken by bacteria under aerobic conditions (Spotte 1970). Nitrate is toxic to fish larvae only at extremely high concentrations, beyond those normally found in

aquaculture (Brownell 1980a). Although nitrite is toxic to fish and has been shown to predispose fish to infectious disease (Langdon 1988), the levels required to reduce feeding success or survival of marine fish larvae are generally well above those found in aquaculture (Brownell 1980a, Holt 1990).

Tookwinas (1986) found the median tolerance limit of *L. calcarifer* (presumably in salt water) to nitrite to be 933.5 mg NO_2-N per litre for 18 day old fish (mean length 7.4 mm). The incipient toxic levels of nitrite are higher for fish in salt water than in freshwater, because chloride ions have a strong protective effect, probably by competing for nitrite uptake by the gills (Langdon 1988). As a consequence, direct nitrite toxicity is unlikely to occur in salt water larval rearing ponds.

Finally, it should be noted that while barramundi generally have wide tolerances to a range of physicochemical parameters in terms of survival, even moderate changes in some of these parameters will depress growth rates substantially. In addition, the effects of different parameters may be additive or even synergistic, so a decline in water quality across several parameters may cause disease or mortality where one alone may not. For example, Bergerhouse (1993) found that hybrid striped bass (striped bass *Morone saxatilis* ♀ × white bass *M. chrysops* ♂) larvae exposed to sub-lethal unionised ammonia concentrations and elevated pH, were subject to higher mortality than those fish subjected to comparable levels of unionised ammonia or pH alone. This suggests an interaction of pH and unionised ammonia which adversely affects the survival of striped bass larvae, possibly through osmotic disturbance (Bergerhouse 1993).

Pond Fertilisation

Because nutrient levels are the primary factors limiting productivity in ponds, fertilisers are added to larval rearing ponds to enhance their productivity (Boyd 1990). The addition of fertilisers to overcome the limiting effect of nutrient availability will eventually result in some other factor becoming limiting, e.g. the amount of solar energy penetrating the pond surface (Geiger 1983b).

Two types of fertilisers, inorganic and organic, are generally used in extensive larval rearing applications. Inorganic and organic fertilisers have different modes of action and affect pond productivity in different ways (Fig. 6.1): inorganic fertilisers stimulate phytoplankton production and organic fertilisers directly encourage growth of bacteria and protozoans (Geiger 1983a,b, Sturmer 1990). Phytoplankton, bacteria and protozoans may all be utilised as food sources by a variety of zooplankton which are in turn utilised as prey by the fish (Geiger 1983a,b, Porter 1984, Rieper 1984, Sturmer 1990).

Inorganic fertilisers used in ponds are similar or identical to those used in agriculture (Boyd 1990). The primary nutrients in inorganic fertilisers are nitrogen, phosphorus and potassium (Boyd 1990). Although nutrient requirements vary considerably between sites (Smith 1984, Boyd 1990), phosphorus is usually the limiting nutrient in freshwater systems whereas nitrogen is usually the limiting nutrient in marine systems (Geiger 1983b, Smith 1984, Sturmer 1990, Boyd and Daniels 1993).

Fertilisation regimes, including types, amounts and frequency of application of fertilisers, differ substantially between different operators, even for culture of a

single species (Colura 1990). Such differences may be based on different water sources and production goals (Colura 1990) and fertilisation regimes may be modified in the light of continuing experience (Rutledge and Rimmer 1991).

Organic fertilisers usually consist of various animal or plant wastes (Boyd 1990) which are decomposed to inorganic carbon (usually CO_2) and inorganic nitrogen by a wide variety of aquatic fungi, bacteria and protozoa (Geiger 1983b). Organic fertilisers may serve as direct food sources for zooplankton or they may decompose and the inorganic nutrients released may support phytoplankton blooms (Boyd 1990). However, organic fertilisers have a very low fertiliser grade and large quantities are needed to supply the nutrients equivalent to those found in a relatively small quantity of inorganic fertiliser (Boyd 1990, Anderson 1993a, Qin *et al.* 1995). As a result, their primary role in extensive rearing is to support populations of decomposer microorganisms.

The decomposition rate of organic fertilisers primarily depends on the particle size and the carbon:nitrogen (C:N) ratio of the fertiliser used. A small particle size allows faster colonisation by bacteria, algae and protozoans and subsequently quicker decomposition and solubilisation of key nutrients (Geiger 1983b).

Initial decomposition rates are usually high as the simple organic compounds break down, but the more resistant organic compounds (cellulose, chitin, etc.) are the last to decompose. Fertilisers with a high C:N ratio (e.g. wheat straw and various hays) decompose more slowly than green crop residues, such as cottonseed meal and alfalfa (lucerne) meal or some of the animal manures (Geiger 1983b, Colura and Matlock 1984, Sturmer 1990). The presence of inorganic

nitrogen also increases the rate of decomposition of organic fertilisers (Sturmer 1990, Boyd and Daniels 1993).

Different organic fertilisers are used in different aquaculture operations based on their efficiency and availability (Anderson 1993a). Colura and Matlock (1984) found that cottonseed meal produced higher densities of zooplankton in ponds than did chicken manure, and Ludwig and Tackett (1991) found that ponds fertilised with rice bran supported higher densities of zooplankton and produced significantly larger striped bass (*Morone saxatilis*) than did ponds fertilised with cottonseed meal.

The decomposition of organic fertilisers is an aerobic process and thus exerts an oxygen demand. As a result, excessive applications may result in depletion of dissolved oxygen (Colura and Matlock 1984, Boyd 1990, Qin *et al.* 1995).

A combination of organic and inorganic fertilisers, compared with the use of only one fertiliser type, generally results in increased production of zooplankton and increased survival or production of fish, probably due to the differential effects of the different fertiliser types on various zooplankton groups (Geiger 1983a,b, Porter and Maciorowski 1984, Colura 1990, Colura *et al.* 1991, Johnson and Schlosser 1991, Qin *et al.* 1995).

Pond Maintenance

A proportion of the phosphorus added to ponds in inorganic fertilisers becomes trapped in the bottom sediments in the form of specific compounds with limited solubilities (Boyd 1990). This phosphorus can be released by drying the pond

substrate thoroughly and applying agricultural lime ($CaCO_3$) to the substrate (Boyd 1990, Boyd and Daniels 1993). Liming increases the pH of the bottom mud and thereby increases the availability of phosphorus for plant growth (Boyd 1990). Another benefit of liming freshwater ponds is increased buffering capacity which obviates drastic changes in water quality, although such buffering is usually unnecessary in marine or brackishwater ponds because of the high buffering capacity of salt water (Boyd 1990, Sturmer 1990, Boyd and Daniels 1993). Boyd (1990) and Boyd and Daniels (1993) provide a detailed discussion of the effects of lime on pond muds, phosphorus availability and application rates for lime in fresh and brackishwater ponds.

Phytoplankton and Cyanobacteria

Nutrient enhancement in managed ponds results in denser phytoplankton communities than is found in unmanaged ponds (Boyd 1990). Phytoplankton species commonly found in fish ponds include representatives of the Chlorophyta, Euglenophyta, Chrysophyta and Cyanophyta (or Cyanobacteria) and algal blooms may appear yellow, brown, green, blue or milky in colour depending on the species involved (Boyd 1990, Hallegraeff 1991).

The phytoplankton communities found in fertilised ponds are usually of low diversity and the effects of dramatic fluctuations in abundance of a single species may have drastic effects on the pond environment (Boyd 1990). Fluctuations in phytoplankton abundance are common and have been attributed to numerous factors, including pH, temperature, nutrient concentrations, light, weather,

diseases, grazing by fish and zooplankton, competition between species, and natural algal toxins (Reynolds 1984, Boyd 1990).

High densities of phytoplankton may generate anoxic conditions resulting from high respiration rates by the algae at night or during dim daylight conditions or, more commonly, from bacterial respiration during decay of the phytoplankton bloom (Boyd 1990, Hallegraeff 1991).

Some algal species may damage fish gills either through mechanical damage or through producing haemolytic substances that destroy the red blood cells in gill tissues (Hallegraeff 1991). Algal species which have been implicated in fish kills in aquaculture and in the wild are listed by Hallegraeff (1991).

The response by phytoplankton to similar management regimes in adjacent ponds often differs substantially, and no management procedure will consistently result in a particular type of phytoplankton community or abundance of phytoplankton (Boyd 1990). The magnitude of variation is even greater between ponds in different physiographic areas (Boyd 1990).

As noted previously, phytoplankton is the major source of dissolved oxygen in larval rearing ponds. A secondary benefit of phytoplankton blooms is the high turbidity associated with high densities of phytoplankton. This reduces light penetration and prevents the proliferation of benthic algae and macrophytes, which may die off and cause deoxygenation problems through decomposition, or which may trap fish during draining and harvesting of the pond (Boyd 1990).

Fertilised ponds provide an ideal environment for the development of high densities of cyanobacteria (blue-green algae) since their abundance is closely

linked to eutrophication, and optimal temperatures for bloom-forming species are generally in the range 25-35°C (Sevrin-Reyssac and Pletikosic 1990, Paerl and Tucker 1995). Cyanobacteria possess several biological criteria which enable them to compete successfully with eukaryotic microalgae in a range of water conditions. Cyanobacteria can withstand extreme light levels at the water surface and are better able to sustain their biomass under low light conditions than are eukaryotic algae (Sevrin-Reyssac and Pletikosic 1990). Many species (those with heterocysts) are able to fix nitrogen, and cyanobacteria generally may be able to store nitrogen which they can use under nitrogen-limiting conditions. Low dissolved oxygen concentrations may also favour cyanobacteria because nitrogen fixation is improved under conditions of low dissolved oxygen concentrations (Sevrin-Reyssac and Pletikosic 1990, Paerl and Tucker 1995).

The abundance of zooplankton grazers and cyanobacterial blooms is often closely correlated. Cyanobacteria have a competitive advantage in the presence of zooplankton grazers because cyanobacteria are generally not grazed as rapidly as other phytoplankton. Many zooplankton, particularly cladocerans, are unable to successfully prey on cyanobacteria because of the relatively large size of the filaments (Sevrin-Reyssac and Pletikosic 1990, Paerl and Tucker 1995), although Culver and Geddes (1993) noted that the cyanobacterium *Anabaena* may support growth and reproduction of the cladoceran *Daphnia* in freshwater larval rearing ponds.

Many species of cyanobacteria undertake vertical migrations, which are carried out by means of turgor-pressure mediated partial collapse and resynthesis of the

gas vesicles in the cells (Sevrin-Reyssac and Pletikosic 1990, Hallegraeff 1991, Paerl and Tucker 1995). Cyanobacteria may form thick mats of cells at the surface as a result of the presence of senescent cells which are not capable of effecting turgor collapse (Sevrin-Reyssac and Pletikosic 1990, Hallegraeff 1991). These blooms may form a layer several centimetres thick which may cover the entire pond surface or drift to the leeward side as the result of wind action (Boyd 1990, Sevrin-Reyssac and Pletikosic 1990). The high density of cells in these aggregations may effectively preclude grazing by zooplankton (Sevrin-Reyssac and Pletikosic 1990).

Cyanobacteria secrete a variety of metabolic substances into their environment. These substances may have an inhibiting effect on other species ('hetero-antagonism') or on the species which secreted them ('self-antagonism'). This latter effect may be responsible for the common phenomenon of rapid death of cyanobacterial blooms after reaching high densities (Sevrin-Reyssac and Pletikosic 1990). These secretions may stimulate development of other species of phytoplankton, and have been shown to inhibit nutrition and reproduction among some rotifers and cladocerans (Sevrin-Reyssac and Pletikosic 1990).

Fish kills are common in the presence of high densities of cyanobacteria. There are several aspects of the biology of cyanobacteria which can cause mortalities in fish and other aquatic organisms. During periods of high light intensity, rapid rates of photosynthesis by algae in surface scums may cause high pH, low CO_2 concentrations and supersaturation with dissolved oxygen. Such conditions may result in massive death of cyanobacteria, apparently through

photo-oxidation (Boyd 1990). Fish kills may then result from oxygen depletion caused by decay of dead cells. In addition, this collapse of the cyanobacterial bloom may be accompanied by a sharp rise in ammonia levels and toxins may be released by the decomposing cells (Sevrin-Reyssac and Pletikosic 1990).

Cyanobacterial toxins may also cause mortalities amongst fish, and the bacteria associated with cyanobacteria may be poisonous if ingested (Sevrin-Reyssac and Pletikosic 1990, Hallegraeff 1991, Paerl and Tucker 1995).

Because they make little contribution to zooplankton production and may cause fish kills, cyanobacteria are regarded as undesirable in larval rearing ponds. Several methods for controlling cyanobacterial blooms have been developed:

1. Algicides (such as copper sulphate and simazine) can be used, but such algicides are non-specific and also kill eukaryotic phytoplankton (Boyd 1990, Sevrin-Reyssac and Pletikosic 1990). (Note: these chemicals are not registered for use in aquaculture in Australia).
2. Increasing the nitrogen:phosphorus (N:P) ratio of the inorganic fertiliser regime to 5:1 or greater can inhibit cyanobacterial blooms, since increased levels of inorganic nitrogen prevent the development of a nitrogen-limited environment which favours the development of nitrogen-fixing cyanobacteria (Geiger 1983b, Sevrin-Reyssac and Pletikosic 1990, Paerl and Tucker 1995).

The routine use of mechanical aeration may also assist in preventing the development of conditions which favour the development of cyanobacterial blooms

i.e. stratification, low dissolved oxygen levels and low nitrogen levels in the pond (Sevrin-Reyssac and Pletikosic 1990, Paerl and Tucker 1995).

Bacteria and Protozoa

Bacteria and protozoa are important food sources for various types of zooplankton including rotifers, copepods and cladocerans (Geiger 1983a,b, Porter 1984, Rieper 1984, Boon and Shiel 1990). The production of bacteria and protozoans in the pond environment is stimulated by the decomposition of organic fertilisers. In addition, much of the organic matter fixed by primary producers enters an extracellular organic pool as algal exudates and as losses during feeding and excretion by heterotrophic microorganisms. As much as 50% of primary production may be utilised in this way and heterotrophic protozoa are major consumers of this microbial production (Porter *et al.* 1985).

Protozoa may also act as intermediaries between phytoplankton and zooplankton, utilising picoplankton (i.e. plankton 0.2-2.0μm in size) which are too small for zooplankton to utilise directly; these protozoa in turn become food for zooplankton (Porter *et al.* 1985).

Zooplankton

Rotifers and copepods are the major zooplankton taxa found in marine or brackishwater ponds used for extensive larval rearing (Sturmer 1990). Cladocerans are an important component of zooplankton communities in

freshwater ponds but have only been recorded from brackishwater ponds at relatively low salinities (<c. 20 ppt) (Rutledge and Rimmer 1991).

The usual source of a zooplankton inoculum for extensive rearing ponds is the water used to fill the pond (Sturmer 1990). Responses by the zooplankton to management practices depend on their feeding habits, reproductive capacities, competition, and predation (Nassogne 1970, Sturmer 1990). Their abundance can also be affected by physicochemical factors such as temperature, salinity, dissolved oxygen, pH and the availability of dissolved nutrients. Because of the large number of uncontrolled variables which affect zooplankton communities in larval rearing ponds, a high degree of variability is often apparent in zooplankton production in extensive rearing applications.

One technique designed to reduce some of this variation is the inoculation of ponds with selected plankton species to provide greater predictability in developing a zooplankton community (Sturmer 1990). Red drum rearing ponds stocked with rotifers in log-phase growth exhibited better fish survival and greater daily production than non-inoculated ponds (Sturmer 1990).

After inoculation, the abundance of each zooplankton group typically increases rapidly to reach a peak density, after which abundance decreases. Because of the different life cycles of different zooplanktonic organisms, different organisms reach peak density at different times. Thus a 'succession' of zooplankton communities is seen, with different groups dominating the zooplankton community at different times of the rearing cycle. Life history parameters of rotifers and copepods which affect patterns of zooplankton succession are summarised in Table

6.1. There is little biological information on the euryhaline cladocerans which occur in brackishwater ponds, but freshwater cladocerans generally have an egg to egg generation time of 7 days at 20°C (Geiger 1983b).

Table 6.1 Life history parameters of major zooplankton taxa found in marine and brackishwater larval rearing ponds (from Sturmer 1990).

Parameter	Temperature (°C)	Rotifer	Copepod
Egg to egg	20	2-3	13-15
generation (days)	25	1.3-1.7	7-8
Total offspring per	20	15-25	250-500
lifespan	25	15-25	500-750
Lifespan (days)	20	12	50
	25	5	40
Peak reproduction (days)	20	3.5	24.0
	25	2.0	17.5

Rotifers, which have relatively short life-cycles and unspecialised feeding habits, usually peak first at 8-10 days after filling and inoculation. Copepods, which have longer life-cycles, greater capacity for selective feeding and greater capacity for predator avoidance, generally peak later in the zooplankton succession at 13-23 days (Legendre *et al.* 1987, Colura *et al.* 1987, Sturmer 1990).

Physicochemical factors such as temperature, salinity, dissolved oxygen and pH can influence zooplankton communities in ponds (Geiger 1983b). Temperature

influences zooplankton growth, filtering rates and reproduction, and temperature-photoperiod interactions have been shown to affect cladoceran life-spans, age at reproductive maturity, and numbers of young produced per day (Geiger 1983b).

Salinity may exert substantial influence on the structure of zooplankton communities and the abundance of different zooplankton groups. Colura *et al.* (1987) obtained substantially higher zooplankton densities in ponds at 10 ppt compared with ponds at 15 and 20 ppt, due primarily to an increased abundance of rotifers. They also found differences in the timing of peak abundance of zooplankton: (9 days at 10 ppt, versus 23 days at 15 and 20 ppt) and concluded that this was associated with dominance of rotifers at the lower salinity compared with copepods at higher salinities (Colura *et al.* 1987).

Rotifers, copepods and cladocerans will only reproduce when the supply of food is in excess of metabolic maintenance requirements (Nassogne 1970, Geiger 1983b). Zooplankton exhibit size selectivity in the food particles ingested. Most rotifers are non-selective suspension feeders which ingest particles in the size range 1-20μm (Geiger 1983b, Sturmer 1990). *Brachionus plicatilis*, which is commonly the dominant rotifer species in marine ponds, selectively filters algae less than c. 12-15μm in size (Walker 1981). Cladocerans are able to utilise a wider range of food particle sizes than rotifers (1-50μm), and this, combined with their higher particle filtering rates, gives cladocerans a competitive advantage over rotifers (Geiger 1983b). Copepods can utilise a wide size range of prey (2-100μm) since they feed by filtering smaller particle sizes and capturing, but only partly ingesting, larger particles (Nassogne 1970, Sturmer 1990). Copepods also

appear to be more selective in the types of food ingested (Geiger 1983b, Sturmer 1990). As food becomes less abundant, copepods have an advantage over rotifers and cladocerans mainly because of their ability to ingest prey of a wider size range (Geiger 1983b). Copepods may also resort to cannibalism of nauplii under conditions of low food availability and high densities of adult copepods (Ohno and Okamura 1988).

The quality of the food source also influences zooplankton populations. Filtration activity, rate of ingestion, adult life span and egg production in copepods are all influenced by the type of unicellular algae supplied as a food source (Nassogne 1970). Colonial algae and many cyanobacteria are poorly assimilated by zooplankters and dominance of the phytoplankton community by such species may result in decreased zooplankton abundance (Geiger 1983b).

Fish predation can exert substantial affects on zooplankton communities, particularly affecting the mean size and species composition of the zooplankton community, although precise affects differ depending on the species cultured, location, etc. (Stenson 1982, Geiger 1983b, Sturmer 1990). Once zooplankton communities are subject to predation by fish, it is difficult to maintain desirable crustacean populations longer than 2-3 weeks (Geiger 1983a).

Zooplankton succession in ponds can be modified by application of chemicals, such as the pesticide trichlorfon, which reduces the abundance of arthropods without adversely affecting phytoplankton or rotifer populations (Opuszynski *et al.* 1984, Sturmer 1990). (Note: this chemical is not registered for use in aquaculture in Australia).

Zooplankton populations can be artificially enhanced, e.g. by adding brine shrimp to ponds. This technique has been successfully used to extensively rear Australian bass (*Macquaria novemaculeata*) larvae. Brine shrimp were introduced to larval rearing ponds because naturally occurring zooplankton responded poorly to pond fertilisation (presumably due to low winter water temperatures) and brine shrimp comprised a major component of the diet of Australian bass larvae in these ponds (Battaglene *et al.* 1992). However, when the same technique was trialled in spotted seatrout (*Cynoscion nebulosus*) larval rearing ponds, no brine shrimp were subsequently found in the guts of spotted seatrout larvae sampled from the ponds to which brine shrimp had been introduced (Porter and Maciorowski 1984). A disadvantage inherent in this technique is that deficiencies in the fatty acid composition of the brine shrimp may affect fish growth and survival (see Chapter 3).

Food Requirements of Marine Fish Larvae

The management strategies which have been developed for extensive larval rearing are largely based on Hjort's hypothesis that larval survival is directly related to the availability of prey organisms at the very earliest stages of fish larval development (Blaxter 1986, Lasker 1981). Extensive rearing techniques thus emphasise provision of maximal densities of suitable zooplankton at the time the fish larvae begin feeding and immediately thereafter (Geiger 1983a,b, Rowland 1983, Sturmer 1990). While there is still little direct evidence to validate Hjort's hypothesis (Lasker 1981), Sturmer (1990) noted that increased fingerling

survival occurs when first-feeding fry are stocked into ponds dominated by zooplankters of a size that can be consumed, i.e. rotifers and first naupliar stages of copepods.

The size of a fish larva at the time of first feeding and the amount of time available to find food before the onset of irreversible starvation are both largely determined by egg size and water temperature (Blaxter and Hempel 1963, Hunter 1981). Most marine fishes have small eggs which produce small larvae with limited yolk reserves and which thus must commence feeding within a relatively short time of hatching. In *L. calcarifer*, the yolk is used up 50 hours after hatching and larvae begin feeding 54.5 to 71 hours after hatching (26-30°C) (Kohno *et al.* 1986, Avila and Juario 1987).

Marine fish larvae are visual feeders, and for this reason most species feed only during daylight hours (Blaxter 1968, 1986, Hunter 1981, Sturmer 1990). Modelling of searching behaviour of fish larvae indicates that the volume of water searched is low, ranging from 0.1-1.8 l/h (Hunter 1981). Marine fish larvae can only perceive prey which is relatively near, e.g. first-feeding marine fish larvae react to prey at distances of 0.2-1.0 body length (Hunter 1981). Consequently, early larvae must be exposed to high densities of prey organisms to ensure that energy gained from digestion of the prey exceeds the energy expended on searching for prey in order to provide energy for growth. Precise prey densities required for optimal survival depend on the species cultured and on the behaviour of the dominant prey species (Buskey *et al.* 1993).

Recommended minimal zooplankton densities for larval rearing ponds are 1,500 to 3,000 organisms per litre at first feed, although higher densities may be necessary if the zooplankton community is largely composed of rotifers, due to the lower calorific value of rotifers compared with crustacean zooplankton (Geiger 1983b, Theilacker and Kimball 1984, Blaxter 1986, Szyper 1989, Sturmer 1990). A minimum concentration of 1,900 brine shrimp per litre is necessary to establish a feeding response among striped bass larvae at first feed (Geiger 1983b).

Feeding success is often low at the onset of feeding, e.g. in northern anchovy (*Engraulis mordax*) larval feeding success increases from 10% at the onset of feeding to 90% by 3 weeks of age (Hunter 1981, Blaxter 1986). This change in success is at least partly attributable to learning, since a change in prey type may be immediately followed by a decrease in feeding success, after which feeding success gradually increases again (Hunter 1981, Blaxter 1986).

Naupliar through adult stage copepods are the typical food of most marine fish larvae in the wild, although most larvae are euryphagous during the earlier stages and often eat organisms such as tintinnids, phytoplankton, mollusc larvae and ciliates as well as copepods (Blaxter 1965, Hunter 1981, Sturmer 1990). This greater variety of prey organisms eaten by fish larvae during the early stages of development may be due to a greater variety of small organisms of the proper size for ingestion by early larvae (Hunter 1981).

Although the provision of prey for the 'critical period' immediately following the commencement of exogenous feeding is the primary aim of extensive pond management techniques, the maintenance of adequate densities of suitably sized

zooplankton throughout the larval rearing phase is necessary to maximise survival and growth rates.

Hunter (1981) reviewed food density - survival experiments for six species of marine fish larvae and concluded that a density of 1,000-4,000 microcopepods per litre is required for high survival rates under laboratory conditions (Hunter 1981). Barramundi larvae fed brine shrimp at densities ranging from 1,000 to 10,000 organisms per litre from days 8 to 15 exhibited similar survival (100%) under hatchery conditions (M. Pearce, unpublished data).

Although there is no published information on minimal prey densities required for survival of barramundi larvae, Thai workers have examined the effects of prey density on food consumption by *L. calcarifer* larvae and juveniles. Pechmanee *et al.* (1986) found that consumption of rotifers by *L. calcarifer* larvae reached a maximum at densities of c. 4,000 rotifers per litre for 4-5 day old larvae, increasing to ≥32,000 rotifers per litre for 13-14 day old larvae. Dhesprasith *et al.* (1986) found that consumption of copepods (*Tigriopus japonicus*) by juvenile *L. calcarifer* reached a maximum when copepod densities reached 2,000 copepods per litre for 14-15 day old fish, increasing to 8,000 copepods per litre for 20-24 day old fish.

Striped bass larvae were found to grow more rapidly when fed brine shrimp at concentrations over 100 organisms per litre compared with larvae fed brine shrimp at concentrations less than 100 organisms per litre (Geiger 1983b), but crustacean prey densities of 10-20 organisms per litre at day 5 (larvae stocked day 4) in extensive rearing ponds are regarded as adequate for this species

(Anderson 1993b). Barramundi larvae achieved maximal growth when fed on brine shrimp at densities of 10,000 organisms per litre from days 8 to 20 (M. Pearce, unpublished data).

Such laboratory studies may not be directly relevant to the conditions found in larval rearing ponds, since such high prey densities are rarely found in ponds and larvae stocked in ponds may be subject to greater physiological and environmental challenges than fish maintained in the laboratory (Geiger 1983b).

Prey size dominates the prey selection patterns of larval fishes (Hunter 1981). Increase in prey size as fish size increases has consistently been recorded for all larval fish species studied, and usually involves some degree of selection for prey in a preferred size range (Blaxter 1965, Hunter 1981). Mouth size (gape and width) increases as fish length increases, and thus allows larger prey organisms to be ingested as the larva grows (Blaxter 1965, Hunter 1981). The maximum prey size generally tends to increase more rapidly than the minimum, thus the range of prey sizes selected also increases with larval length (Hunter 1981). Speed, capture success rates and perceptive distance also increase with increasing length or age (Hunter 1981). Other factors which influence prey selection are: prey visibility (particularly contrast against the background), the encounter rate of predator with prey (which is a function of prey density, larval swimming ability, and prey locomotory behaviour), prey escape response, and prey swimming pattern (Buskey *et al.* 1993).

Larvae of various species cultured in ponds, including red drum, spotted sea trout and cod (*Gadus morhua*), show similar patterns of prey selection. Larvae

commence feeding on smaller zooplankton (rotifers and copepod nauplii), then eat progressively larger zooplankters (copepod adults) and, later, insect larvae, amphipods, polychaetes and small shrimp (Geiger 1983b, Kvenseth and Oiestad 1984, Porter and Maciorowski 1984, Sturmer 1990).

Feeding on larger prey organisms is energetically more efficient, e.g. an increase of 2.5 times the cephalothorax width of copepods represents a tenfold increase in dry weight (Hunter 1981). In addition, a change in preferred prey type may also increase energetic efficiency, e.g. the calorific value of copepods is higher than that of rotifers (Theilacker and Kimball 1984, Szyper 1989).

Predators

Hunter (1981) reviewed the literature on predation of marine fish larvae in the wild and noted that early larvae are less capable of avoiding predation than older larvae which have better developed sensory and locomotor systems.

Few predation studies on marine fish larvae reared in ponds have been carried out. The presence of large numbers of hydromedusae was found to be associated with poor survival of cod in ponds but it is not clear whether this was the result of predation on fish larvae or competition for zooplankton (Kvenseth and Oiestad 1984, Huse 1991). Apparent total mortality of barramundi larvae during an extensive rearing trial (P1, undertaken by W.P. Rutledge) was associated with high densities of comb jellyfish (Phylum Ctenophora) and arrow-worms (Phylum Chaetognatha), and the arrow-worms were observed to attack and kill newly hatched barramundi larvae (Rutledge and Rimmer 1991). Odonata (dragonfly)

larvae have been found to be important predators of juvenile barramundi (< c. 20 mm TL) in freshwater ponds (Barlow *et al.* 1991). Changes in the behaviour of juvenile barramundi, from a 'roving zooplanktivore' to a 'lurking predator' habit, which occurs when the fish reach about 18-20 mm TL, reduce predation opportunities for dragonfly larvae (Barlow *et al.* 1991).

Extensive Larval Rearing of Barramundi

The research described in Part Two of this thesis expands on the research by Rutledge and Rimmer (1991) which demonstrated the practicability of extensive larval rearing for barramundi. The overall objective of this research was to determine which factors affect survival and growth of extensively reared barramundi larvae. As noted earlier in this chapter, both growth and survival are primarily dependent on the provision of a stable environment with regard to physicochemical parameters, and the provision of suitable prey organisms for larval and juvenile fish. Chapter 7 provides details of individual pond trials, with emphasis on the results of a basically standardised management regime on survival and growth of larvae. Chapter 8 examines the diet and feeding behaviour of extensively reared larval and juvenile barramundi with particular respect to the type and size of prey organisms utilised at different stages of growth. Chapter 9 synthesises these results and examines management strategies which may assist in increasing the efficiency of extensive larval rearing of barramundi by increasing survival and growth.

CHAPTER 7

Extensive Rearing of Barramundi Larvae

Introduction

There have been previous attempts to rear barramundi larvae in fertilised salt or brackishwater ponds in India (De 1971, Patnaik and Jena 1976, Ghosh and Pandit 1979), in Thailand (Chungyampin *et al.* 1986) and in Australia (S. Coco, pers. comm.). These attempts seem to have largely met with poor survival (c. 5%) and consequently the technique has not been widely adopted.

The techniques used to rear barramundi in earthen ponds were based on those used in the United States to rear the larvae of marine fish species, particularly red drum (*Sciaenops ocellata*), because of similarities in the larval and juvenile biology of red drum and barramundi. Larval and juvenile red drum utilise coastal salt marshes as nursery habitats (Bass and Avault 1975). Likewise, larval and juvenile barramundi (5 mm TL and larger) move into coastal nursery swamps within the mangrove zones of tropical estuaries. The barramundi larvae remain in these swamps until they reach 150-300 mm TL when they move from these nursery swamps into adjacent coastal streams (Moore 1982, Davis 1985a, Russell and Garrett 1985).

Rutledge and Rimmer (1991) showed that the extensive larval rearing techniques developed for red drum culture could be used to rear barramundi larvae, albeit with some modifications. The objective of the research described in this chapter was to expand on the results of Rutledge and Rimmer (1991) and to examine the factors affecting survival and growth of barramundi larvae in

extensive rearing in order to develop management methodologies to maximise these parameters.

The early trials were undertaken with minimal resources and equipment, but after the success of the initial trials more resources were allocated to the development of extensive rearing techniques and additional equipment was purchased to assist with data collection and analysis. In addition, sampling procedures were continually refined in response to the provision of additional resources. Consequently, there are some slight modifications in sampling methodology throughout the trials described in this chapter.

Since this research was intended to develop extensive rearing procedures suitable for commercial application, several methods were evaluated to determine whether simplified sampling techniques could be applied to commercial operations without a need for exact precision. These were:

1. Comparison of Secchi disk visibility and chlorophyll *a* concentration to determine phytoplankton abundance.
2. Comparison of plankton net and tube sampler estimates to determine zooplankton abundance.
3. Estimation of the density of larval and juvenile barramundi in the pond to predict survival.

In addition, two changes in the recommended management regime for larval rearing ponds were examined to detemine the affects on pond management procedures and survival and growth of fish in the pond:

1. Drying and liming of the pond prior to use was suspended for two pond trials in the 1991-92 season (P9 and P10), in order to determine whether carrying out these operations between trials had a marked effect on pond productivity. This strategy simulates the conditions which could be expected to occur in commercial aquaculture when farmers attempt to produce as many fish as possible before the onset of the wet season.
2. Aeration was used throughout pond trial P12 to determine whether this treatment had any beneficial or detrimental affects on routine pond management procedures.

Because only a single pond was available for this research, no replicated experiments were possible. In practice, experimental trials using replicated ponds often produce highly variable results because of the difficulty in controlling the large number of variables which interact in the pond environment (Boyd 1990).

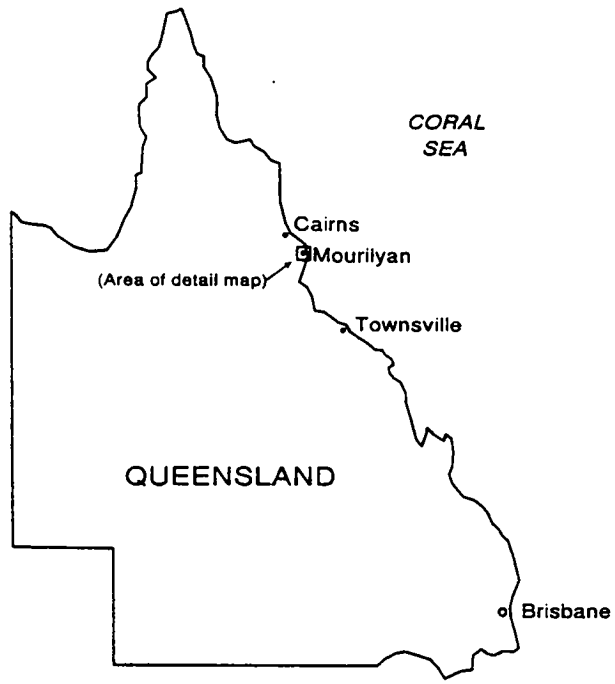
Study Site, Materials and Methods

Pond

The pond used for these trials was a 1350 m^2 (0.14 ha) earthen pond constructed at Mourilyan, Queensland (Fig. 7.1). The pond was roughly triangular in shape, and ranged in depth from 1.8 to 1.2 m (average depth 1.5 m) (Fig. 7.2). An aerial view of the pond is shown in Figure 7.3a. A raceway (2 m wide and 0.4 m deep) was originally formed in the bottom of the pond. This was replaced prior to the 1990-91 season with a concrete raceway using half-pipe sections 0.9 m wide and 0.4 m to 0.2 m deep (Fig. 7.3b).

Water was supplied from the adjacent Moresby River through a supply channel. The inflowing water was screened first through 1 mm mesh, then through a filter 'sock' with 300 μm mesh screen to exclude eggs and larvae of other fishes as well as invertebrate predators (Rutledge and Rimmer 1991).

Figure 7.1 Maps showing location of study site for the pond trials described in this thesis, near Mourilyan, far northern Queensland.


CORAL
SEA
Cairns
Mourilyan
(Area of detail map)
Townsville
QUEENSLAND
Brisbane

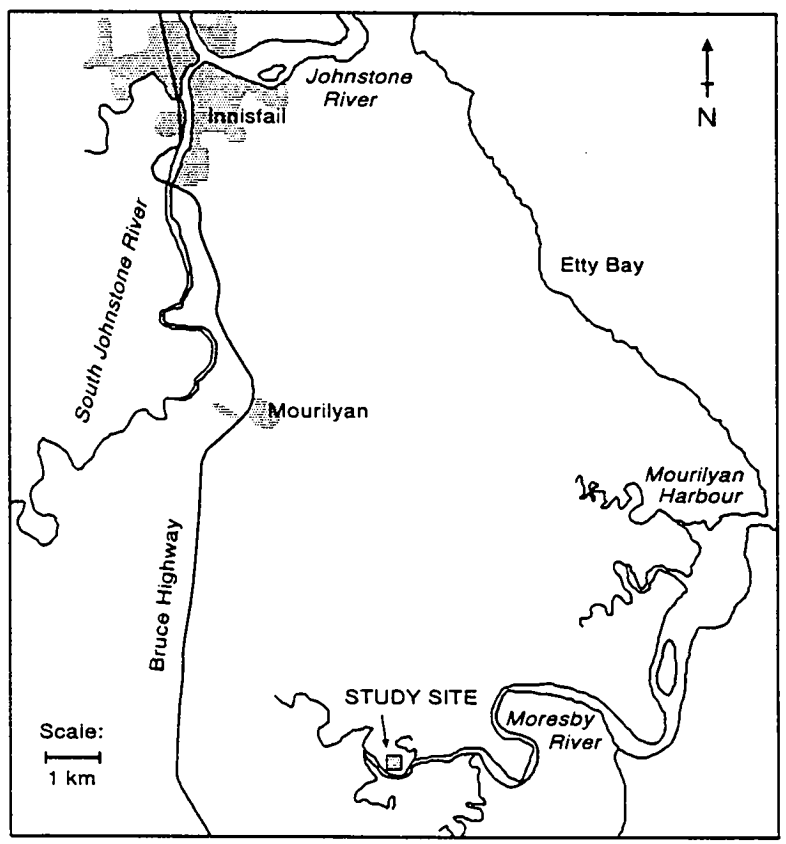

Johnstone
River
Innisfail
N
South Johnstone River
Etty Bay
Mourilyan
Mourilyan
Harbour
Bruce Highway
STUDY SITE
Moresby
River
Scale:
1 km

Figure 7.2 (a) Plan of the pond used for extensive rearing trials with barramundi larvae.

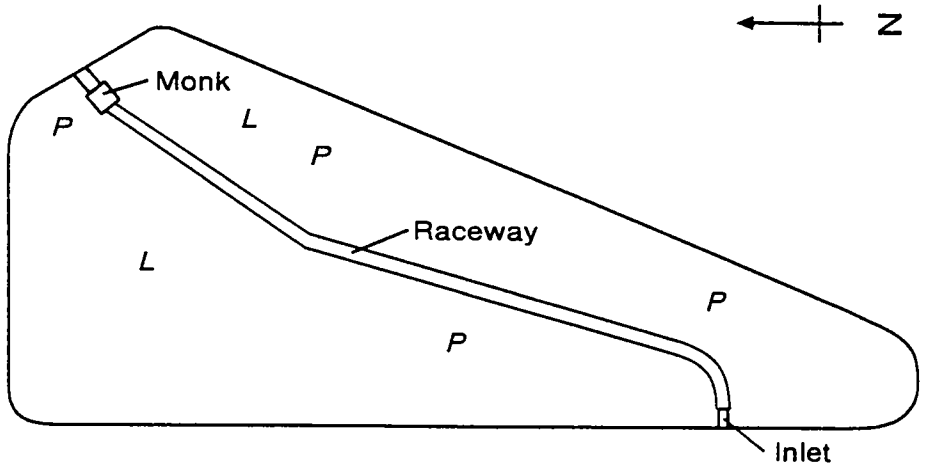
Key to abbreviations:

P: sites sampled for plankton using vertical tube sampler.

L: lift net locations.

(b) Cross-sectional view of the pond used for extensive rearing trials with barramundi larvae. (Not to scale).

a


N
Monk
P
L
P
Raceway
L
P
P
Inlet

b

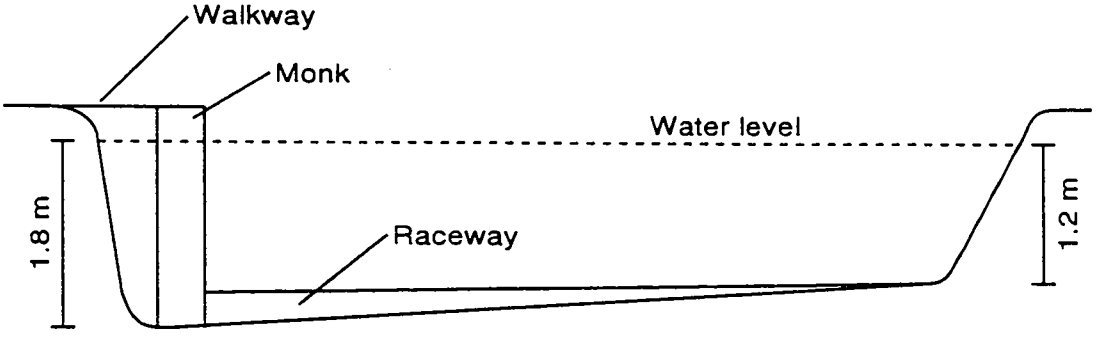

Walkway
Monk
Water level
1.8 m
Raceway
1.2 m

Figure 7.3 (a) Aerial view of the pond used for extensive rearing trials with barramundi larvae (the empty pond at lower right).

(b) View of empty pond showing concrete raceway for harvesting fingerlings.

Fertilisers

Diammonium phosphate (DAP) (18% N, 19% P) was used as the inorganic fertiliser in these trials. The DAP was dissolved by adding the fertiliser to the filter sock used to screen inflowing water. Application rates were calculated to provide approximately 1 mg/l phosphorus and and 1 mg/l nitrogen based on total pond volume (Rimmer and Rutledge 1991). Lucerne pellets were used as the organic fertiliser, applied at 900 kg/ha. The lucerne pellets were broadcast throughout the pond using a modified fertiliser spreader. The schedule for application of the fertilisers is shown in Table 7.1. Note that although lucerne pellets were generally applied as indicated by the schedule, additional applications of DAP or of urea (45% N) were made when Secchi disk visibility increased above about 80 cm, regardless of whether such applications were scheduled. Such *ad hoc* additions of inorganic fertiliser were necessary to maintain the phytoplankton bloom at maximal density.

Pond Drying and Liming

Pond trials were generally timed to allow for a maintenance period between trials to permit drying of the pond substrate and application of lime in order to release phosphorus trapped in the bottom sediments (Boyd 1990). Approximately 250 kg of agricultural lime (39% Ca) was applied to the pond between trials. Complete drying of the pond substrate between trials was not always possible, due to wet weather conditions. In addition, the site used was subject to considerable tidal variation in salinity, so pond filling was timed to coincide with the highest

tides available in order to obtain the highest salinity for larval rearing trials. In some cases, this resulted in a relatively short interval between the end of one trial and the start of the next, often only a few days. Consequently, there was some variation in between-trial pond preparation.

The requirement for drying and liming marine saltwater ponds was tested during the 1991-92 barramundi breeding season. Drying and liming of the pond was suspended prior to pond trials P9 and P10 and reinstated for trials P11 and P12.

Table 7.1 Fertilisation schedule used in pond trials P3 - P12 (from Rutledge and Rimmer 1991).

Day	
1	Fill pond
2	DAP (5.3 kg/Ml)
3	Lucerne pellets (450 kg/ha)
6	DAP (5.3 kg/Ml)
10	Lucerne (150 kg/ha)
12	DAP (5.3 kg/Ml)
14	Lucerne (150 kg/ha)
18	DAP (5.3 kg/Ml)
20	Lucerne (150 kg/ha)

Water Quality and Weather

Water quality data were recorded with several instruments, according to the availability of equipment: sampling details are listed in the relevant figure legends.

Daily measurements of temperature, conductivity (subsequently converted to salinity), pH, turbidity, and dissolved oxygen were taken using an Horiba Model U-7 Water Checker meter. These readings were taken early each morning (c. 0600 h) at the pond bottom adjacent to the monk.

Commencing with the 1990-91 season trials, Great Lakes Instruments conductivity and temperature probes were installed just above the pond bottom adjacent to the monk and readings recorded on a Yew 3087 chart recorder at 2 hourly intervals. These data were averaged to provide daily mean bottom temperature and conductivity measurements. The conductivity data were subsequently converted to salinity using a computer program based on the UNESCO equations for conversion of conductivity to salinty (UNESCO 1981).

From Trial P5, a Yeo-Kal Model 606 submersible data logger (SDL) was also used to record depth, temperature, salinity, pH and dissolved oxygen at hourly intervals. This unit was positioned just above the pond bottom adjacent to the monk. Temperature, salinity and pH data were averaged to provide mean daily values, while minimum daily dissolved oxygen values were used. Not all functions were available during all trials due to equipment malfunction.

Turbidity was measured with a Secchi disk as part of the zooplankton sampling schedule. pH of the water sample used for plankton density estimates was

measured on a Metrohm E558 laboratory pH meter. Temperature and salinity were also measured with a mercury thermometer and a Yeo-Kal salinty meter respectively; these samples were used to check the accuracy of the remote recording equipment and to provide additional samples of surface conditions.

Surface and bottom water temperature and salinity data are available for most extensive larval rearing trials. In most trials there was little or no variation between surface and bottom water temperature and salinity, and so only one data set (usually bottom samples) has been presented for each trial, to avoid unnecessarily complicating the graphical data. In trials where stratification caused dramatic differences between surface and bottom samples (e.g. pond trial P7) both data sets have been presented.

Air temperature and cloud cover (recorded at 0300, 0600, 0900 and 1500 h EST each day) and daily rainfall data (to 0900 h) were obtained from the nearest Bureau of Meteorology recording station at Innisfail, approximately 13 km north of the study site. Air temperature from dry bulb readings taken at 0300 h and 1500 h each day were used to approximate daily minima and maxima respectively, and cloud cover at 1500 h was used as a measure of daily cloud cover.

Aeration

Air was supplied by a centrifugal blower to a 40 m length of finely perforated irrigation hose ('WaterWik') which was weighted with wire rope and placed in the concrete raceway. This aeration system provided a flow of extremely fine air

bubbles which generated mild turbulence throughout the pond. With the exception of pond trial P12, aeration was used only during harvesting to ensure dissolved oxygen levels remained above critical values (>2 mg/l). The same aeration system was used throughout pond trial P12 to examine the effects of aeration on pond management procedures and pond productivity.

Chlorophyll a

Chlorophyll *a* concentrations were determined using Holm-Hansen and Riemann's (1978) modification of the technique described by Strickland and Parsons (1968). A water sample was filtered through 37μm mesh screen to remove zooplankters, then 25-100 ml of the sample was filtered under vacuum through Whatman GF/C filter paper. The sample was sealed in aluminium foil and stored at -20°C. All samples were analysed within 3 weeks to preclude degradation of chlorophyll pigments (Holm-Hansen and Riemann 1978).

Each sample was thawed, placed in 10 ml 90% methanol in a centrifuge tube and heated in a water bath at 68-70°C for 3-4 minutes. The sample was allowed to cool, the filter paper removed and the total volume adjusted to 10 ml using 90% methanol. After centrifuging at 3,500 rpm for 5 minutes, the supernatant was transferred to a 1 cm spectrophotometer cell and the absorbance of the sample read at 665nm and 750nm, using 90% methanol as the blank.

Chlorophyll *a* data were compared with Secchi disk visibility readings taken at the same time to determine whether Secchi disk visibility could be used as an accurate estimator of phytoplankton density. Correlation between chlorophyll *a*

concentration and Secchi disk visibility was analysed using the statistical programs Genstat and Statistix. Since maximum Secchi disk visibility was c. 150 cm (i.e. to the pond bottom), all readings of chlorophyll *a* obtained when Secchi disk visibility was ≥150 cm were omitted from the analysis.

Zooplankton

Zooplankton samples were collected 2-3 times each week using a plankton net 15 cm in diameter and 25 cm in length with 25 μm mesh. A single vertical tow immediately adjacent to the monk allowed sampling of the full depth range of the pond. The samples were preserved in formalin acetic alcohol and made up to 100 ml total volume. Three subsamples of 1 ml were removed and the numbers of rotifers, copepod nauplii, copepods (i.e. copepodites and copepod adults), cladocerans and ostracods were counted. The average of the three counts was used to calculate the density of each zooplankton group in the pond. (Note: because of the difficulty in differentiating the naupliar stages of copepods from those of other marine crustaceans, counts of 'copepod nauplii' on occasions also included the nauplii of barnacles (Class Cirrepedia) and ostracods (Class Ostracoda). However, copeopd nauplii consistently dominated the naupliar fauna in the pond. As barramundi larvae presumably do not discriminate between different crustacean taxa, the inclusion of some other crustacean naupliar stages in these counts does not affect the results obtained in this study.

Commencing in the 1990-91 season (Trial P5), plankton samples were collected 2-3 times each week using a tube sampler of 50mm diameter to sample

the entire water column. A check valve at the base of the sampler ensured that the sample was retained as the sampler was withdrawn. Four replicate samples were taken at each of 4 sites within the pond (Fig. 7.3). The combined sample was filtered through plankton mesh screens of 37μm, 100μm, 250μm, 500μm and 1000μm width mesh and the total water volume recorded. Plankton retained on each screen were preserved in formalin acetic alcohol. Each sample was made up to 100 ml volume and 3 subsamples of 1 ml were removed and the number of organisms in each major group (rotifers, copepod nauplii, copopedites and copepod adults, cladocerans and other organisms were counted. These data were expressed as the density of zooplankton in each size class (abbreviated to $>37\mu$m, $>100\mu$m, $>250\mu$m, $>500\mu$m and $>1000\mu$m) and the relative proportions of the various major groups of organisms in each size class.

Because of the more accurate estimation of zooplankton densities obtained using the tube sampler (Graves and Morrow 1988, DeVries and Stein 1991), this technique was used to provide zooplankton data from trial P5 onwards.

Because plankton nets are preferred for their ease of use in commercial aquaculture applications, use of the 25μm mesh plankton net was continued to permit a comparison of the tube sampler and plankton net data to determine whether the latter technique is suitable for routine commercial applications. Results from the 25μm plankton net samples for pond trials P5-P12 are contained in Appendix 1.

Fish

Fertilised barramundi eggs were obtained from induced spawnings of broodstock held at NFC, Cairns (Garrett and Connell 1991). The eggs were placed in black 400 litre fibreglass tanks connected to a filtered recirculating seawater system in the NFC hatchery prior to hatching, which generally occurred 12-15 hours after fertilisation. Day 2 larvae (where day 1 is the day of hatching) were counted in 5-10 samples of 75 ml of water taken from the tank containing the hatched larvae. The mean of these counts was used to provide an estimate of larval density and a volume of water corresponding to the required number of larvae was withdrawn from the tank.

No assessment of larval 'quality' was made apart from estimations of fertilisation rate and hatching rate for each batch of larvae. Fertilisation and hatching rates exceeded 70% and 80% respectively for all batches of larvae used in these trials. Most batches came from a population of 7 broodfish held in a single tank at NFC, in order to minimise the effects of parental variation on the trials.

Stocking density varied between trials due to the differing availability of barramundi larvae, and concerns that zooplankton densities in some trials were inadequate to support large populations of juvenile fish in the pond. The availablity of larvae varied at this time because spawning induction methods were still being refined to their present high degree of reliability, and because NFC was selling barramundi larvae to commercial hatcheries, in part to support this research.

Larvae were transported to the pond in plastic bags inside insulated plastic containers and tempered before stocking by mixing pond water with the water in the plastic bags to reduce the stress associated with rapid changes in water quality. Larvae used in some trials were tempered for salinity in the hatchery in the period between hatching and stocking so that further tempering by the pond was reduced to a minimum.

Larval barramundi were sampled from the pond using a plankton net 40 cm in diameter, 80 cm long, with 300μm mesh, and a light trap which contained a 40 cm long 12 V fluorescent light in a perspex trap measuring 30 cm square at the top and tapering to a 25 mm tap fitting to allow draining of the trap (Fig. 7.4). The light was switched on, using a timer, from 0100 - 0500 on the day the sample was taken. Juvenile barramundi were sampled with 1 m^2 lift nets made with 1 mm width mesh. The lift nets were constructed with a vertical 'skirt' which lifted into place when the net was raised to prevent fish escaping from the sides of the net. The number of fish on each of the nets was counted and a subsample retained for measurement of total length (TL).

Size independent growth rate (specific growth rate) for each sample interval was derived using the equation:

$$SGR = 100 (\ln TL_2 - \ln TL_1) / (t_2 - t_1)$$

where SGR is specific growth rate (%/day), and TL_1 and TL_2 represent mean TL (mm) of fish sampled at time t_1 and t_2 (days) respectively (Wootton 1992). Degree-days were determined by summing the mean daily water temperatures for the period between fish samples.

Samples of barramundi larvae and juveniles were examined for symptoms of protozoan infestations, particularly white spot *Cryptocaryon irritans*, or other health problems. Detailed examinations of barramundi sampled from the pond at irregular intervals were undertaken by I. Anderson and N. Stewart (QDPI Veterinary Laboratory, Oonoonba, Townsville).

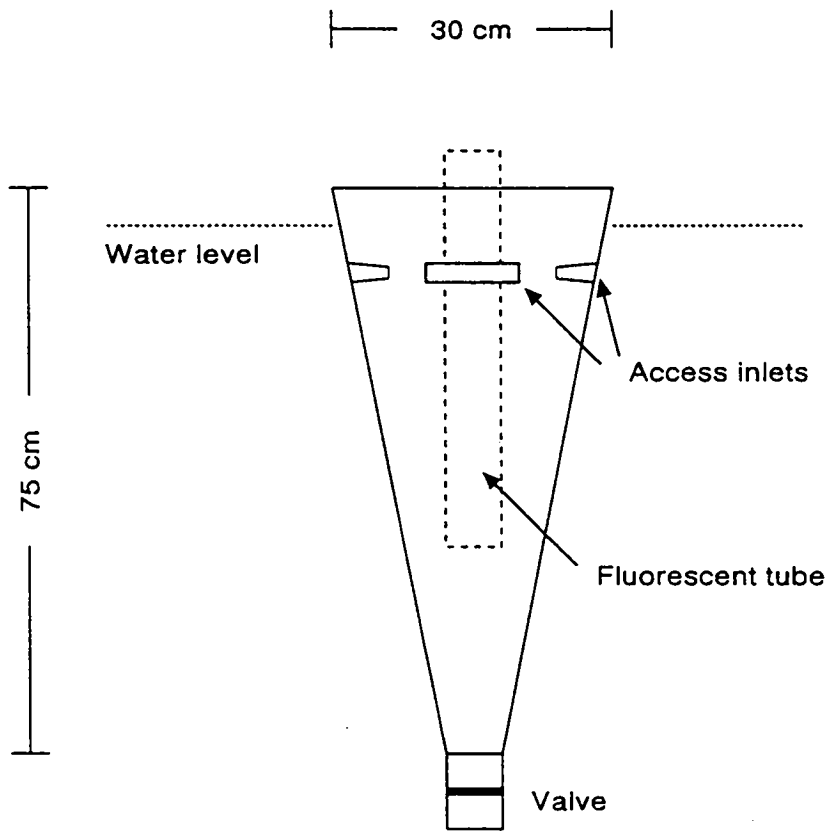


Figure 7.4 Diagram of light trap used to collect samples of larval and juvenile barramundi from the pond.

Harvesting

One to three days prior to harvesting, a length of perforated irrigation pipe ('WaterWik') connected to an air vane pump was placed in the raceway in order to maintain high dissolved oxygen levels in the pond during draining (Fig. 7.5a). The pond was drained slowly to avoid trapping fish on the outlet screen (Fernandez 1990). Harvesting usually took place during the night or early morning to avoid high daytime water temperatures and associated stress problems (Fernandez 1990). During the 1989-90 season (Trials P3-P4), harvesting took place by draining the pond down to the level of the raceway and then netting the fish from the drain end of the raceway. The pond was modified prior to the 1990-91 season by installing a concrete raceway which allowed a water flow down the raceway once the pond had been drained. Commencing with trial P7, a trap was placed in the raceway facing downstream (Fig. 7.5b), and a flow of water directed down the raceway. The barramundi juveniles swam 'upstream' into the current until they reached the trap and were then netted from the raceway, placed in aerated 20 litre plastic buckets, then transferred to 800 l transport tanks fitted to 1 tonne utility trucks (Fig. 7.5a).

When less than 2,000 fish were harvested from the pond, fish were directly counted to determine survival. In other cases, all fish were weighed, in batches, immediately after removal from the pond to provide a total weight of fish harvested. Five to ten samples of 50 fish were then weighed to provide an estimate of mean fish weight which was used to estimate the number of fish harvested from the pond. These samples were taken throughout the harvest

process to avoid any bias in the event that different size classes of fish were removed from the pond at different stages of the harvest.

Aquarium Observations

A glass aquarium (155 cm long, 45 cm wide, 38 cm high; capacity c. 250 litres) was used to observe the distribution of barramundi larvae in the water column, in an attempt to shed light on their possible distribution within the pond. The aquarium was filled with filtered seawater and fertilised with DAP and lucerne pellets according to the schedule listed in Table 7.1. Samples of water taken from the larval rearing pond at Mourilyan were added to the aquarium to provide an inoculum of phytoplankton and zooplankton. A 150 W metal halide light was placed above the aquarium and was switched on, using a timer, for 12 hours each day to stimulate phytoplankton growth. Black plastic was placed around the aquarium to prevent light penetrating from the sides, in order to more closely simulate the pond environment. Approximately 1,000 barramundi larvae (day 2 from hatching) were stocked into the aquarium on day 10.

Figure 7.5 Harvesting barramundi fingerlings from larval rearing pond.

(a) Pond draining for harvest; note aeration along raceway.

(b) Pond raceway showing harvesting trap in operation.

Statistical Analyses

Because of the large number of variables examined in these trials, a multivariate approach similar to that described by Pauly and Hopkins (1983) was used. Data from all pond trials were combined and subjected to multiple correlation and regression analysis using the statistical programs Statistix and Genstat. Where applicable, logistic regressions were undertaken using the maximum likelihood estimation procedures of the Statistix statistical program. This procedure transforms proportional data (such as survival) using the logit transformation: ln(p/(1-p)) where p is a proportion (Anon 1992). Other data transformations were undertaken where applicable, and these are noted in the Results and Discussion section.

Results and Discussion

Pond Trials

Trials P1 and P2 were carried out by W.P. Rutledge and the results are not included in this thesis, although summary data have been included in Tables 7.2 and 7.3 for comparative purposes. Details of these early trials were published by Rutledge and Rimmer (1991).

The results of extensive rearing trials P1 - P12 and water quality data for each trial are summarised in Tables 7.2 and 7.3 respectively. Water quality data for days 2-9 after hatching (i.e. the first week after stocking, when the larvae are presumably least tolerant to changes in water quality conditions) for pond trials P3 - P12 are summarised in Table 7.4. Zooplankton densities at stocking and maximum zooplankton densities for each trial, both factors which may influence larval survival, are listed in Table 7.5.

Table 7.2 Summary of survival and growth data for barramundi in pond trials P1-P12. Key to abbreviations: TL: Total Length; SGR: Specific Growth Rate.

Trial	Start	Finish	No. of larvae stocked	Stocking density (no/m^2)	No. of fish harvested	Survival	Mean TL (mm)	Growth (mm/day)	SGR (%/day)	Degree-days
P1*	Oct 89	Oct 89	50,000	37	0	0%	-	-	-	-
P2*	Nov 89	Dec 89	100,000	74	20,000	20%	38.8	1.6	-	-
P3	2 Jan 90	2 Feb 90	100,000	74	0	0%	-	-	-	-
P4	5 Feb 90	5 Mar 90	100,000	74	40,000	40%	35.5	1.6	12.8	677.4
P5	14 Nov 90	11 Dec 90	150,000	111	45,000	30%	23.6	1.1	11.4	615.2
P6	3 Jan 91	24 Jan 91	70,000	52	60	0.1%	11.4	0.7	12.0	451.8
P7	29 Jan 91	27 Feb 91	70,000	52	0	0%	-	-	-	-
P8	8 Oct 91	11 Nov 91	90,000	67	63,000	70%	28.8	1.1	10.3	697.5
P9	14 Nov 91	11 Dec 91	70,000	52	1,244	2%	21.5	1.3	12.4	589.2
P10	12 Dec 91	7 Jan 92	70,000	52	0	0%	-	-	-	-
P11	14 Jan 92	14 Feb 92	70,000	52	14,000	20%	40.6	1.7	12.8	677.5
P12	18 Feb 92	20 Mar 92	100,000	74	86,000	86%	33.2	1.4	11.7	659.7

*Data from Rutledge and Rimmer (1991).

Table 7.3 Summary of water quality and rainfall data for pond trials P1-P12. Missing water quality values are the result of equipment malfunction.

Trial	Survival	Temperature (°C)		Salinity (ppt)		Dissolved Oxygen (mg/l)		pH		Total Rainfall (mm)
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	
P1*	0%	25.2	28.7	26.0	28.0	7.1	13.7	7.7	8.3	-
P2*	20%	28.9	35.6	18.0	27.0	8.3	16.8	7.8	8.9	-
P3	0%	27.8	37.4	12.7	18.7	5.6	18.9	7.6	8.9	171
P4	40%	28.4	36.5	16.3	23.6	4.5	14.0	8.1	9.2	246
P5	30%	29.0	31.7	27.6	30.6	4.3	11.3	8.0	9.0	24
P6	0.1%	30.7	37.4	12.0	26.4	5.6	10.4	7.7	8.9	913
P7	0%	28.4	38.2	3.8	22.3	4.3	6.4	7.6	8.9	1,254
P8	70%	27.2	29.9	31.8	33.8	5.6	7.2	7.4	8.1	9
P9	2%	29.4	33.7	28.0	31.6	4.6	6.2	7.8	8.5	131
P10	0%	31.2	32.8	20.0	27.3	-	-	7.4	9.2	22
P11	20%	30.4	35.1	15.0	30.3	4.4	8.9	7.5	8.6	224
P12	86%	29.5	33.0	16.1	29.9	3.7	7.1	7.6	8.7	268

*Data from Rutledge and Rimmer (1991).

Table 7.4 Summary of water quality data recorded during the first week following stocking (i.e. days 2-9 from hatching) for pond trials P3-P12. Missing water quality values are the result of equipment malfunction.

Trial	Survival	Temperature (°C)		Salinity (ppt)		Dissolved Oxygen (mg/l)		pH	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
P3	0%	31.3	37.4	13.1	17.8	9.4	18.9	7.6	8.9
P4	40%	30.6	33.0	17.4	23.6	6.8	11.7	8.4	8.9
P5	30%	29.9	30.6	27.6	28.3	5.7	9.5	8.1	8.7
P6	0.1%	32.8	34.5	13.4	16.9	8.4	10.4	8.1	8.5
P7	0%	36.7	38.2	19.4	22.3	4.3	6.1	8.0	8.9
P8	70%	28.1	29.0	31.8	32.3	5.8	7.2	7.6	8.1
P9	2%	30.6	33.7	28.6	31.0	-	-	8.1	8.5
P10	0%	31.5	32.8	20.0	24.1	-	-	8.5	8.7
P11	20%	30.4	31.2	27.3	30.3	4.4	4.8	8.2	8.6
P12	86%	29.5	31.7	22.3	24.8	4.9	5.6	7.6	7.9

Table 7.5 Day of stocking, zooplankton densities at stocking and maximum zooplankton density in pond trials P3-P12. Numbers in square brackets indicate the day on which maximum zooplankton densities was recorded. Data from trials P3 and P4 are from 25μm net samples; data from trials P5 to P12 are from vertical tube samples. Note that rotifer densities were not estimated in pond trial P3.

Trial	Survival	Stocking				Maximum		
		Day	Zooplankton Density (no./l)			Zooplankton Density (no./l) [day]		
			Nauplii	Rotifers	Copepods	Nauplii	Rotifers	Copepods
P3	0%	17	39	-	24	198 [30]	-	148 [8]
P4	40%	8	177	241	32	410 [9]	2,334 [16]	245 [16]
			>37μm	>100μm	>250μm	>37μm	>100μm	>250μm
P5	30%	8	636	94	1	2,659 [13]	915 [13]	7 [20]
P6	0.1%	9	3,263	463	8	3,263 [9]	1,156 [7]	59 [14]
P7	0%	11	735	1,108	21	2,103 [4]	1,108 [11]	170 [18]
P8	70%	10	148	450	86	1,897 [18]	3,548 [16]	86 [10]
P9	2%	8	92	101	3	611 [12]	510 [16]	17 [14]
P10	0%	8	143	259	2	4,133 [16]	6,003 [16]	80 [26]
P11	20%	10	4,283	2,673	24	9,813 [21]	10,871 [18]	433[25]
P12	86%	10	1,981	1,769	11	4,218 [7]	4,036 [7]	202 [18]

Trial P3: 2 January - 2 February 1990

Water quality data for this trial indicate that salinity was low throughout the trial, ranging from 12.7 to 18.7 ppt (Fig. 7.6). This was caused by heavy rainfall prior to the trial which substantially diluted the salt water used to fill the pond. Cloud cover was heavy throughout the trial (Fig. 7.7) and rainfall totalled 171 mm for the duration of this trial (Table 7.3, Fig. 7.7). Water temperature ranged from 27.8 to a peak of 37.4°C on day 18 (Fig. 7.6); corresponding air temperatures are shown in Figure 7.7. The extremely high water temperatures recorded on days 18 and 20, 37.4°C and 36.0°C respectively, may have resulted from measurement error or instrument malfunction. There appears to be no particular reason for the drastic and rapid temperature changes which occurred between days 17 and 21 in this trial. Daily water temperature values generally exceeded air temperature at 1500 h, and this same pattern appeared consistently during other pond trials. Evaporative cooling is an important heat loss mechanism for water bodies, and the consistently high humidity of the Mourilyan district may have reduced evaporative cooling, leading to retention of heat by the pond water.

Dissolved oxygen remained high throughout this trial, ranging from 5.6 to 18.9 ppt, and pH ranged from 7.6 to 8.9 (Fig. 7.6).

Peak densities of copepod nauplii were expected to occur around day 14 (Rutledge and Rimmer 1991), so 100,000 barramundi larvae were stocked into the pond on day 17 to coincide with this expected peak. However, in this trial the density of copepod nauplii peaked at or before day 8 (Fig. 7.8), then declined before beginning a gradual increase towards the end of the trial. The density of

copepodites and copepod adults peaked at about the same time, then decreased from day 15 and remained at low density for the rest of the trial. Cladocerans increased in density from day 15 to day 17, then decreased before peaking a second time around day 25. Rotifer densities were not measured in this trial. Increases in Secchi disk visibility were associated with the peaks in cladoceran density (Fig 7.8), suggesting that phytoplankton density was particularly affected by cladoceran predation.

No barramundi survived in this trial. Factors which may have contributed to the mortality of barramundi in this trial are: high water temperature (31.3 to 37.4°C) and low salinity (13.1 to 17.8 ppt) during the first week after stocking (Table 7.4) and low densities of zooplankton at stocking (Table 7.5).

Figure 7.6 Water quality parameters for pond trial P3 (2 January - 2 February 1990): temperature, salinity, dissolved oxygen and pH sampled from near the bottom of the pond each morning. The water temperature values for days 18 and 20 appear to be anomalous, possibly due to instrument error. The day of stocking is marked with an arrow.

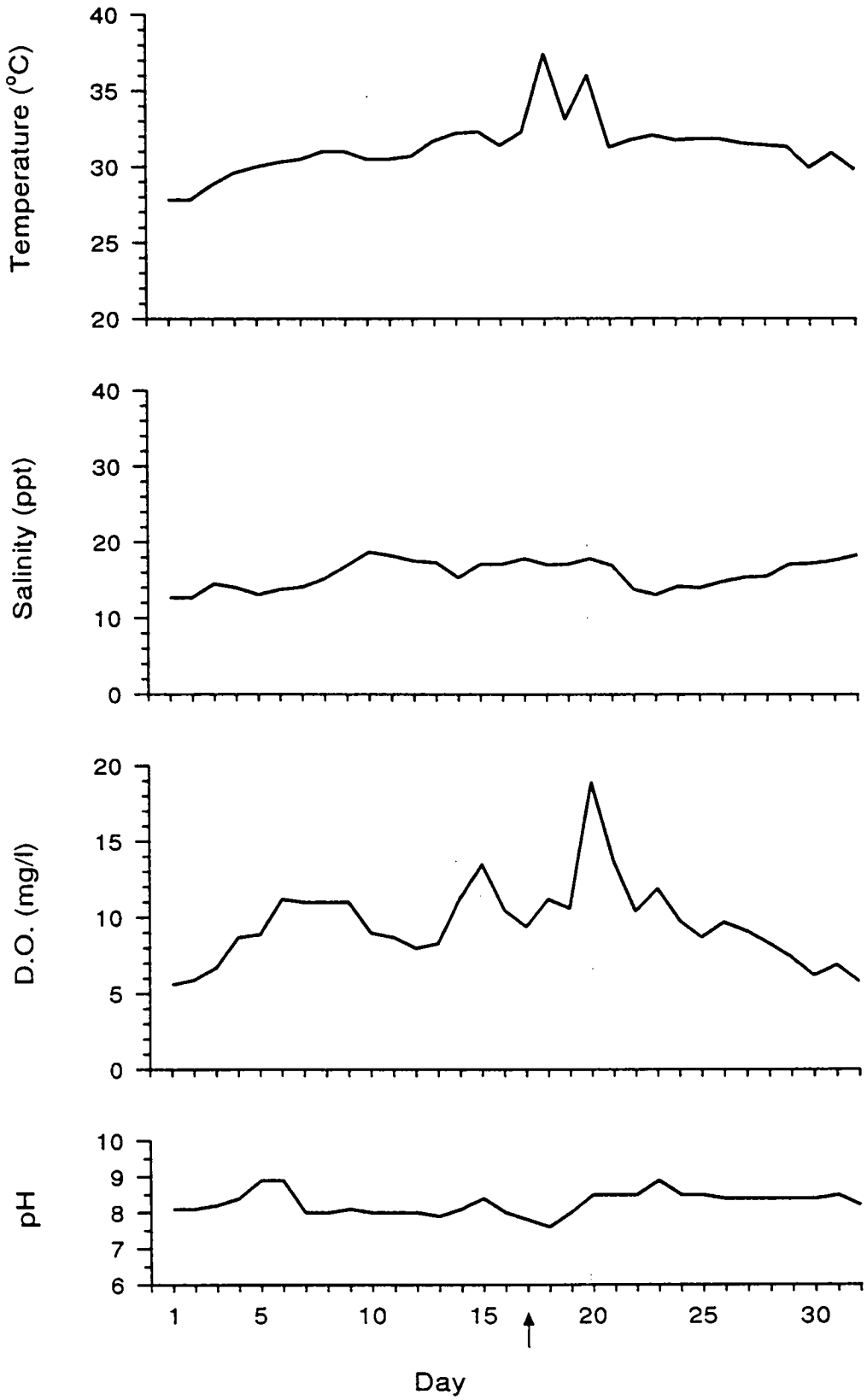

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
30
Day

Figure 7.7 Air temperature at 0300 and 1500 h, cloud cover at 1500 h and daily rainfall for Innisfail during pond trial P3 (2 January - 2 February 1990). The day of stocking is marked with an arrow.

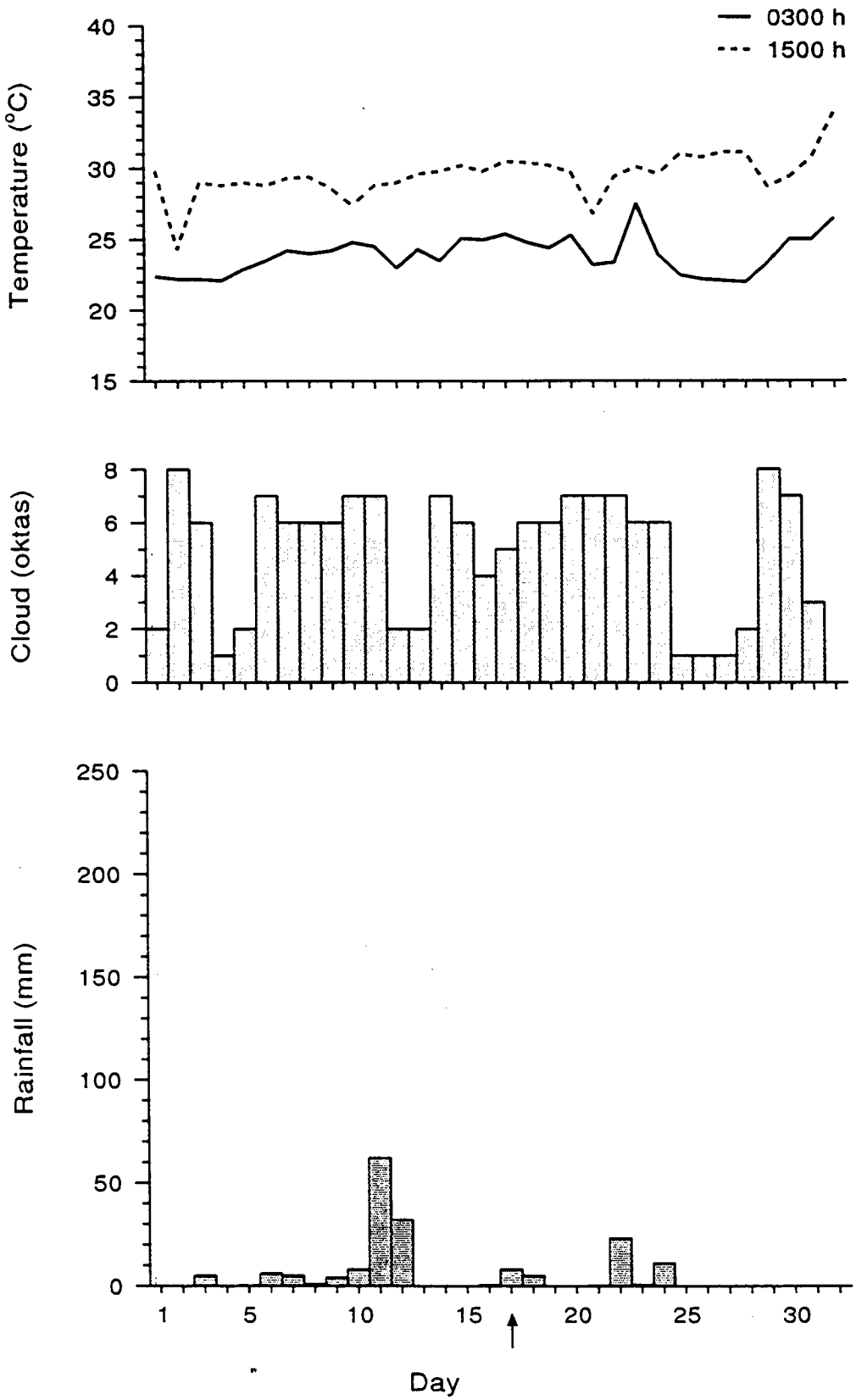
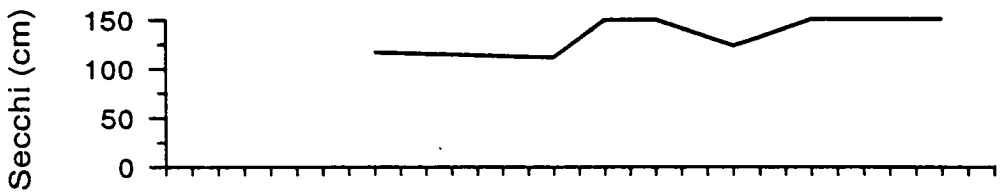
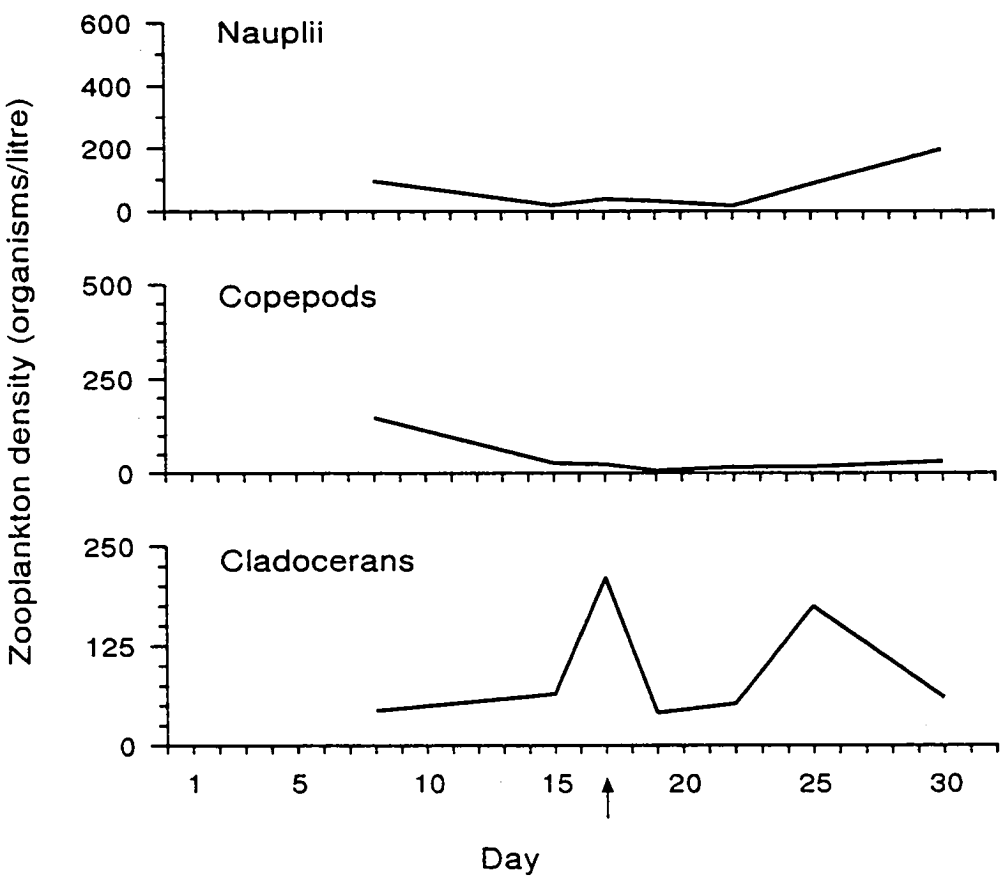

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
30
Day

Figure 7.8 Secchi disk visibility and densities of copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P3 (2 January - 2 February 1990). Rotifer densities were not measured during this trial. The day of stocking is marked with an arrow.


Secchi (cm)
150
100
50
0


Zooplankton density (organisms/litre)
Nauplii
600
400
200
0
Copepods
500
250
0
Cladocerans
250
125
0
1
5
10
15
20
25
30
Day

Trial P4: 5 February - 5 March 1990

Salinities recorded during this trial were consistently higher than those experienced in the previous trial, ranging from 16.3 to 23.6 ppt throughout the trial (Fig. 7.9) and 17.4 to 23.6 ppt during the first week after stocking (Table 7.4). Temperature ranged from 28.4 to a peak of 36.5°C on day 16 (Fig. 7.9). Dissolved oxygen ranged from 4.5 to 14.0 ppt and pH from 8.1 to 9.2 during the trial (Table 7.3, Fig. 7.9).

Cloud cover was light for the first 2 weeks of this trial, with no rainfall recorded over that period (Fig. 7.10). During the second 2 weeks of the trial cloud cover was heavier and the 246 mm of rainfall recorded during this trial fell during this time (Fig. 7.10).

Densities of copepod nauplii peaked at day 9 during this trial, while densities of rotifers, copepodites and copepod adults, and cladocerans peaked simultaneously on day 16 (Fig. 7.11). Secchi disk visibility increased substantially following the decline in zooplankton densities which occurred after about day 20 (Fig. 7.11) and remained high for the rest of the trial. Densities of all zooplankton groups were very low from day 23 onwards (Fig. 7.11).

Because densities of copepod nauplii peaked on or before day 8 in the previous trial, the day of stocking was brought forward to coincide with this expected peak density. For this trial 100,000 barramundi larvae were stocked into the pond on day 8. Larvae were first captured on day 18 using a lift net, and were subsequently readily sampled using the same technique. Growth rates averaged 1.6 mm/day over the duration of the trial, but growth in the period from day 18

to day 26 was much faster (2.4 mm/day) than that from day 8 (when the larvae were stocked) to day 18 (1.1 mm/day) (Fig. 7.12a). Growth from day 26 to day 29 (when the fish were harvested) was markedly less than that of the previous 8 days (1.1 mm/day), presumably due to the low densities of zooplankton prey available during this period (Fig. 7.11). Specific growth rate decreased steadily from 17.2 %/day in the period from day 8 (day 2 from hatching) to day 18 (day 12 from hatching) to 3.1 %/day in the period from day 26 to day 29 (Fig. 7.12b); overall SGR for this trial was 12.8 %/day (Table 7.2).

Approximately 40,000 juvenile barramundi were harvested at the end of this trial, a survival rate of 40%. Harvested fish averaged 35.5 mm TL (range 22.0 - 48.5 mm TL) (Fig. 7.12c) at day 23 from hatching (677.4 degree-days).

Figure 7.9 Water quality parameters for pond trial P4 (5 February - 5 March 1990): temperature, salinity, dissolved oxygen and pH sampled from near the bottom of the pond each morning. The day of stocking is marked with an arrow.

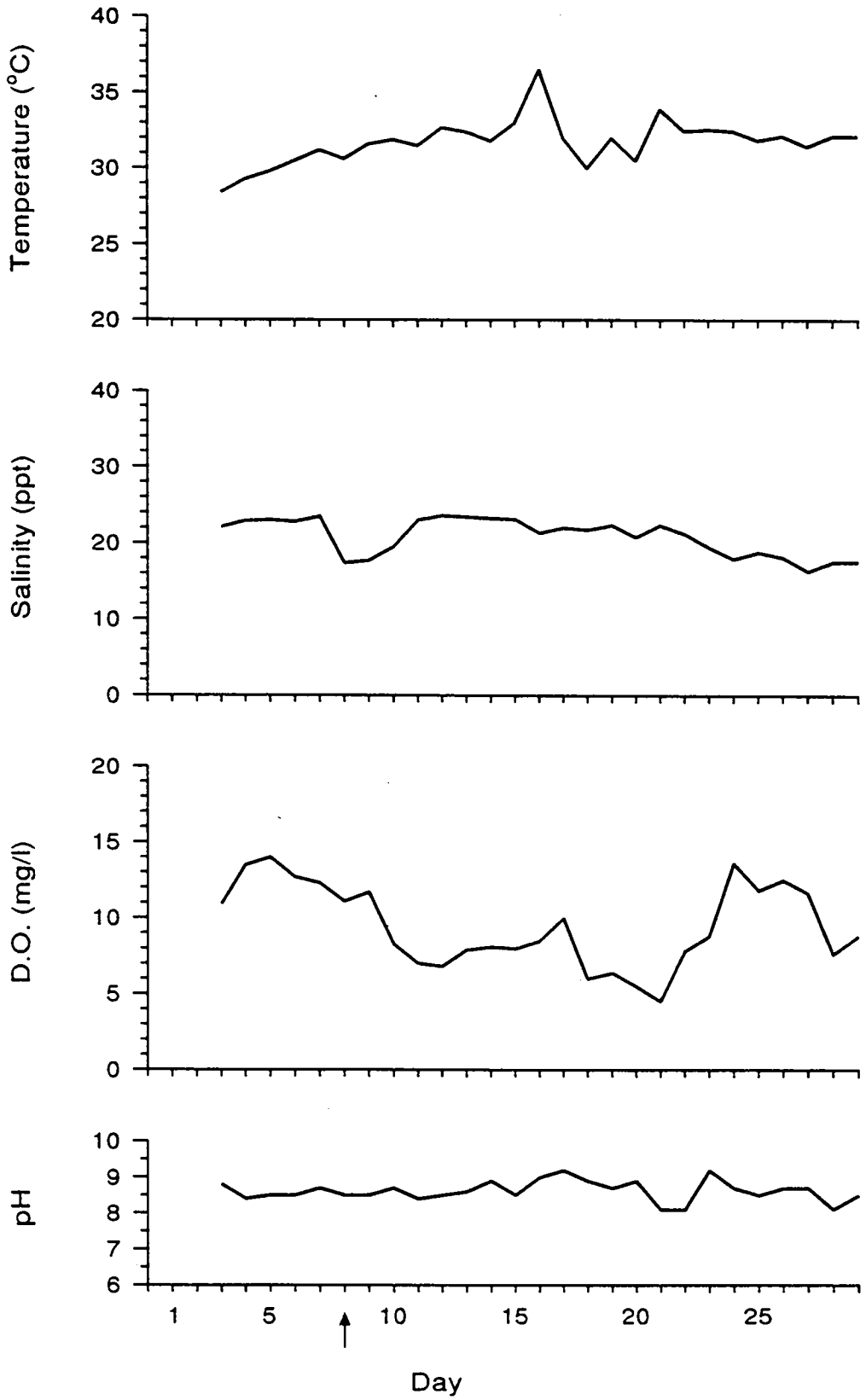

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
Day

Figure 7.10 Air temperature at 0300 and 1500 h, cloud cover at 1500 h and daily rainfall for Innisfail during pond trial P4 (5 February - 5 March 1990). The day of stocking is marked with an arrow.

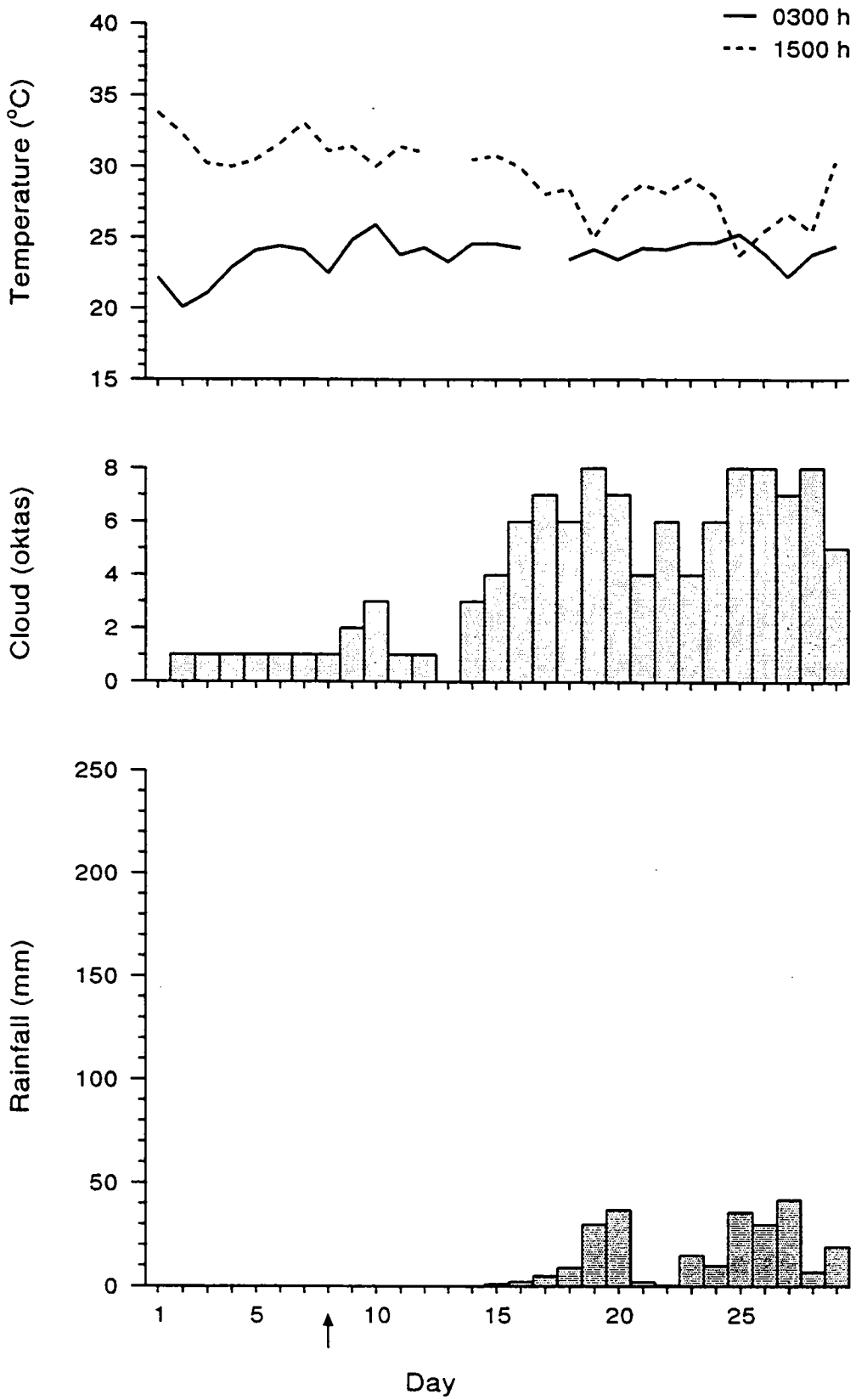

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
Day

Figure 7.11 Secchi disk visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P4 (5 February - 5 March 1990). The day of stocking is marked with an arrow.

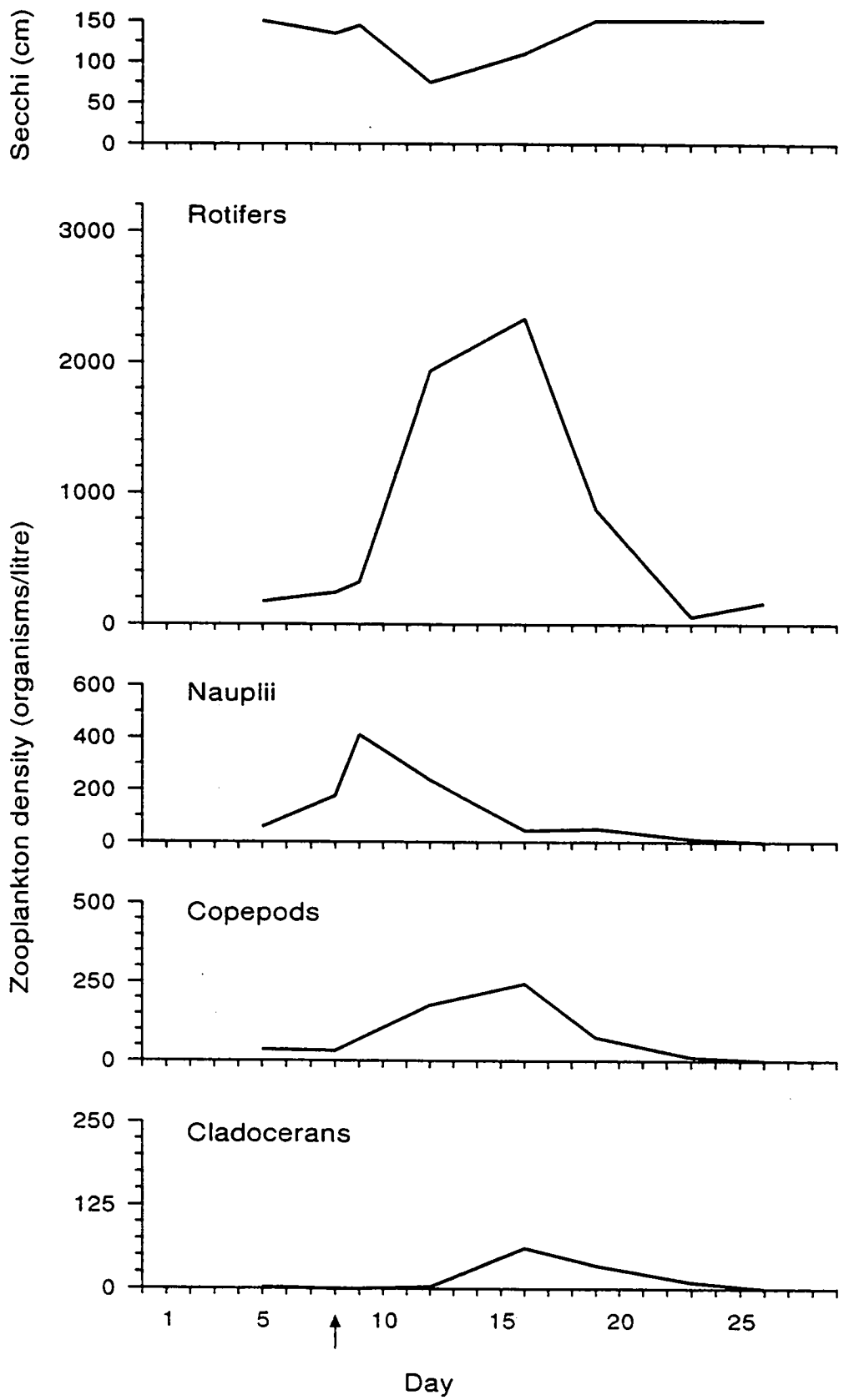
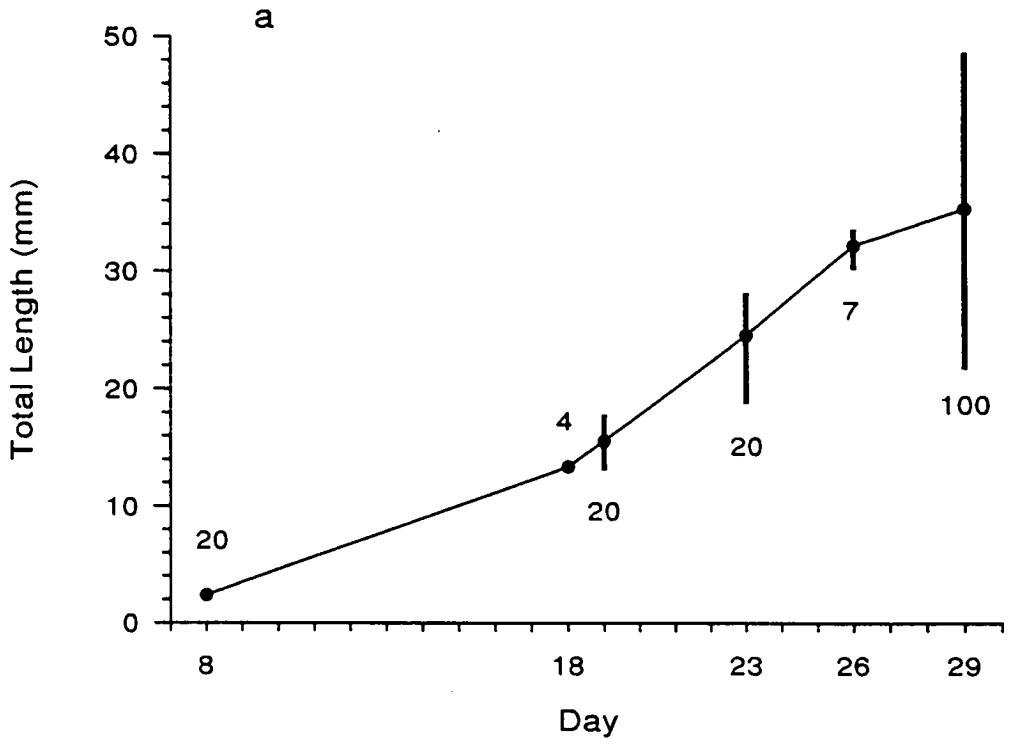
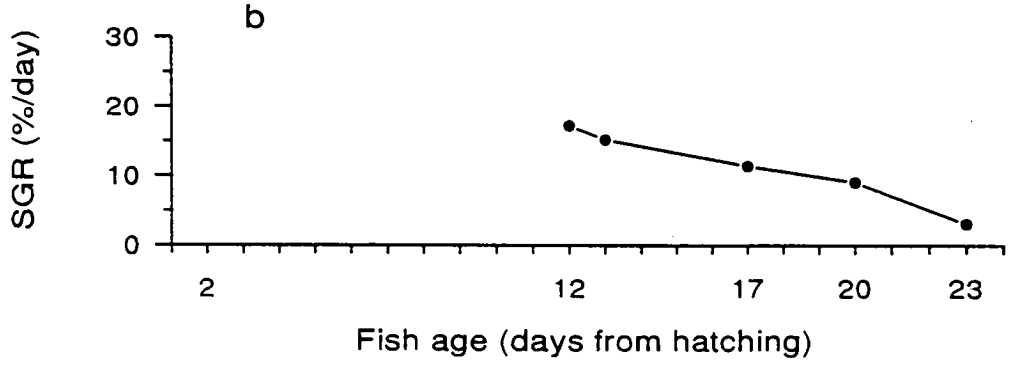
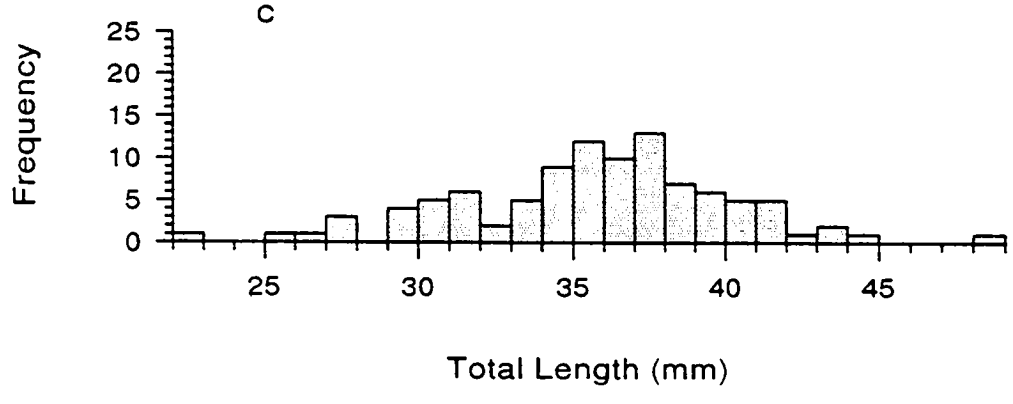

Secchi (cm)
150
100
50
0
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
500
250
0
Cladocerans
250
125
0
Zooplankton density (organisms/litre)
1
5
10
15
20
25
Day

Figure 7.12 (a) Growth of barramundi during pond trial P4 (5 February - 5 March 1990). Mean and range of each sample shown; numbers above or beneath bars represent sample size. (b) Specific growth rate (SGR) of barramundi during pond trial P4. (c) Size frequency of barramundi harvested at the end of pond trial P4 (n=100).


a
Total Length (mm)
50
40
30
20
10
0
20
4
20
20
7
100
8
18
23
26
29
Day


b
SGR (%/day)
30
20
10
0
2
12
17
20
23
Fish age (days from hatching)


c
Frequency
25
20
15
10
5
0
25
30
35
40
45
Total Length (mm)

Trial P5: 14 November - 11 December 1990

Temperature and salinity were relatively stable during the course of this trial, ranging from 29.0 to 31.7°C and 27.6 to 30.6 ppt respectively (Fig. 7.13). Dissolved oxygen ranged from 4.3 to 11.3 mg/l and pH from 8.0 to 9.0 during the trial (Table 7.3).

Cloud cover was generally light throughout the trial (Fig. 7.14) and a total of only 24 mm of rainfall was recorded (Table 7.3, Fig. 7.14).

Data from the vertical tube samples indicated that the smallest zooplankton size class ($>37\mu$m) peaked at day 13 (Fig. 7.15). This size class was composed mainly of rotifers and copepod nauplii (Fig. 7.16). The other zooplankton size classes reached maximal densities from day 10 to day 13 ($>100\mu$m) and day 15 to day 20 ($>250\mu$m). Both these size classes were composed mainly of copepods (copepodites and copepod adults) (Fig. 7.16). The $>37\mu$m and $>100\mu$m size classes of zooplankton were found in much higher densities than the larger size classes (Fig. 7.15). Densities of all zooplankton groups were very low from day 22 onwards (Fig. 7.15). Secchi disk visibility generally decreased when zooplankton densities were low (Fig. 7.15), suggesting that zooplankton grazing of phytoplankton was a major factor affecting phytoplankton density.

150,000 barramundi larvae were stocked into the pond on day 8. Zooplankton densities in the smaller size classes ($>37\mu$m and $>100\mu$m) were low at stocking (636 and 94 organisms/litre respectively [Table 7.5]) but increased rapidly immediately after stocking (Fig. 7.15). Larvae were first captured on day 17 using lift nets. Growth rates averaged 1.1 mm/day over the duration of the trial,

with faster growth in the period from day 17 to day 24 (2.0 mm/day) than from day 8 (when the larvae were stocked) to day 17 (0.8 mm/day) (Fig. 7.17a). There was no increase in total length of barramundi from day 24 to day 28 (when the fish were harvested), during which time zooplankton densities were extremely low (Fig. 7.15). Specific growth rate in the period from day 8 (day 2 from hatching) to day 17 (day 11 from hatching) was 15.8 %/day; it then decreased steadily to 2.7 %/day by harvest (Fig. 7.17b); overall SGR for this trial was 11.4 %/day (Table 7.2).

The density of barramundi found on the lift nets averaged 45.1 fish/m^2 during the course of the trial, giving a predicted population of 60,000 fish in the pond. Approximately 45,000 juvenile barramundi were harvested at the end of this trial, a survival rate of 30%. Harvested fish averaged 23.6 mm TL (range 17.0 - 31.4 mm TL) (Fig. 7.17c) at day 22 from hatching (615.2 degree-days).

Figure 7.13 Water quality parameters for pond trial P5 (14 November - 11 December 1990): daily mean temperature and salinity sampled hourly from near the pond bottom; dissolved oxygen sampled from near the pond bottom each morning; and pH of the water sample used for plankton density estimates. The day of stocking is marked with an arrow.

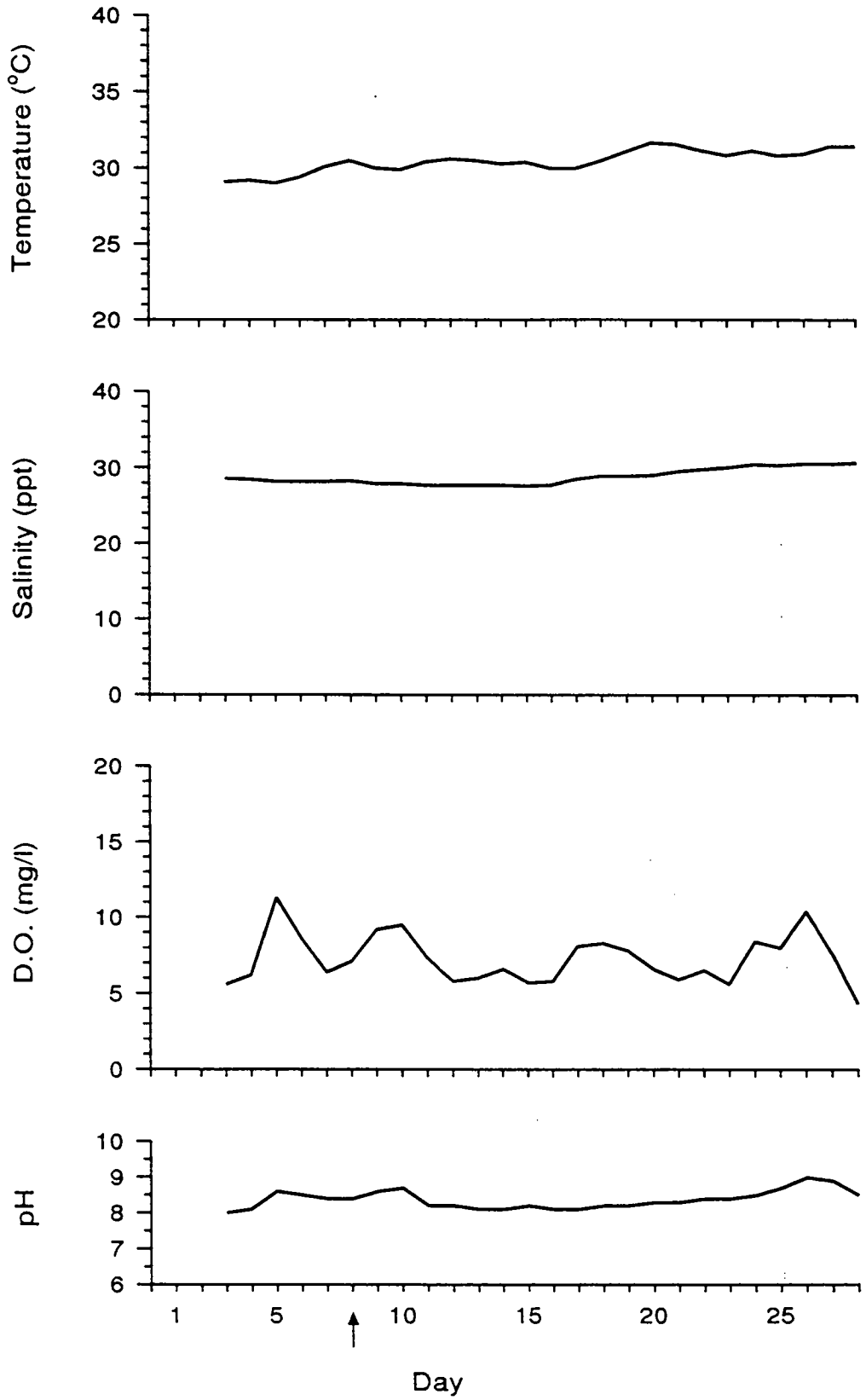

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
Day

Figure 7.14 Air temperature at 0300 and 1500 h, cloud cover at 1500 h and daily rainfall for Innisfail during pond trial P5 (14 November - 11 December 1990). The day of stocking is marked with an arrow.

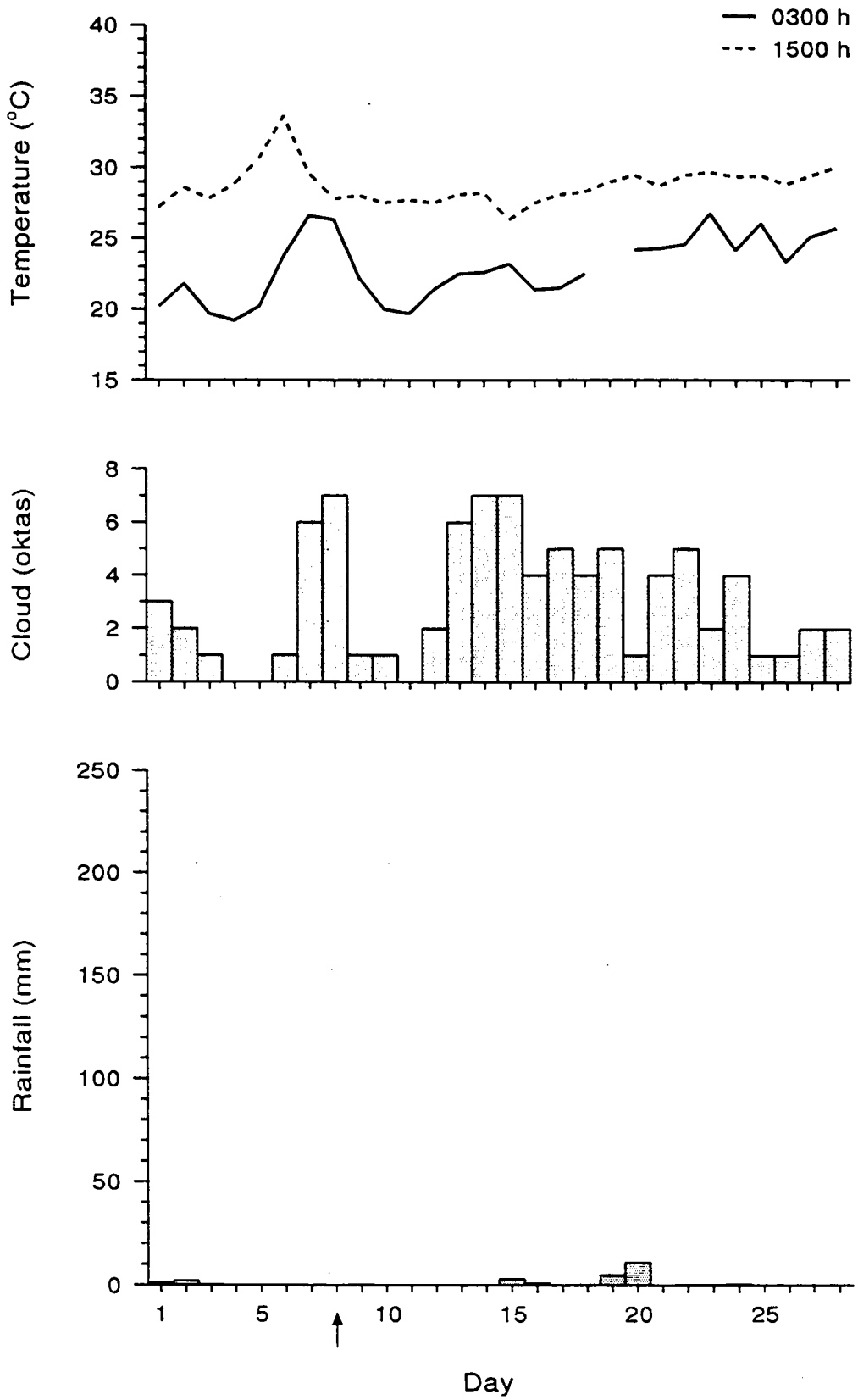

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
Day

Figure 7.15 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P5 (14 November - 11 December 1990). The day of stocking is marked with an arrrow.

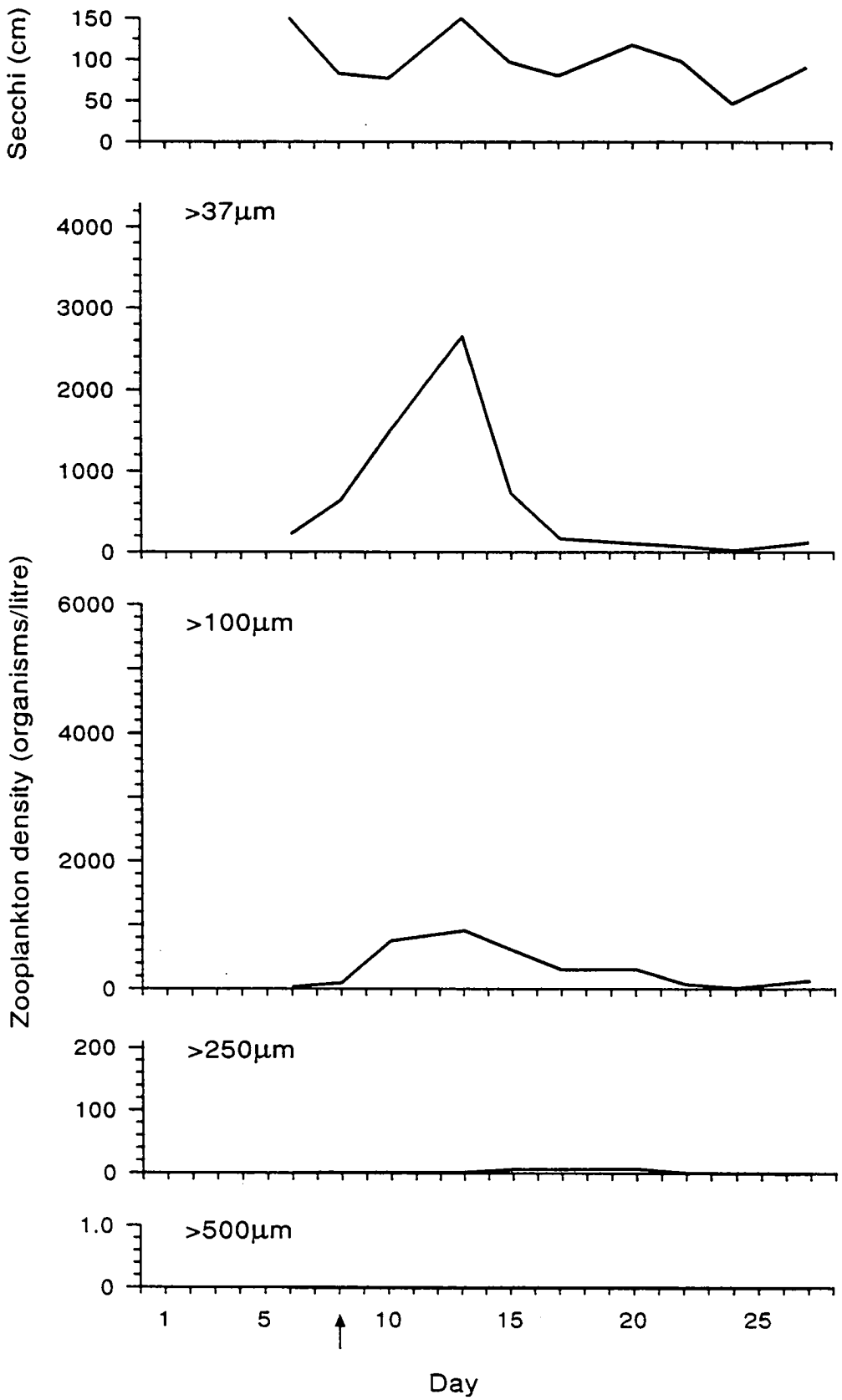

Secchi (cm)
150
100
50
0
>37µm
4000
3000
2000
1000
0
Zooplankton density (organisms/litre)
>100µm
6000
4000
2000
0
>250µm
200
100
0
>500µm
1.0
0
1
5
10
15
20
25
Day

Figure 7.16 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other zooplankton in sieved samples (Fig. 7.15) from pond trial P5 (14 November - 11 December 1990). The day of stocking is marked with an arrrow.

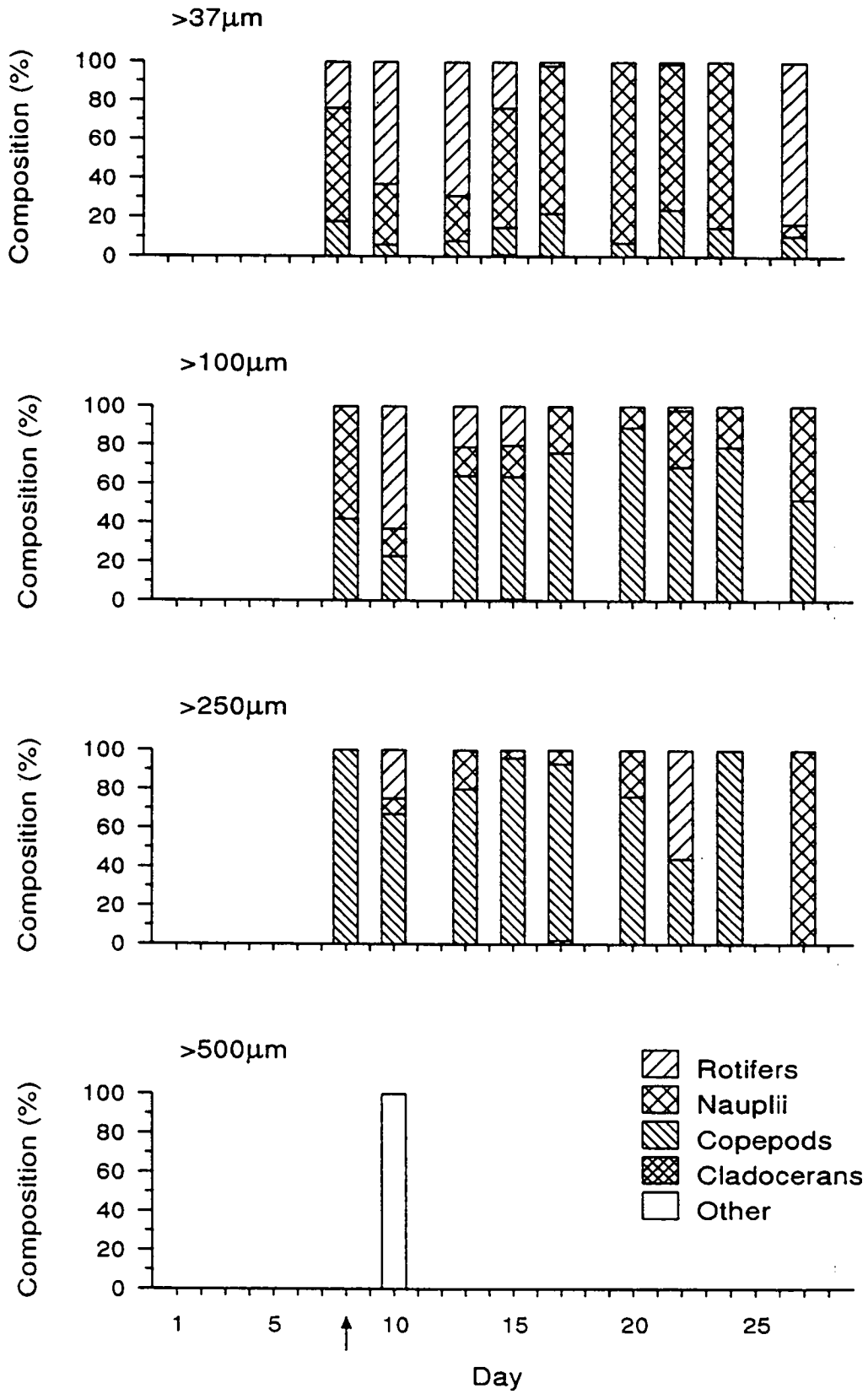
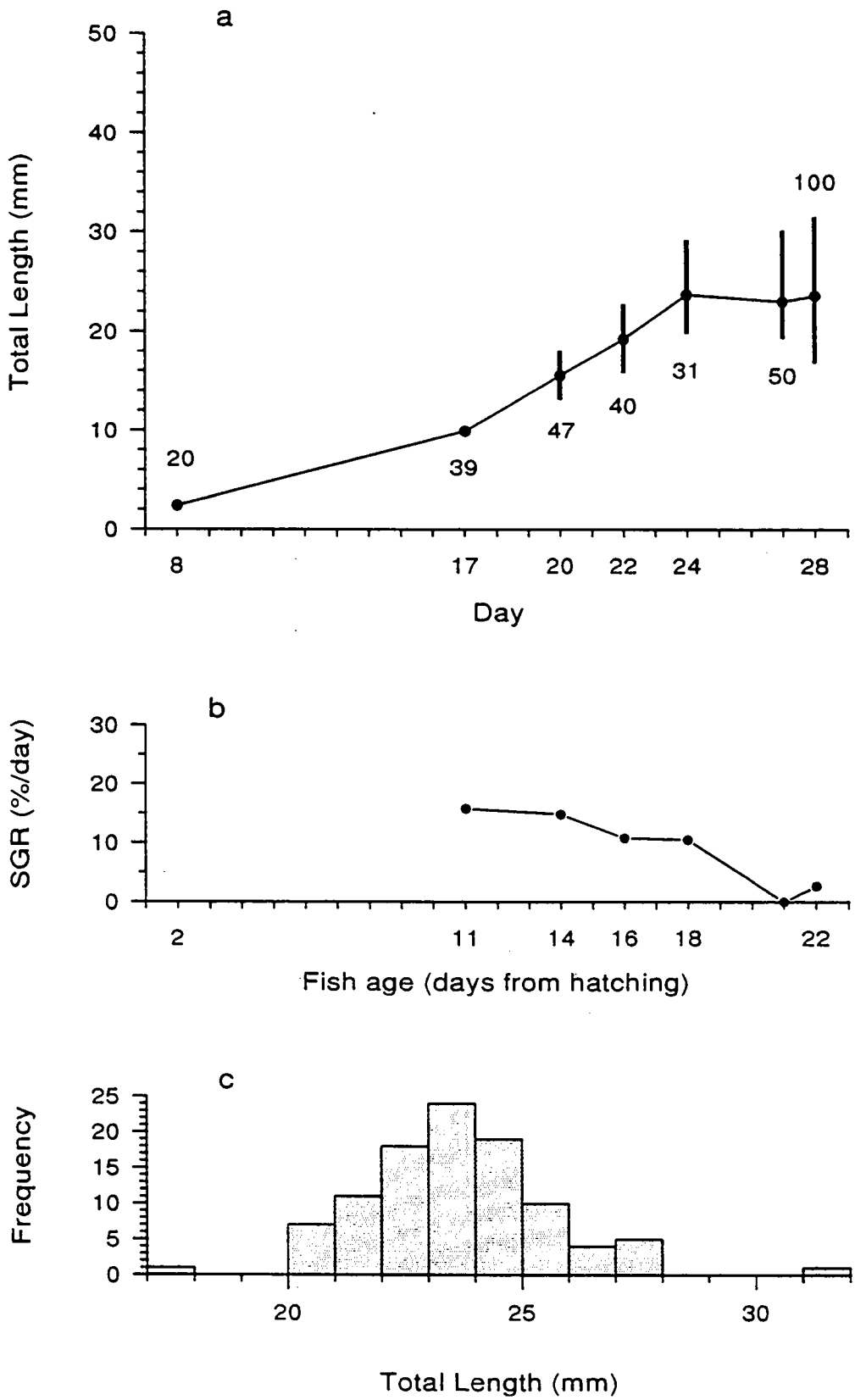

>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Other
1
5
10
15
20
25
Day

Figure 7.17 (a) Growth of barramundi during pond trial P5 (14 November - 11 December 1990). Mean and range of each sample shown; numbers above or beneath bars represent sample size. (b) Specific growth rate (SGR) of barramundi during pond trial P5. (c) Size frequency of barramundi harvested at the end of pond trial P5 (n=100).


a
Total Length (mm)
50
40
30
20
10
0
20
39
47
40
31
50
100
8
17
20
22
24
28
Day
b
SGR (%/day)
30
20
10
0
2
11
14
16
18
22
Fish age (days from hatching)
c
Frequency
25
20
15
10
5
0
20
25
30
Total Length (mm)

Trial P6: 3 January - 24 January 1991

Water temperature increased steadily during this trial, from 30.7°C at the beginning of the trial to 37.4°C by day 22 when the trial was terminated (Fig. 7.18). Cloud cover was heavy throughout the trial (Fig. 7.19) and a total of 913 mm of rain was recorded during this trial (Table 7.3) with heavy rainfall occurring from day 8 to day 15 (Fig. 7.19). This heavy rainfall resulted in a thin (<30 cm) layer of freshwater forming at the surface of the pond. The increase in water temperature throughout the trial may have been caused by the same phenomenon observed during pond trial P7. However, the drastic differences in water temperature between the two layers which was recorded in P7 were not recorded in P6; the maximum temperature difference between the two layers in this trial was only 2.1°C and was usually less than 1.0°C.

Bottom salinity decreased steadily from 26.4 ppt at the beginning of the trial to 12.0 ppt at harvest (Fig. 7.18). Surface salinity decreased rapidly from 12.5 ppt on day 7 to 2.8 ppt on day 9 and remained low at 1.4-3.0 ppt for the rest of the trial (Fig. 7.18). Dissolved oxygen ranged from 5.6 to 10.4 mg/l and pH from 7.7 to 8.9 during the trial (Table 7.3, Fig. 7.18).

Data from the vertical tube samples (Fig. 7.20) indicated that the smallest zooplankton size class (>37μm) peaked at day 9, and the >100μm size class at day 7. These two size classes were mainly composed of rotifers and copepod nauplii (Fig. 7.21). The >250μm size class, which comprised mainly copepodites, copepod adults and cladocerans (Fig. 7.21), peaked at day 14 (Fig. 7.20). The largest size class (>500μm) was made up of copepod adults and

cladocerans found only at extremely low densities (Fig. 7.20). As in previous trials, the smaller size classes of zooplankton were found in much higher densities than the larger size classes (Fig. 7.20). Secchi disk visibility generally increased when zooplankton densities were high (Fig. 7.20).

70,000 barramundi larvae were stocked into the pond on day 9. Zooplankton densities in the smaller size classes were high at stocking: 3,263 and 463 organisms/litre for the $>37\mu m$ and $>100\mu m$ size classes respectively (Table 7.5). Salinity was low during the first week after stocking, ranging from 13.4 to 16.9 ppt (Table 7.4); temperature ranged from 32.8 to 34.5°C over the same period. Although some barramundi were captured on the lift nets after day 16, the low sample sizes available precluded any estimates of density. Due to the evident poor survival of fish in this trial, the pond was harvested early (day 22) to enable it to be prepared for a further rearing trial. A total of 60 fish were harvested at the end of this trial, representing a survival rate of only 0.1%. Harvested fish averaged 11.4 mm TL (range 8.0 - 18.0 mm TL) (Fig. 7.22) at day 15 from hatching (451.8 degree-days), representing a mean growth rate of 0.7 mm/day and a specific growth rate of 12.0 %/day (Fig. 7.22b).

Factors which may have contributed to the poor survival of barramundi in this trial were the low salinity and moderately high water temperature immediately after stocking (Table 7.4). The high densities of zooplankton at stocking suggest that food availability was not a factor in the poor survival of barramundi in this trial.

Figure 7.18 Water quality parameters for pond trial P6 (3 January - 24 January 1991). Solid lines: daily mean temperature and salinity sampled hourly from near the pond bottom; dissolved oxygen sampled from near the pond bottom each morning; and pH of the water sample used for plankton density estimates. Broken lines: surface salinity sampled opportunistically throughout the trial. Missing data are the result of equipment malfunction. The day of stocking is marked with an arrow.

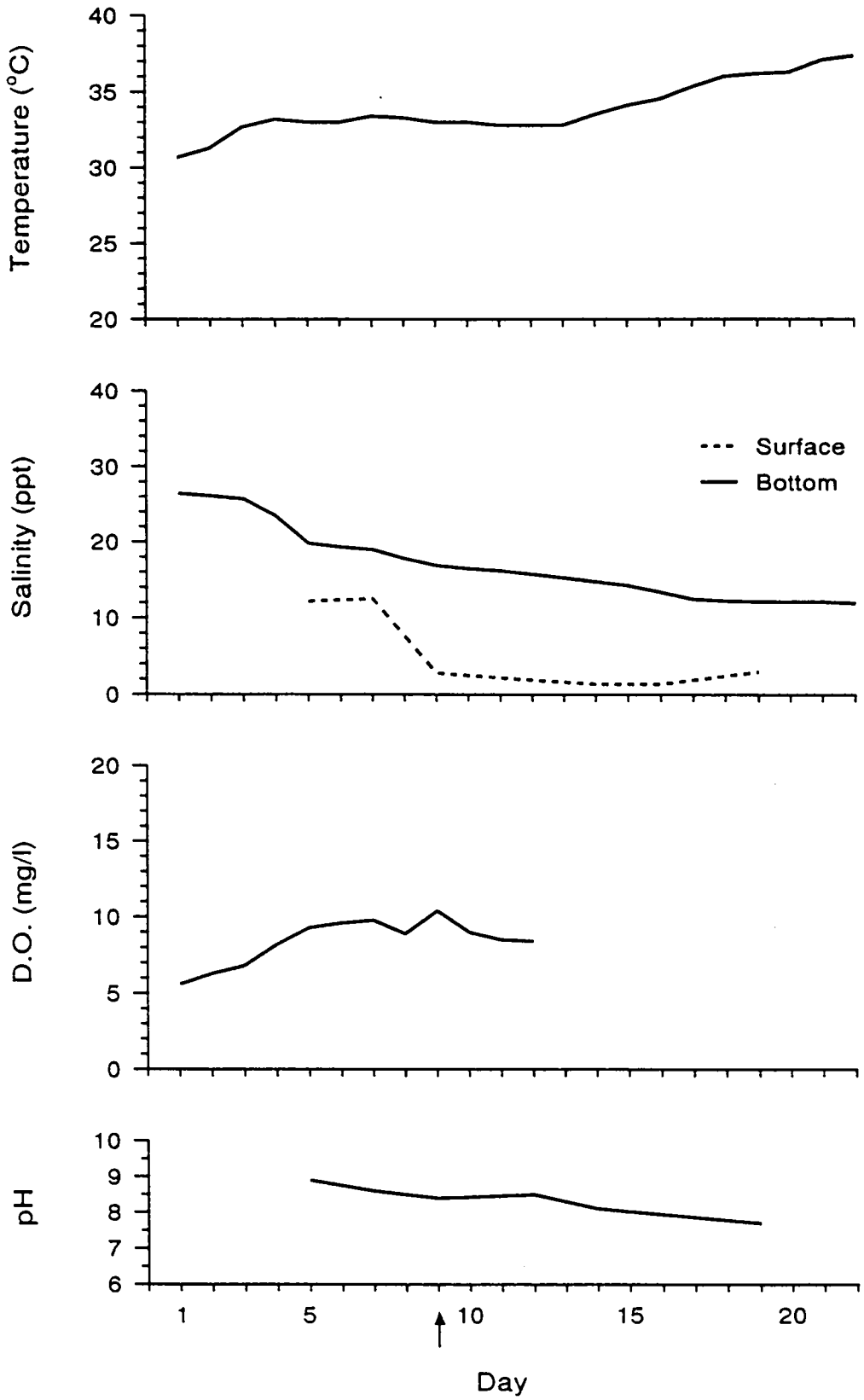

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
Surface
Bottom
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
Day

Figure 7.19 Air temperature at 0300 and 1500 h, cloud cover at 1500 h and daily rainfall for Innisfail during pond trial P6 (3 January - 24 January 1991). The day of stocking is marked with an arrow.

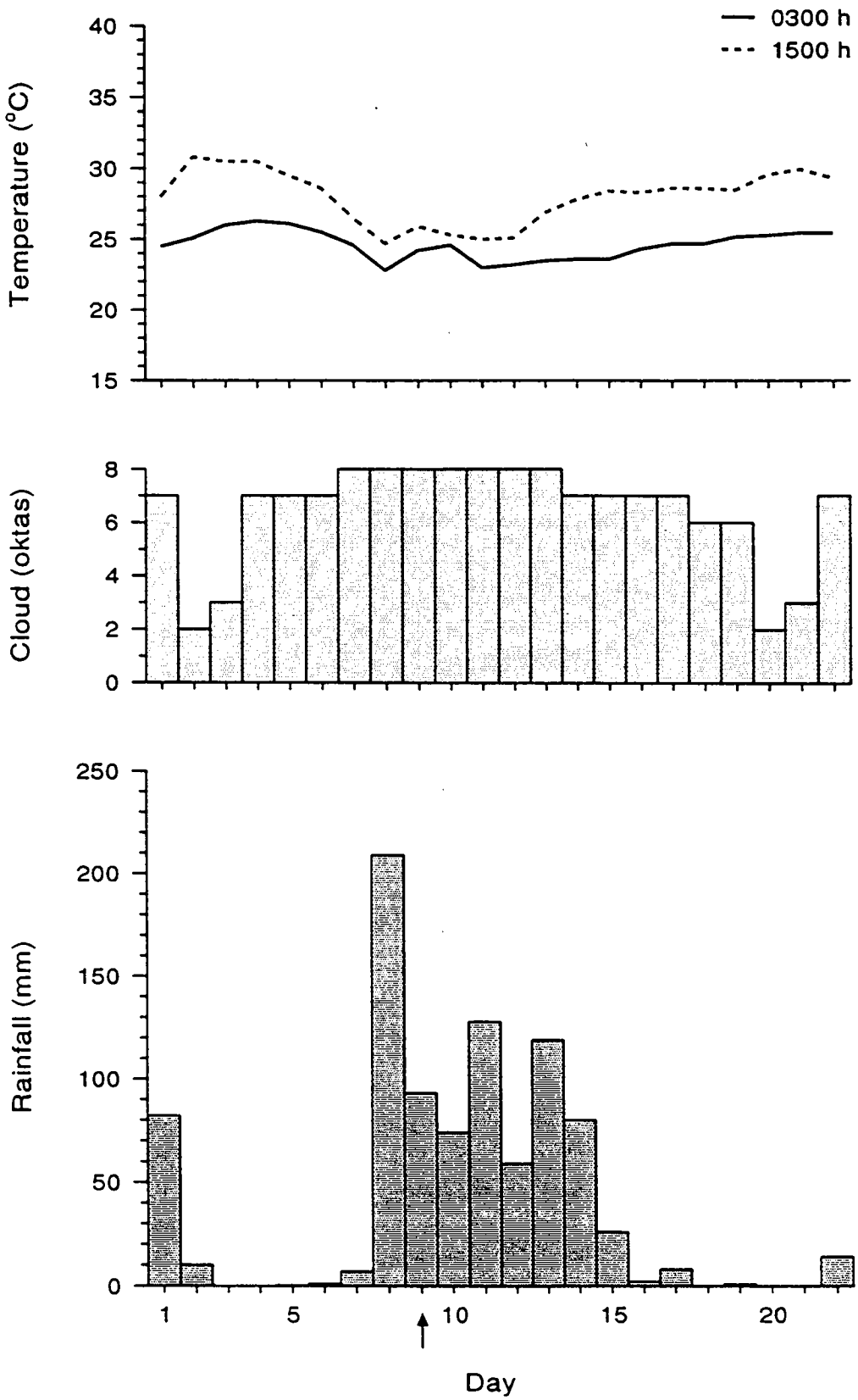

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
Day

Figure 7.20 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P6 (3 January - 24 January 1991). The day of stocking is marked with an arrow.

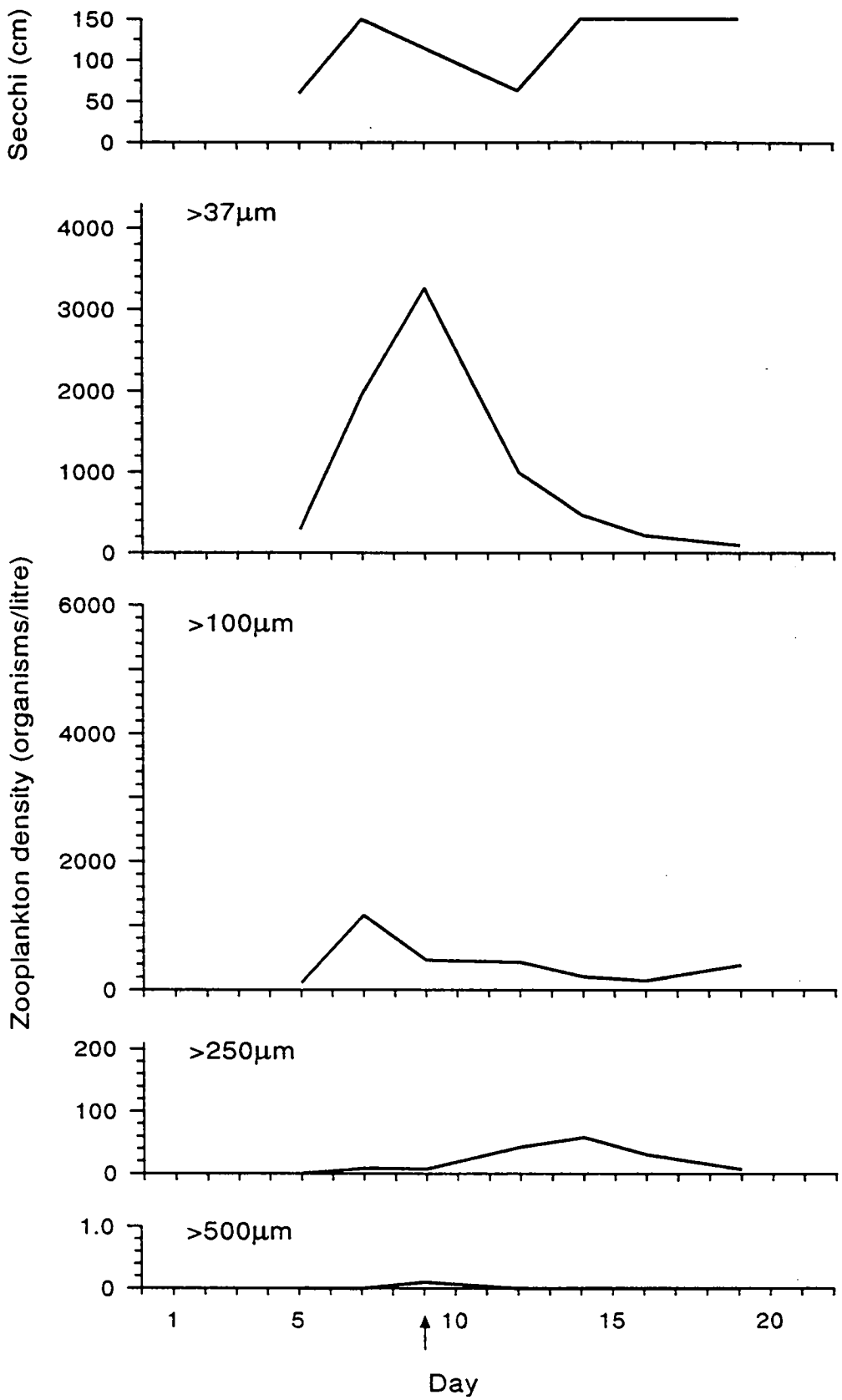

Secchi (cm)
150
100
50
0
>37µm
4000
3000
2000
1000
0
Zooplankton density (organisms/litre)
>100µm
6000
4000
2000
0
>250µm
200
100
0
>500µm
1.0
0
1
5
10
15
20
Day

Figure 7.21 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other organisms in sieved samples (Fig. 7.20) from pond trial P6 (3 January - 24 January 1991). The day of stocking is marked with an arrow.

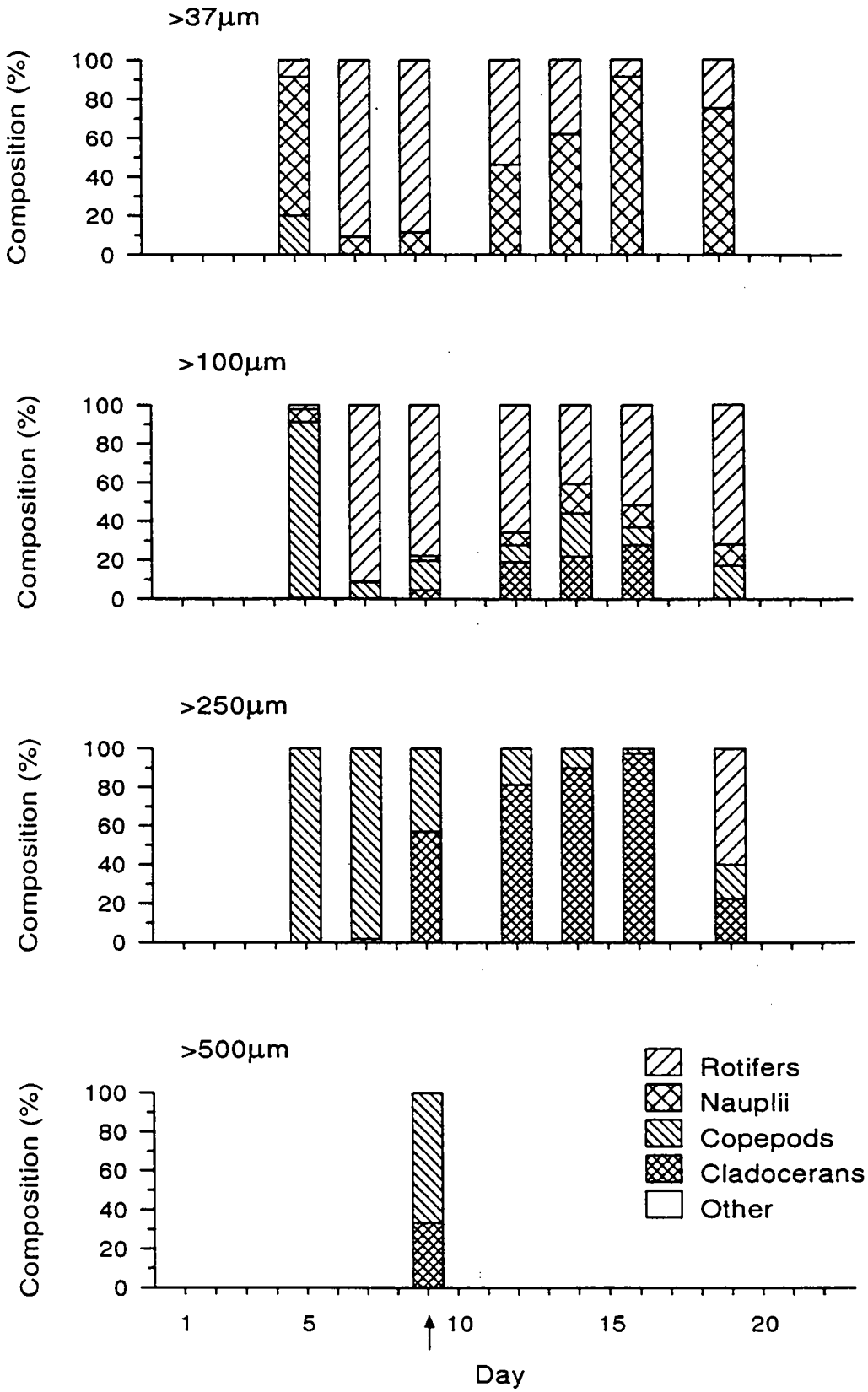
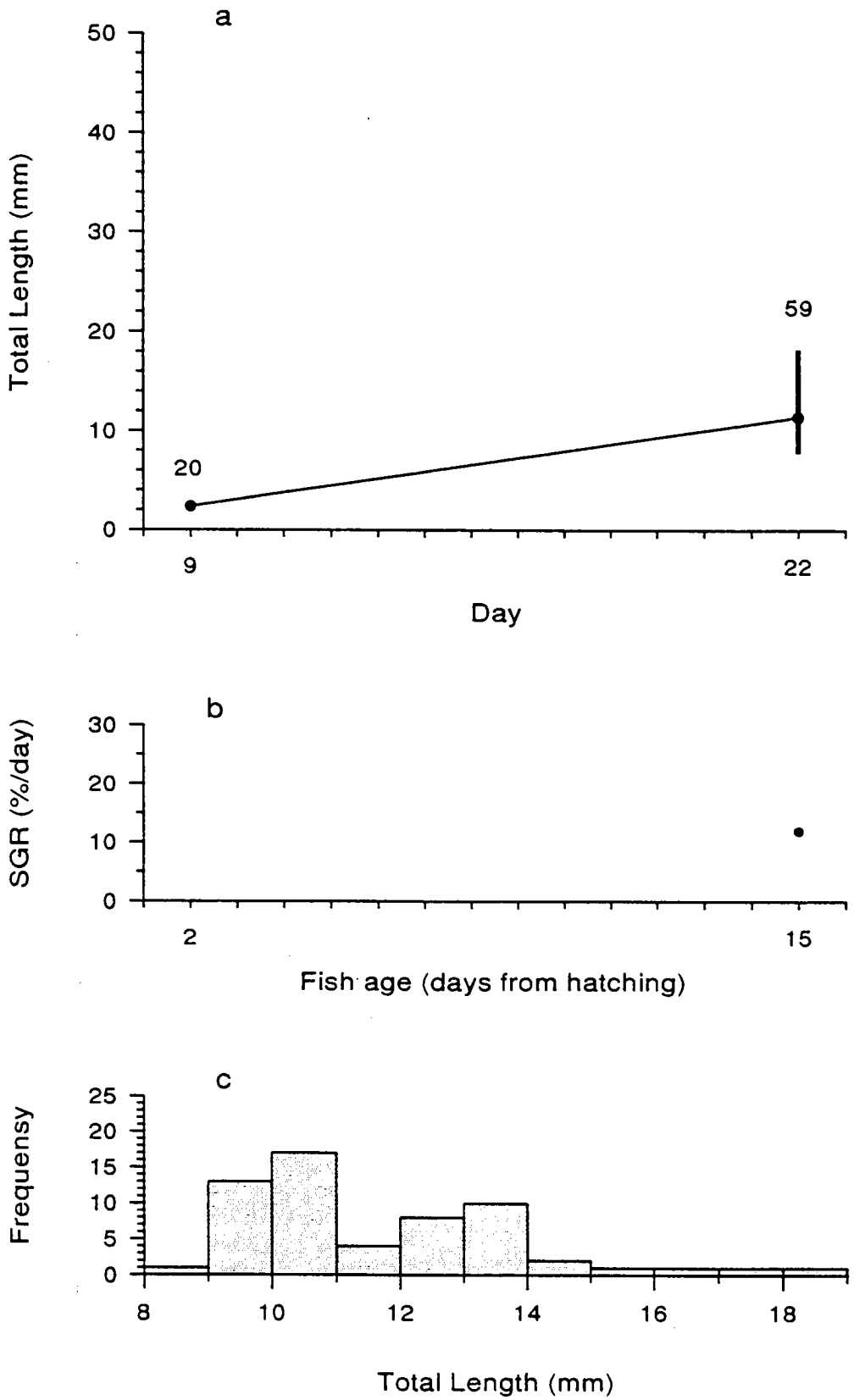

>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
1
5
10
15
20
Day
Rotifers
Nauplii
Copepods
Cladocerans
Other

Figure 7.22 (a) Growth of barramundi during pond trial P6 (3 January - 24 January 1991). Mean and range of each sample shown; numbers above or beneath bars represent sample size. (b) Specific growth rate (SGR) of barramundi during pond trial P6. (c) Size frequency of barramundi harvested at the end of pond trial P6 (n=59).


a
Total Length (mm)
50
40
30
20
10
0
59
20
9
22
Day
b
SGR (%/day)
30
20
10
0
2
15
Fish age (days from hatching)
c
Frequensy
25
20
15
10
5
0
8
10
12
14
16
18
Total Length (mm)

Trial P7: 29 January - 27 February 1991

Heavy rainfall began on day 3 of this trial and continued throughout the trial with a total of 1,254 mm of rain recorded over the 30 days of the trial (Table 7.3, Fig. 7.24). The pond quickly stratified with a layer of fresh water (the mixolimnion) overlying the more saline water (c. 22 ppt) which had been used to fill the pond (the monimolimnion). This form of stratification is termed ectogenic meromixis, i.e. the phenomenon in which some external event (in this case, heavy rainfall) brings salt water into a freshwater body or fresh water into a saline water body (Wetzel 1975, Cole 1979). By day 7 of the trial, the salinity of the mixolimnion had decreased to about 5 ppt while that of the monimolimnion remained at about 22 ppt (Fig 7.23). The temperature of the monimolimnion increased steadily after stratification, from 32.8 to 38.2°C. In contrast, the temperature of the mixolimnion stayed relatively constant at 29-31°C over the same period. The temperature of the monimolimnion during this period was much higher than the air temperature at 1400 h (25-31.5°C) (Fig. 7.24). Consistent overcast weather during this period (Fig. 7.24) prevented the development of a phytoplankton bloom and the pond remained relatively clear with Secchi disk visibility ranging from 80-150 cm (Fig. 7.25). The steadily increasing temperature of the monimolimnion may have been due to the energy from solar radiation heating only the monimolimnion, possibly due to the higher absorbance of the darker pond bottom in comparison with the water. In addition, the monimolimnion would have been unable to lose heat by evaporative cooling because of the presence of the overlying mixolimnion. A similar example of

increased water temperature caused by ectogenic meromixis has been described for Hot Lake, a shallow saline lake in north central Washington, where the energy of solar radiation accumulates as heat in the underlying monimolimnion (Wetzel 1975).

70,000 barramundi larvae were stocked into the monimolimnion of the pond on day 11 of the trial, when the temperature of the monimolimnion was 37°C and the salinity was 22.0 ppt, while the temperature and salinty of the mixolimnion were 29°C and 5.5 ppt respectively (Fig. 7.23). The temperature of the monimolimnion ranged from 36.7 to 38.2°C and salinity from 19.4 to 22.3 ppt during the first week after stocking (Table 7.4, Fig. 7.23). Densities of zooplankton in the size classes $>37\mu$m and $>100\mu$m were 735 and 1,108 organisms/litre respectively at stocking.

On day 19 river water was pumped into the pond and the resulting agitation caused mixing of the two layers. Temperature and salinity in the now homogeneous pond were relatively stable for the remainder of the trial at 29-31°C and 3.8-4.6 ppt respectively (Fig. 7.23). Dissolved oxygen ranged from 4.3 to 6.4 mg/l and pH from 7.6 to 8.9 throughout the trial (Table 7.3, Fig. 7.23).

All the plankton size classes sieved from the vertical tube samples peaked in density twice during the trial, with smaller sized organisms peaking early in the trial and the larger size classes peaking later (Fig. 7.25). Relative proportions of the various size classes of plankton are shown in Figure 7.26. Secchi disk visibility increased following peaks in zooplankton density, suggesting substantial grazing of phytoplankton by zooplankton (Fig. 7.25).

No barramundi larvae were captured despite intensive sampling, and no barramundi were found when the pond was drained on day 30. Both the mixolimnion and the monimolimnion were probably unsuitable for survival of barramundi larvae at the time of stocking, because of the low salinity of the mixolimnion (5.5 ppt) and the high temperature of the monimolimnion (37°C). The densities of zooplankton at stocking for this trial (Table 7.5) were within the range of densities which were associated with survival of barramundi in other trials, suggesting that the availability of prey organisms was not a factor in the mortality of barramundi in this trial. The physicochemical conditions prevailing in the pond at the time of stocking are the most likely causes of the total mortality of barramundi in this trial.

Figure 7.23 Water quality parameters for pond trial P7 (29 January - 27 February 1991). Solid lines: daily mean temperature, daily mean salinity and daily minimum dissolved oxygen sampled hourly from near the pond bottom, and pH of the water sample used for plankton density estimates. Broken lines: surface temperature and salinity sampled opportunistically throughout the trial. The day of stocking is marked with an arrow; the thin dashed vertical line indicates the day when additional water was pumped into the pond (day 19).

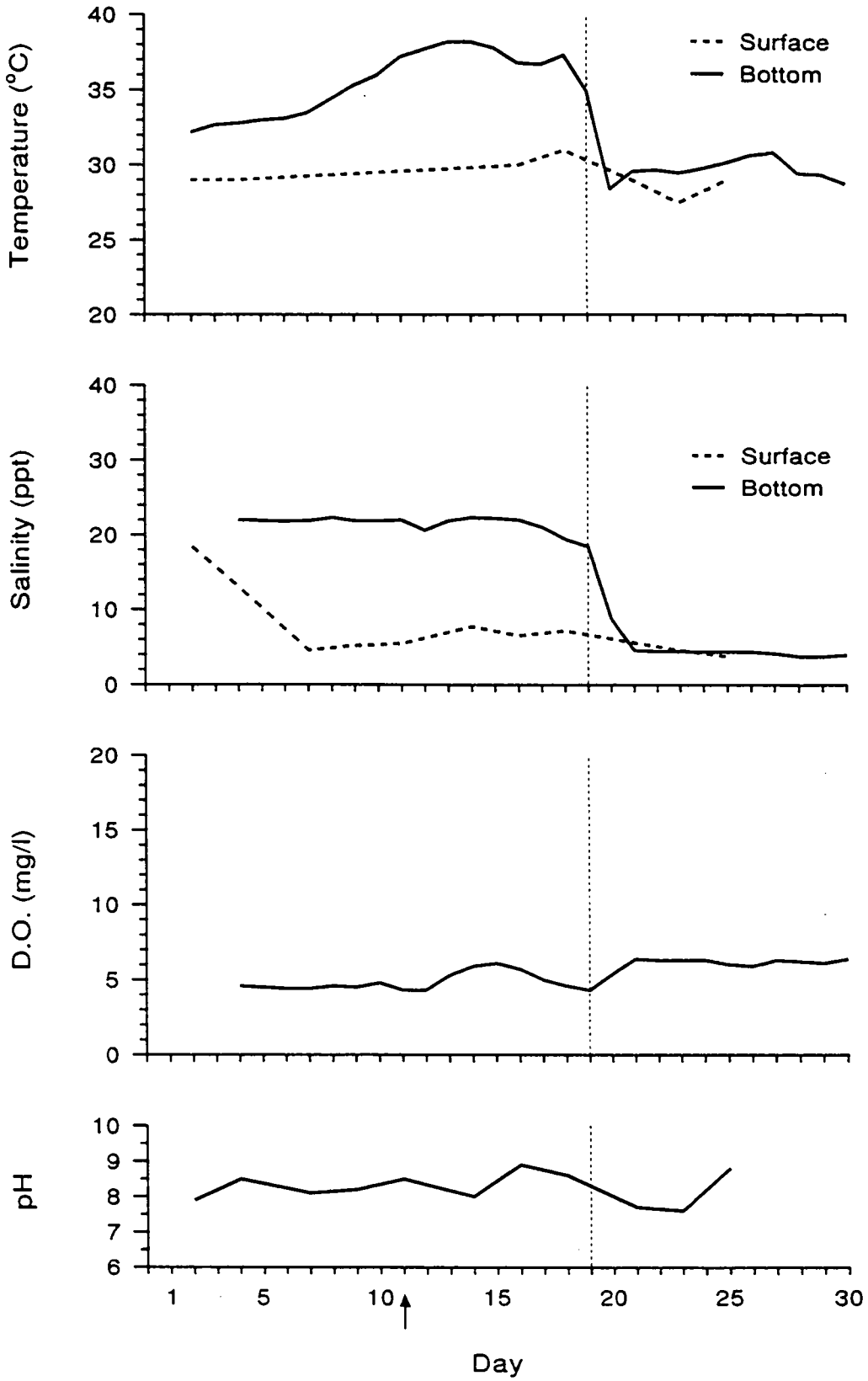

Temperature (°C)
40
35
30
25
20
Surface
Bottom
Salinity (ppt)
40
30
20
10
0
Surface
Bottom
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
30
Day

Figure 7.24 Air temperature at 0300 and 1500 h, cloud cover at 1500 h and daily rainfall for Innisfail during pond trial P7 (29 January - 27 February 1991). The day of stocking is marked with an arrow.

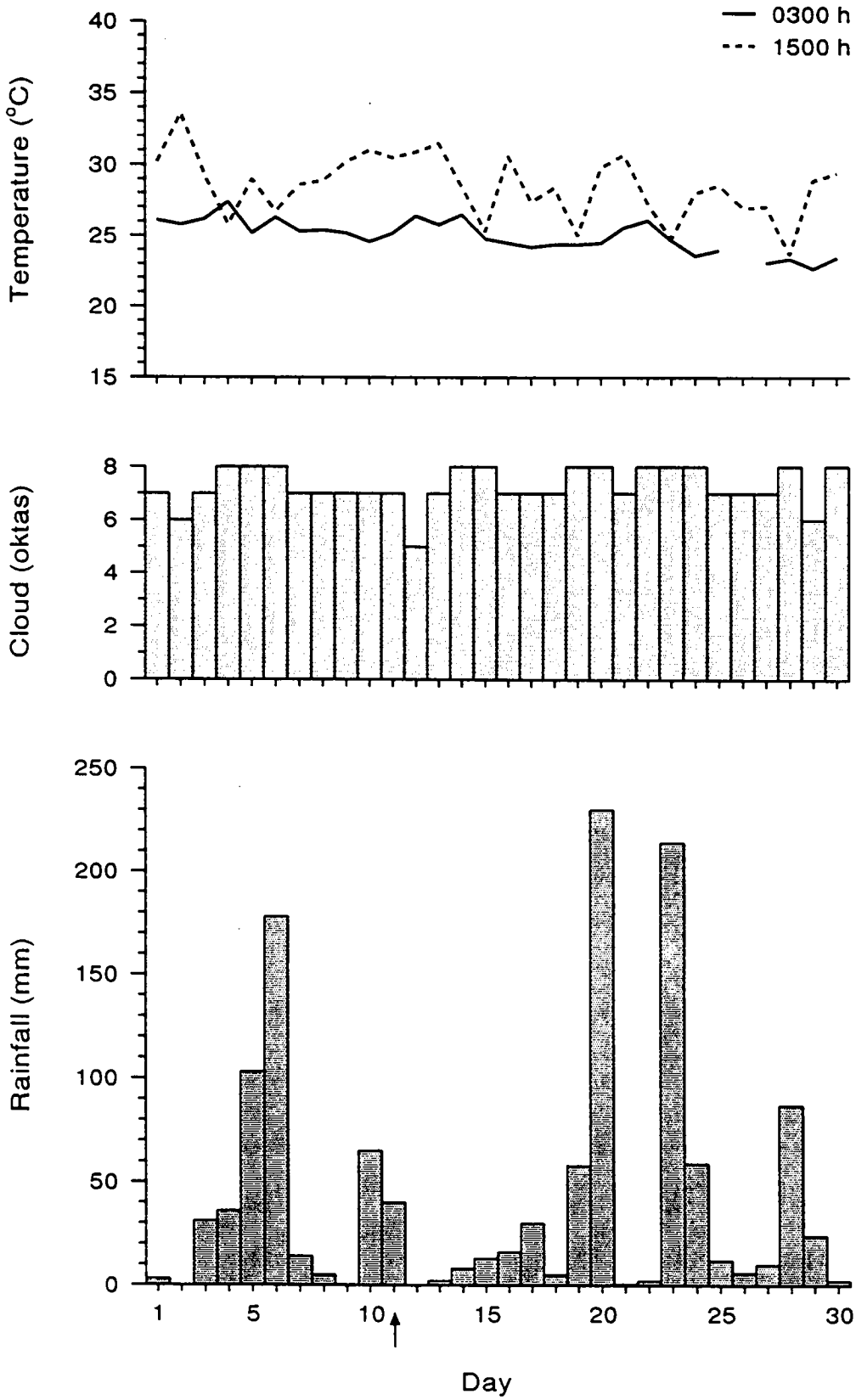

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
30
Day

Figure 7.25 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P7 (29 January - 27 February 1991). The day of stocking is marked with an arrow.

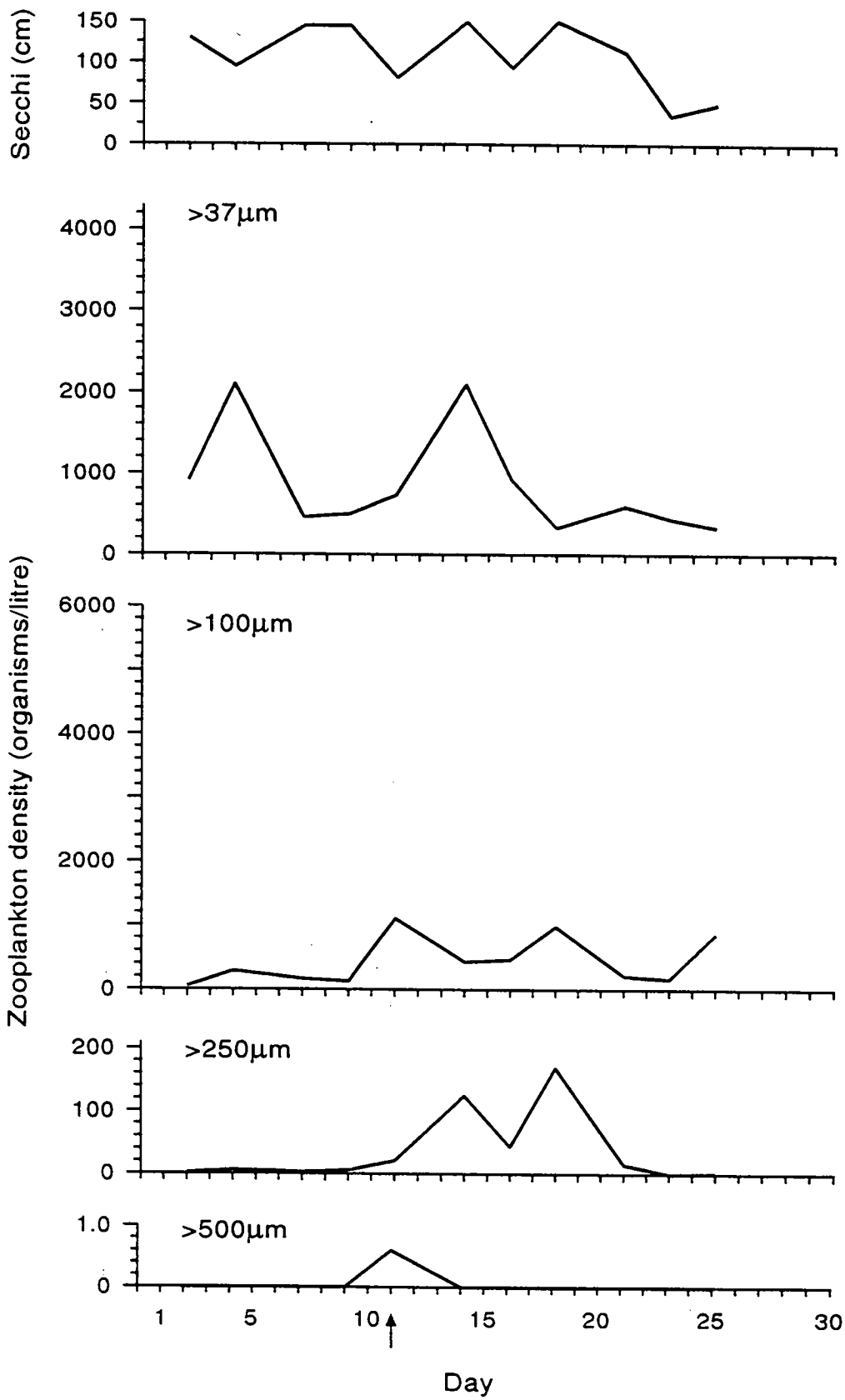
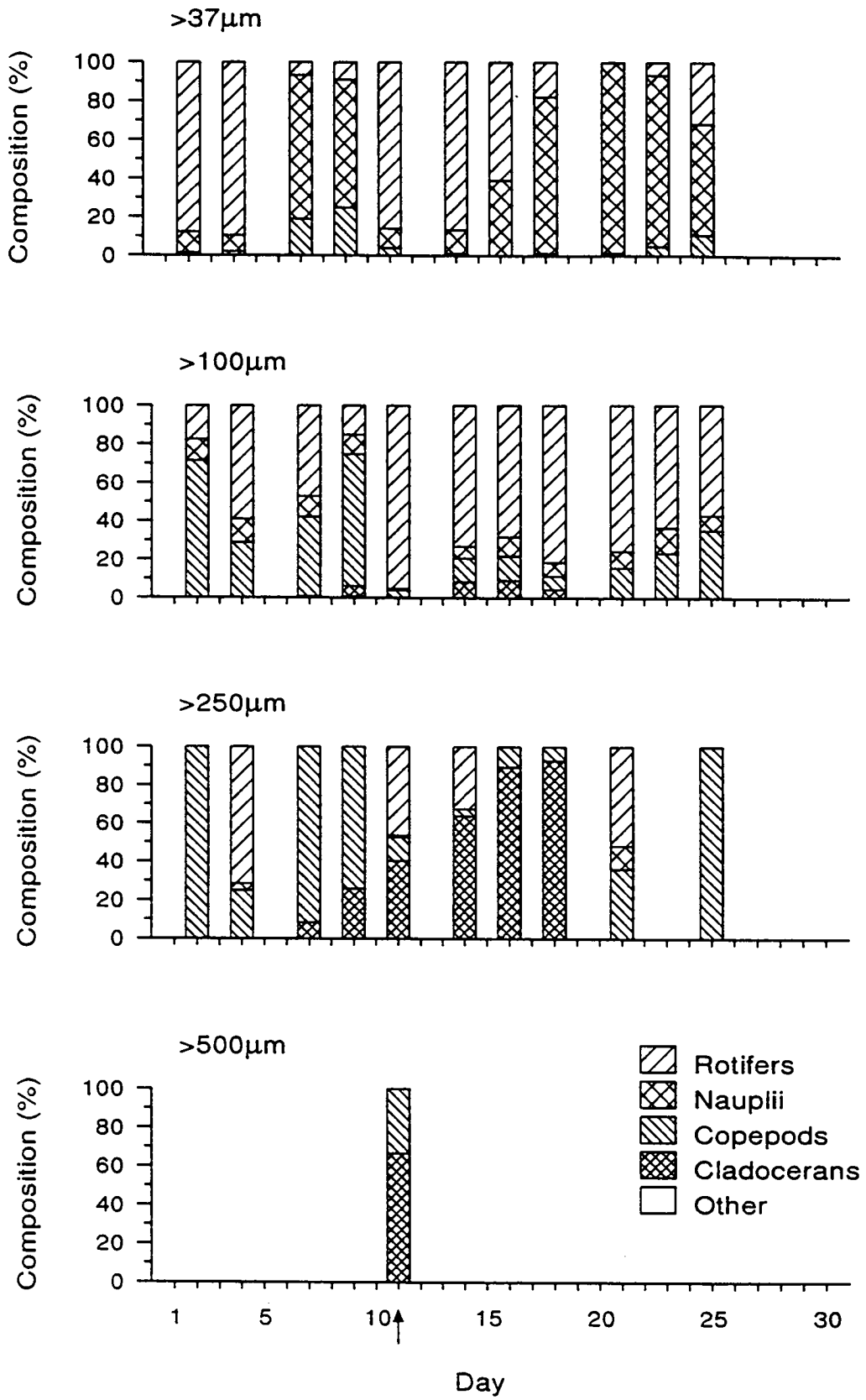

Secchi (cm)
150
100
50
0
>37µm
4000
3000
2000
1000
0
Zooplankton density (organisms/litre)
>100µm
6000
4000
2000
0
>250µm
200
100
0
>500µm
1.0
0
1
5
10
15
20
25
30
Day

Figure 7.26 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other organisms in sieved samples (Fig. 7.25) from pond trial P7 (29 January - 27 February 1991). The day of stocking is marked with an arrow.


>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
1
5
10
15
20
25
30
Day
Rotifers
Nauplii
Copepods
Cladocerans
Other

Trial P8: 8 October - 11 November 1991

All water quality parameters were relatively stable during this trial. Bottom temperature and salinity data were limited to only days 11 to 18 due to malfunction of the SDL and biofouling of the SDL sensors caused by heavy growth of barnacles; consequently surface temperature and salinity data have been plotted in Figure 7.27. Mean daily water temperature increased gradually from 27.2 to 29.9°C and salinity from 31.8 to 33.8 ppt during the course of the trial (Fig. 7.27). Very little rain fell during this trial (Fig. 7.28), with a total of only 9 mm recorded for the whole trial (Table 7.3, Fig. 7.28). Dissolved oxygen data were available for only a few days due to malfunction and biofouling of the SDL, but these data indicate that dissolved oxygen remained near saturation values during this time, ranging from 5.6 to 7.2 mg/l (Table 7.3, Fig. 7.27). pH varied little throughout the trial, ranging from 7.4 to 8.1 (Fig. 7.27).

Densities of zooplankton in the $>37\mu m$ and $>100\mu m$ size classes were low at stocking (148 and 450 organisms/litre respectively [Table 7.5]) but the density of zooplankton in both these size classes increased rapidly following stocking and peaked at days 16 - 18 (Fig. 7.29). Both the $>37\mu m$ and the $>100\mu m$ size classes were made up primarily of copepod nauplii during the first week of the trial, but were dominated by rotifers during the second week (Fig. 7.30). Copepod nauplii again predominated during the later part of the trial. The $>250\mu m$ size class of zooplankton peaked relatively early on day 10 (Fig. 7.29); large copepod nauplii predominated in this size class at this time (Fig. 7.30).

During later stages of the trial this size class was dominated by larger copepod nauplii, copepodites and adult copepods (Fig. 7.30).

70,000 barramundi larvae were stocked into the pond on day 10, and an additional 20,000 (10,000 on day 10 and 10,000 on day 11) were stocked into the experimental enclosures being used for sampling larvae for gut contents (see Chapter 8). The enclosures overflowed and the larvae presumably entered the pond, giving a total of 90,000 larvae stocked for this trial. (Because all larvae stocked in the pond for this trial were of the same age, and the exact time of the second introduction is not known, this second 'stocking' of barramundi larvae is not marked separately on the graphs for this trial). Barramundi larvae were first captured on day 18 using a 300μm mesh plankton net. Growth rates averaged 1.1 mm/day over the duration of the trial, with faster growth in the period from day 21 to day 32 than from day 10 (when the larvae were stocked) to day 18 (Fig. 7.31a). No growth was recorded between days 24 and 27 during which time the density of zooplankton of all size classes was extremely low (Fig. 7.29). Specific growth rate increased from 11.9 %/day to a maximum of 16.5 %/day to day 23 (day 15 from hatching), before decreasing to about 9%/day for the rest of the trial (Fig. 7.31b); overall SGR for this trial was 10.3 %/day (Table 7.2).

The density of barramundi found on the lift nets averaged 38.1 /m^2 during the course of this trial, giving a predicted population of 51,000 fish in the pond. Approximately 63,000 juvenile barramundi were harvested, representing a survival rate of 70%. Harvested fish averaged 28.8 mm TL (range 20.4 - 40.1 mm TL) (Fig. 7.31c) at day 27 from hatching (697.5 degree-days).

Figure 7.27 Water quality parameters for pond trial P8 (8 October - 11 November 1991): daily mean temperature sampled near the surface at two-hourly intervals; salinity sampled opportunistically near the surface; daily minimum dissolved oxygen sampled hourly from near the pond bottom; and pH of the water sample used for plankton density estimates. Missing data are the result of equipment malfunction and severe biofouling of the SDL sensors by barnacles. The day of stocking is marked with an arrow.

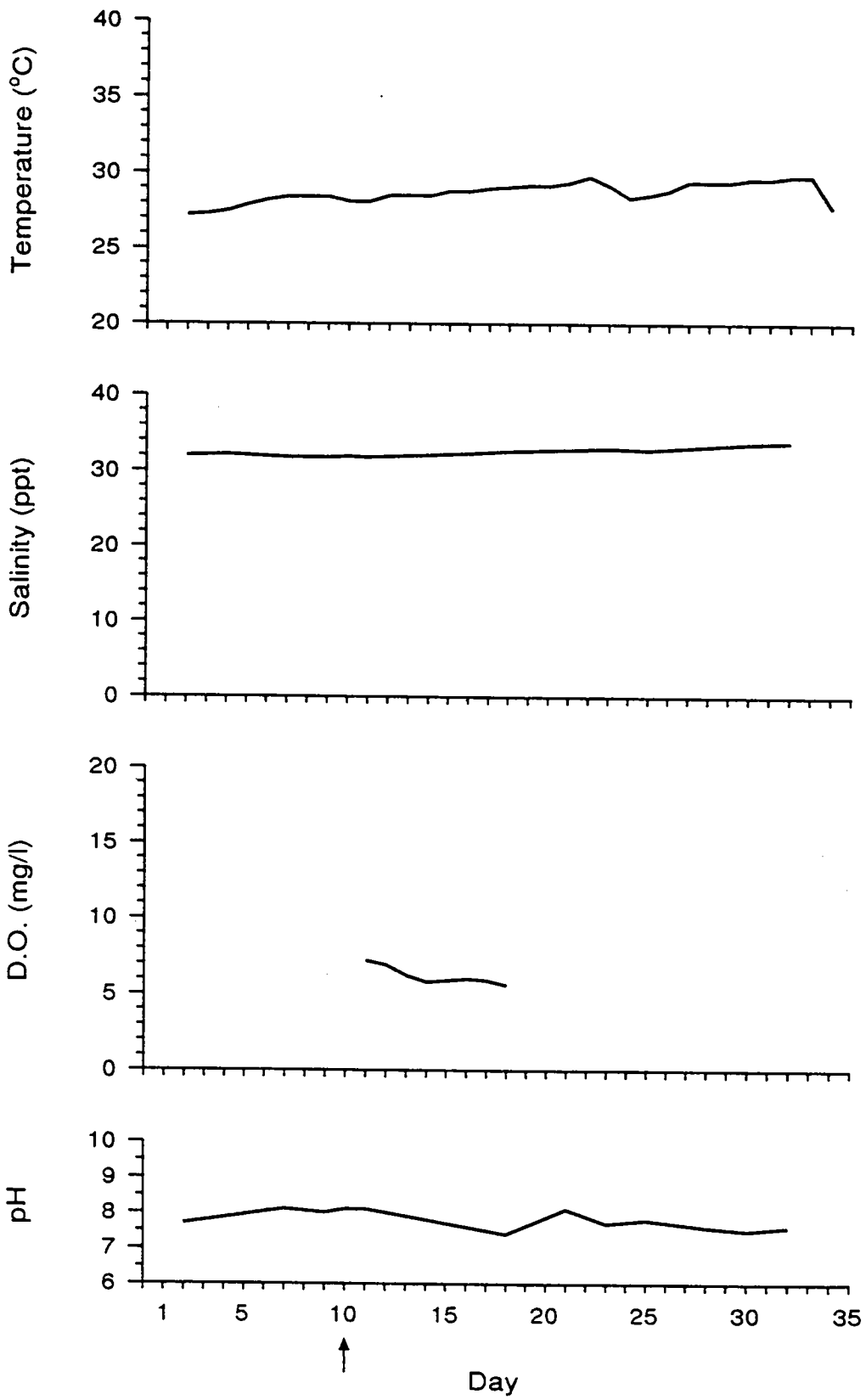

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
30
35
Day

Figure 7.28 Air temperature at 0300 and 1500, cloud cover at 1500 and daily rainfall for Innisfail during pond trial P8 (8 October - 11 November 1991). The day of stocking is marked with an arrow.

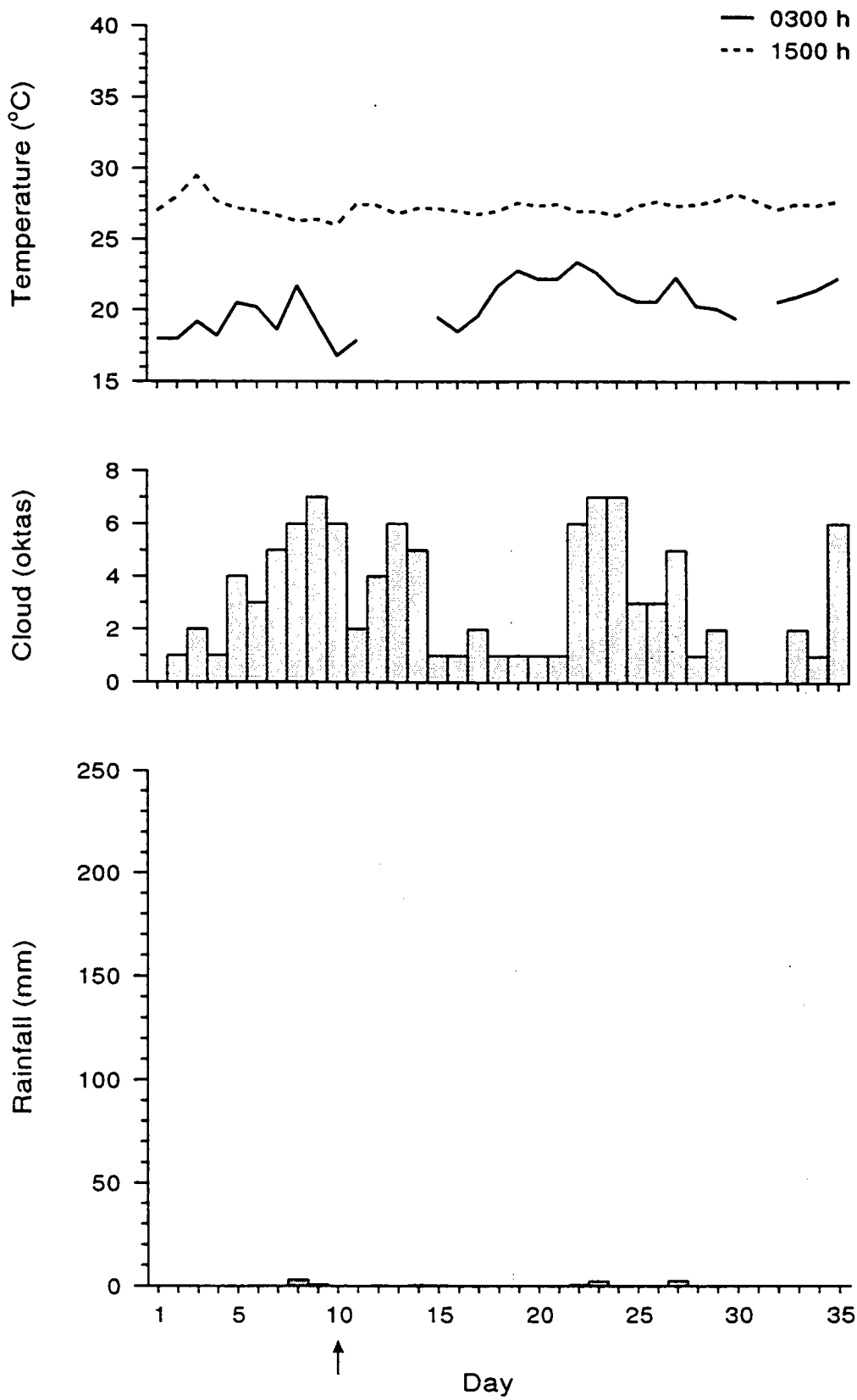

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
30
35
Day

Figure 7.29 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P8 (8 October - 11 November 1991). The day of stocking is marked with an arrow.

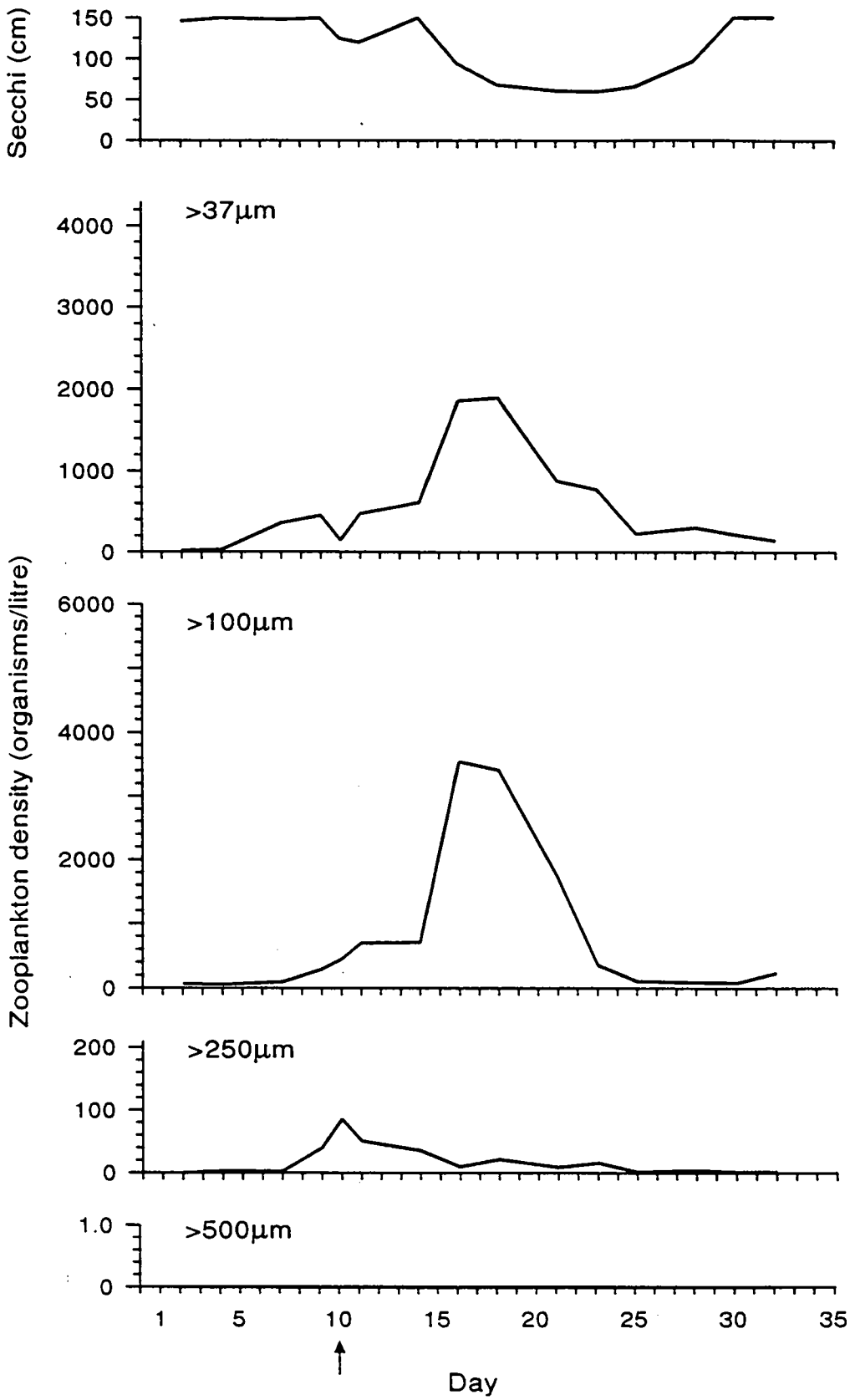

Secchi (cm)
150
100
50
0
>37µm
4000
3000
2000
1000
0
Zooplankton density (organisms/litre)
>100µm
6000
4000
2000
0
>250µm
200
100
0
>500µm
1.0
0
1
5
10
15
20
25
30
35
Day

Figure 7.30 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other organisms in sieved samples (Fig. 7.29) from pond trial P8 (8 October - 11 November 1991). The day of stocking is marked with an arrow.

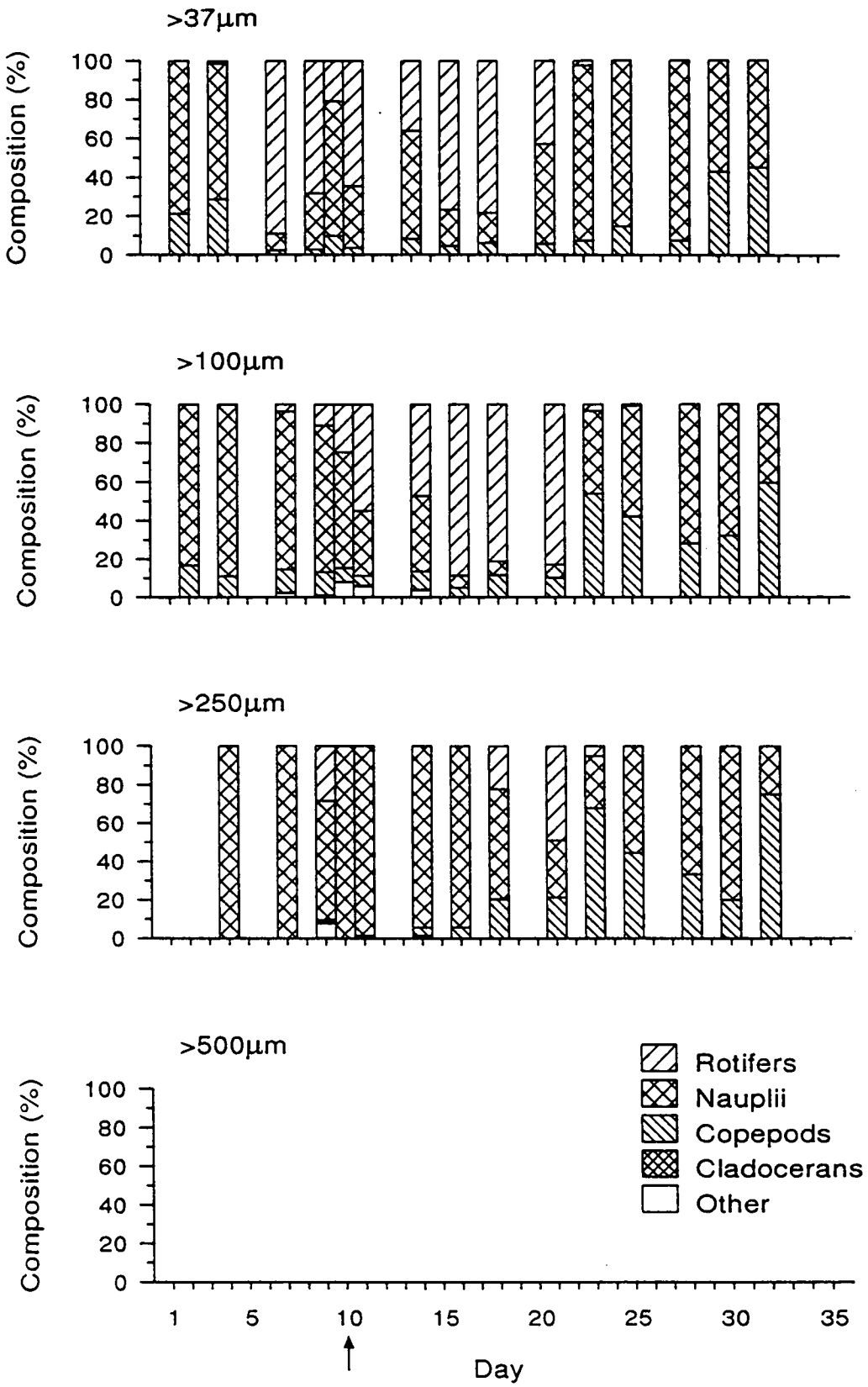
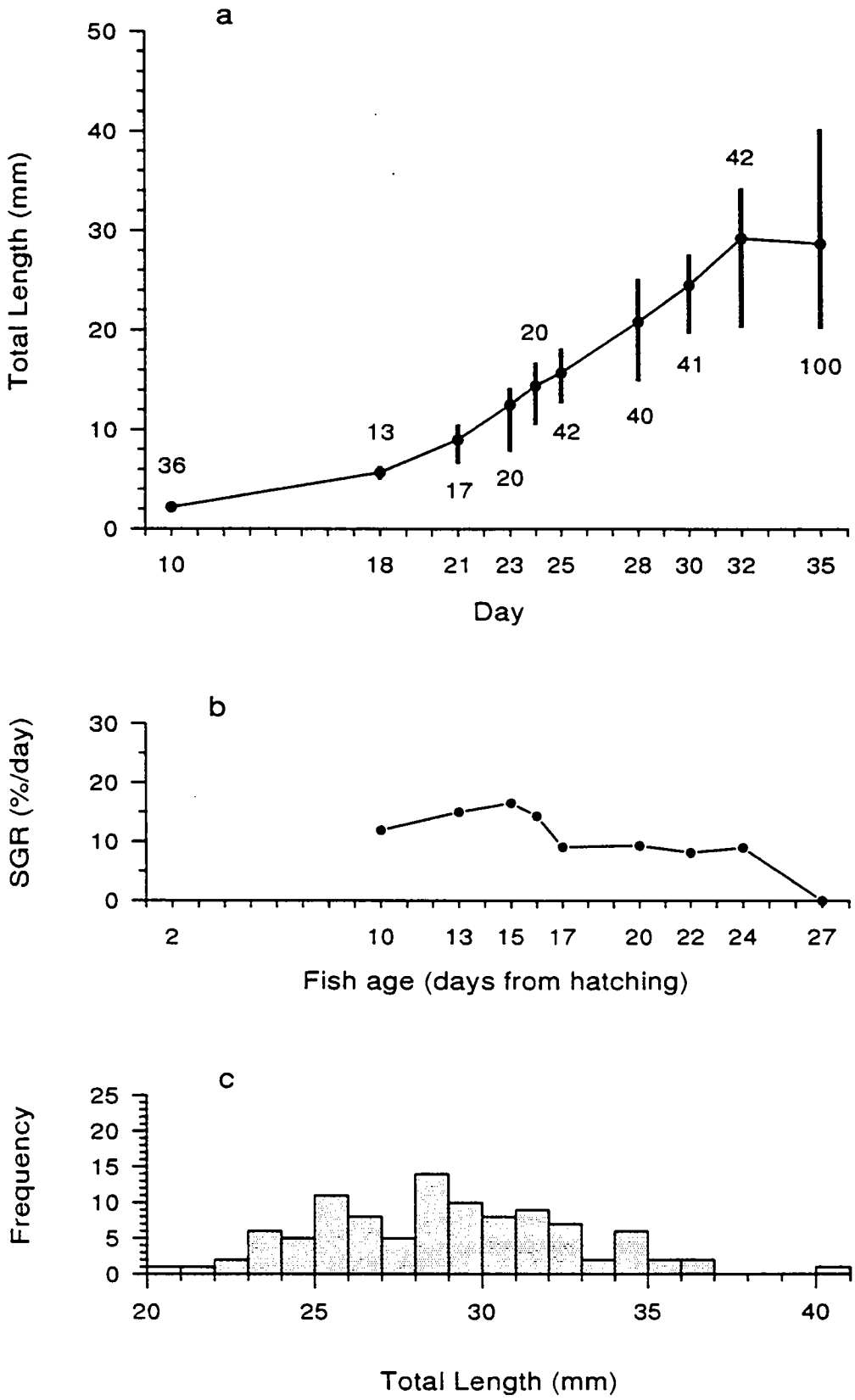

>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
1
5
10
15
20
25
30
35
Day
Rotifers
Nauplii
Copepods
Cladocerans
Other

Figure 7.31 (a) Growth of barramundi during pond trial P8 (8 October - 11 November 1991). Mean and range of each sample shown; numbers above or beneath bars represent sample size. (b) Specific growth rate (SGR) for barramundi during pond trial P8. (c) Size frequency of barramundi harvested at the end of pond trial P8 (n=100).


a
Total Length (mm)
50
40
30
20
10
0
36
13
17
20
20
42
40
42
41
100
10
18
21
23
25
28
30
32
35
Day
b
SGR (%/day)
30
20
10
0
2
10
13
15
17
20
22
24
27
Fish age (days from hatching)
c
Frequency
25
20
15
10
5
0
20
25
30
35
40
Total Length (mm)

Trial P9: 14 November - 11 December 1991

This trial commenced only 3 days after the previous trial was terminated. Consequently, the pond substrate was not completely dried in the period between the end of pond trial P8 and the start of P9.

All water quality parameters were relatively stable during this trial. As in the previous trial, malfunction of the SDL and biofouling of the SDL sensors limited the amount of bottom water quality data available; hence surface temperature and salinity data have been plotted in Figure 7.32. Mean daily water temperature ranged from 29.4 to 33.7°C and salinity decreased gradually from 31.6 to 28.0 ppt during the course of the trial (Fig. 7.32). This gradual decrease in salinity was attributable to the rain which fell throughout this trial (Fig. 7.33); total rainfall for the trial was 131 mm (Table 7.3). Dissolved oxygen data were available for only a few days due to malfunction and biofouling of the SDL, but the data indicate that dissolved oxygen ranged from 4.6 to 6.2 mg/l (Table 7.3). pH varied little throughout the trial (Fig. 7.32), ranging from 7.8 to 8.5 (Table 7.3).

A bloom of an unidentified filamentous cyanobacterium (blue-green alga) occurred during this trial, from about 1 December to 7 December (days 18 to 24). The bloom formed a thin film, bright green in colour, on the surface of the pond. The bloom covered up to 50% of the pond, but was usually restricted to a smaller area on the leeward side of the pond due to wind action. Additional water was added to the pond whenever the bloom accumulated near the monk, so that overflowing water would flush the floating cyanobacterial cells from the pond; an

estimated 50% of the bloom (surface area) could be removed using this procedure. The cyanobacterial bloom had dissipated by 8 December. No adverse effects on water quality were recorded during the duration of this cyanobacterial bloom (Fig. 7.32).

Densities of zooplankton were extremely low throughout this trial (Fig. 7.34). Relatively small peaks in density in the $>37\mu m$ and $>100\mu m$ size classes occurred on days 12 and 16 respectively (Fig. 7.34). Both these peaks are attributable to rotifers which predominated in both these size classes at this time (Fig. 7.35). Copepod nauplii generally predominated in the $>250\mu m$ size class (Fig. 7.35) which peaked in density on day 14 (Fig. 7.34). All size classes of zooplankton appeared to be increasing rapidly in density just prior to the end of the trial on day 28 (Fig. 7.34).

70,000 barramundi larvae were stocked into the pond on day 8. Densities of zooplankton in the $>37\mu m$ and $>100\mu m$ size classes were only 92 and 101 organisms/litre respectively at stocking (Table 7.5). Larvae were first captured on day 19 but were found in only small numbers throughout the trial. Both crude growth and specific growth rates varied considerably throughout this trial (Fig. 7.36). Crude growth rate ranged from 0 to 3.8 mm/day (the highest growth rate observed during these extensive rearing trials) and SGR ranged from 0 to 18.4%/day (Fig. 7.36b). Growth rate averaged 1.3 mm/day over the duration of the trial and the overall SGR for this trial was 12.4 %/day (Table 7.2).

The density of barramundi found on the lift nets averaged 2.2/m^2 during the course of the trial, giving a predicted population of 3,000 fish in the pond. 1,244 juvenile barramundi were harvested at the end of this trial, a survival rate of only 2%. Harvested fish averaged 28.0 mm TL (range 21.5 - 32.3 mm TL) (Fig. 7.36c) at day 22 from hatching (589.2 degree-days).

Figure 7.32 Water quality parameters for pond trial P9 (14 November - 11 December 1991): daily mean temperature sampled near the surface at two-hourly intervals; salinity sampled opportunistically near the surface; daily minimum dissolved oxygen sampled hourly from near the pond bottom; and pH of the water sample used for plankton density estimates. Missing data are the result of equipment malfunction. The day of stocking is marked with an arrow.

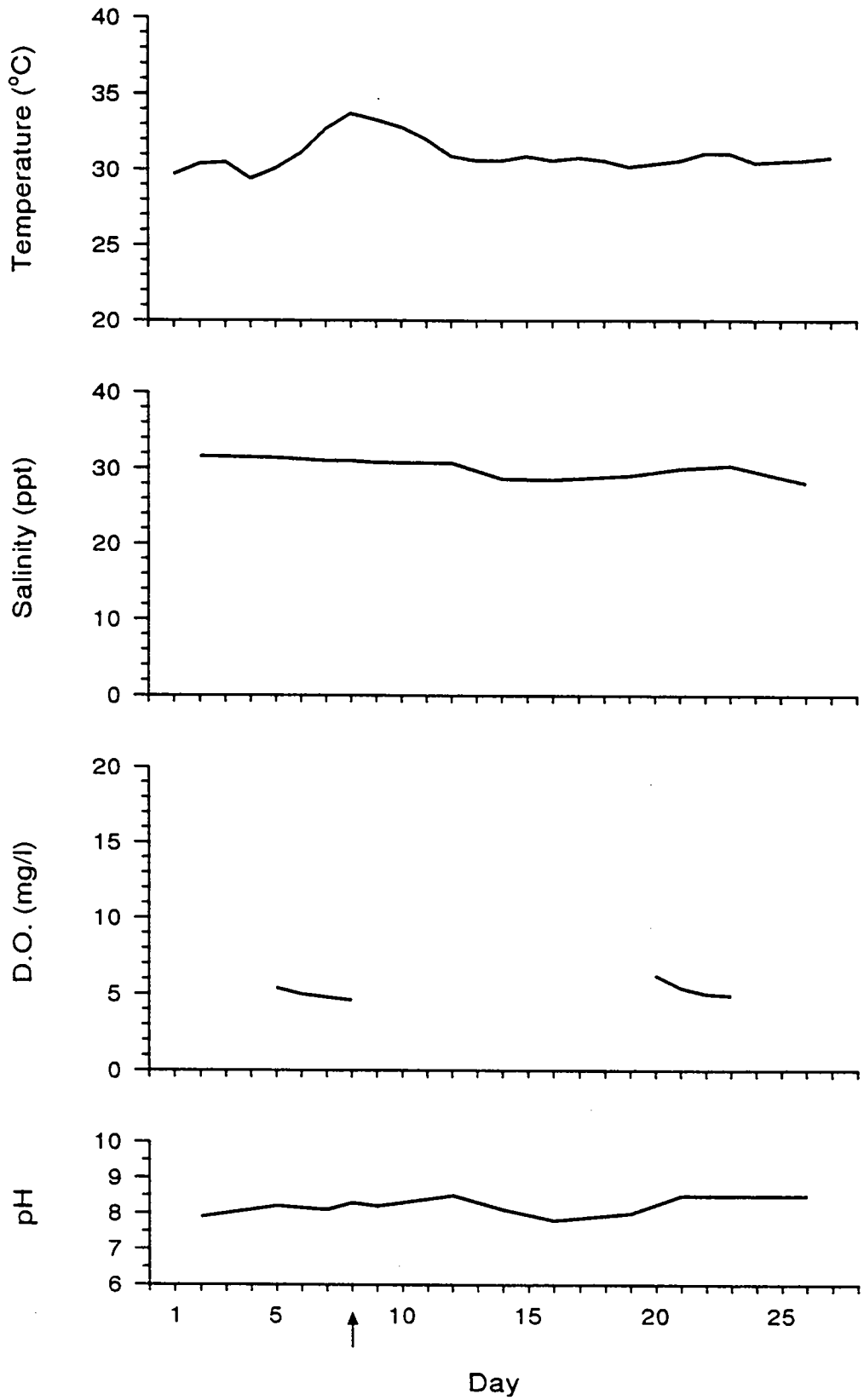

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
Day

Figure 7.33 Air temperature at 0300 and 1500, cloud cover at 1500 and daily rainfall for Innisfail during pond trial P9 (14 November - 11 December 1991). The day of stocking is marked with an arrow.

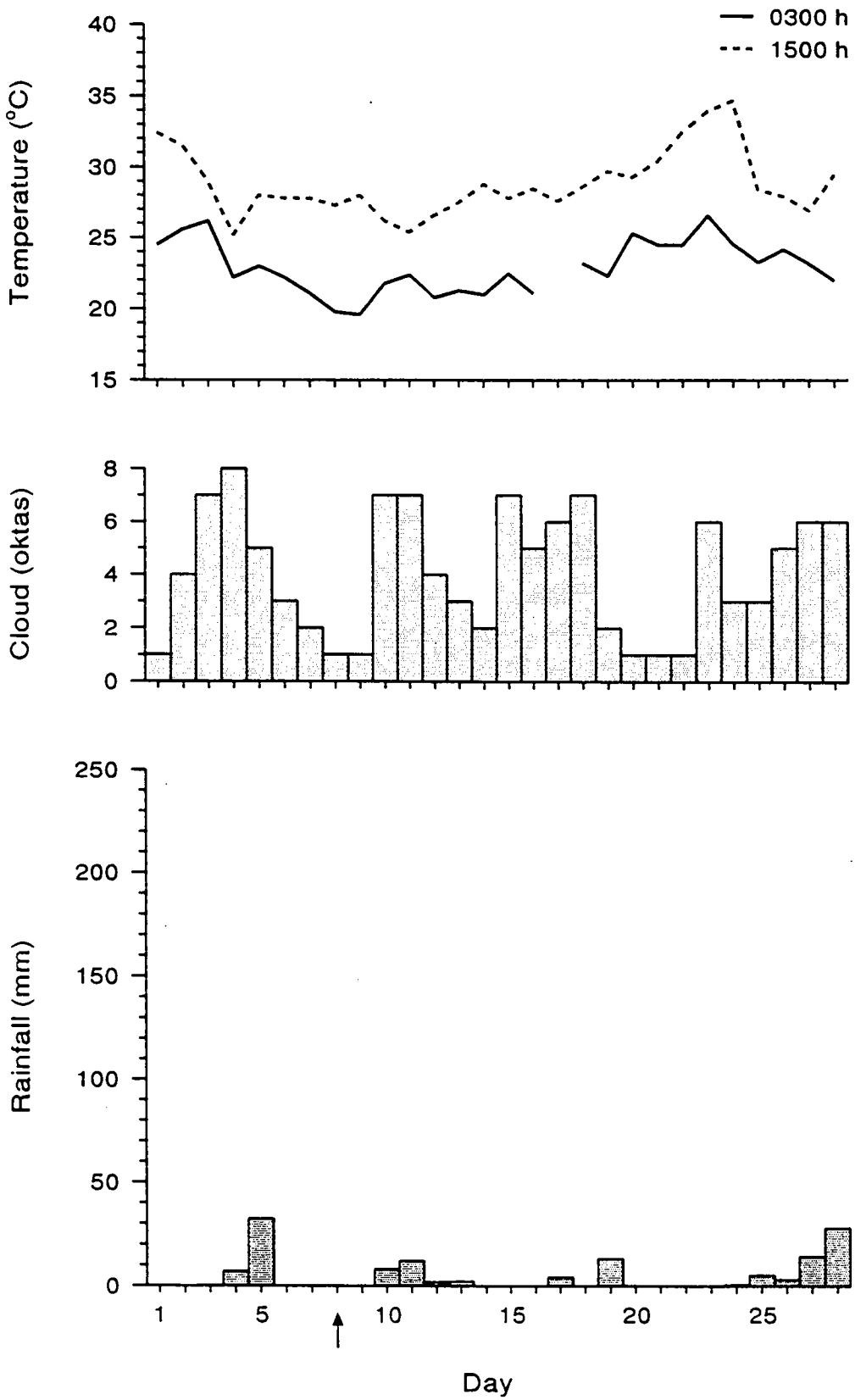

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
Day

Figure 7.34 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P9 (14 November - 11 December 1991). The day of stocking is marked with an arrow.

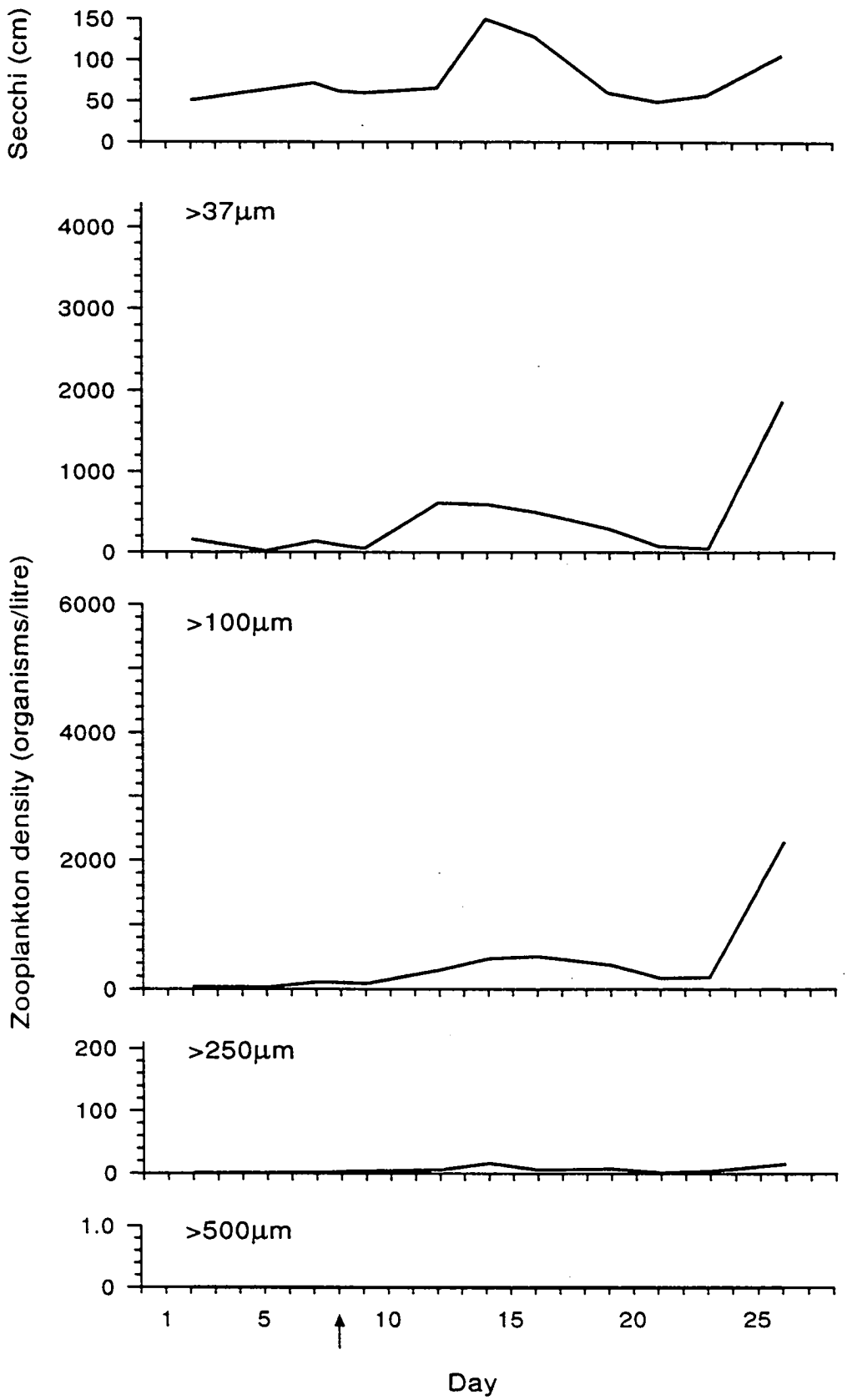

Secchi (cm)
150
100
50
0
>37µm
4000
3000
2000
1000
0
Zooplankton density (organisms/litre)
>100µm
6000
4000
2000
0
>250µm
200
100
0
>500µm
1.0
0
1
5
10
15
20
25
Day

Figure 7.35 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other organisms in sieved samples (Fig. 7.34) from pond trial P9 (14 November - 11 December 1991). The day of stocking is marked with an arrow.

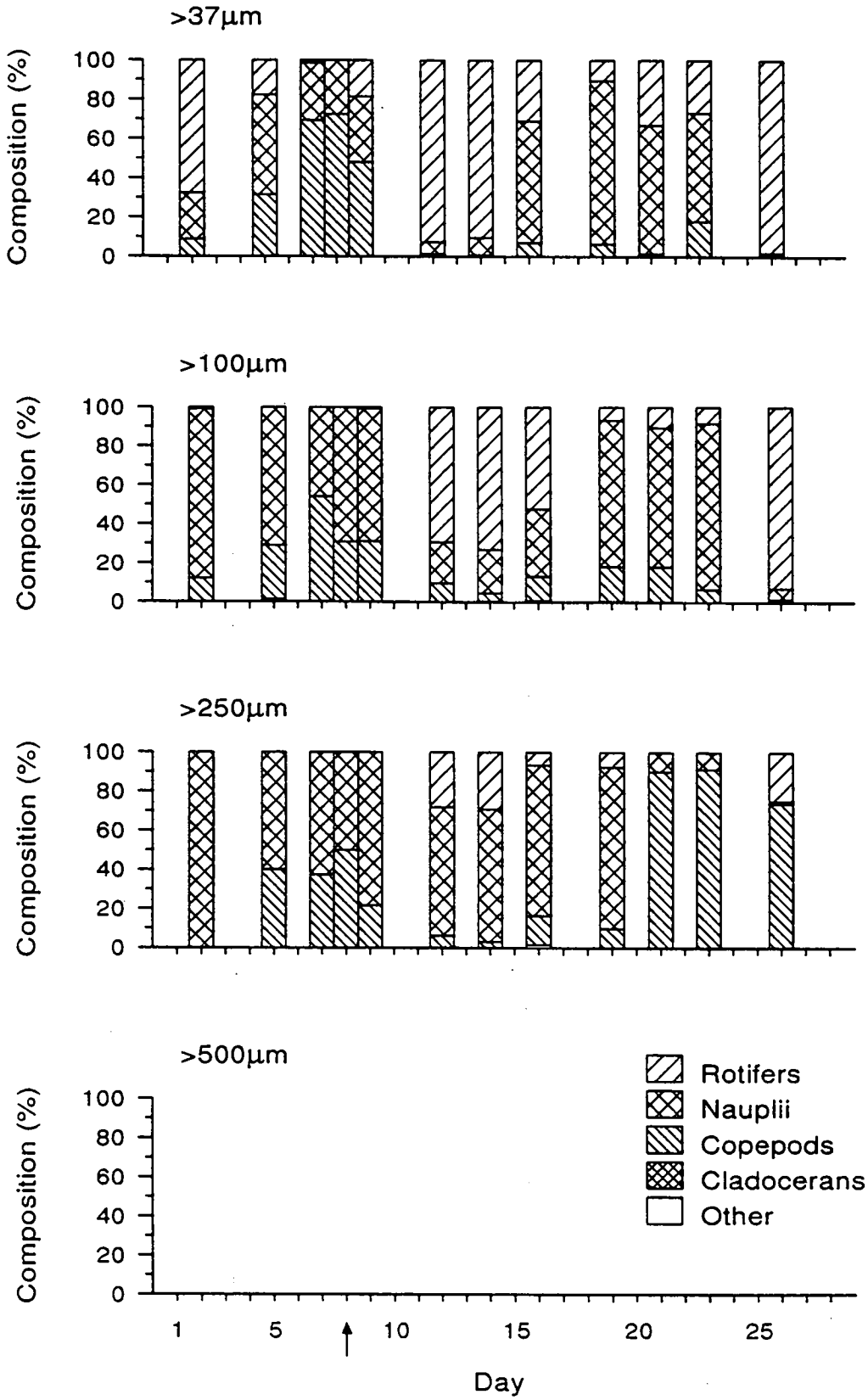
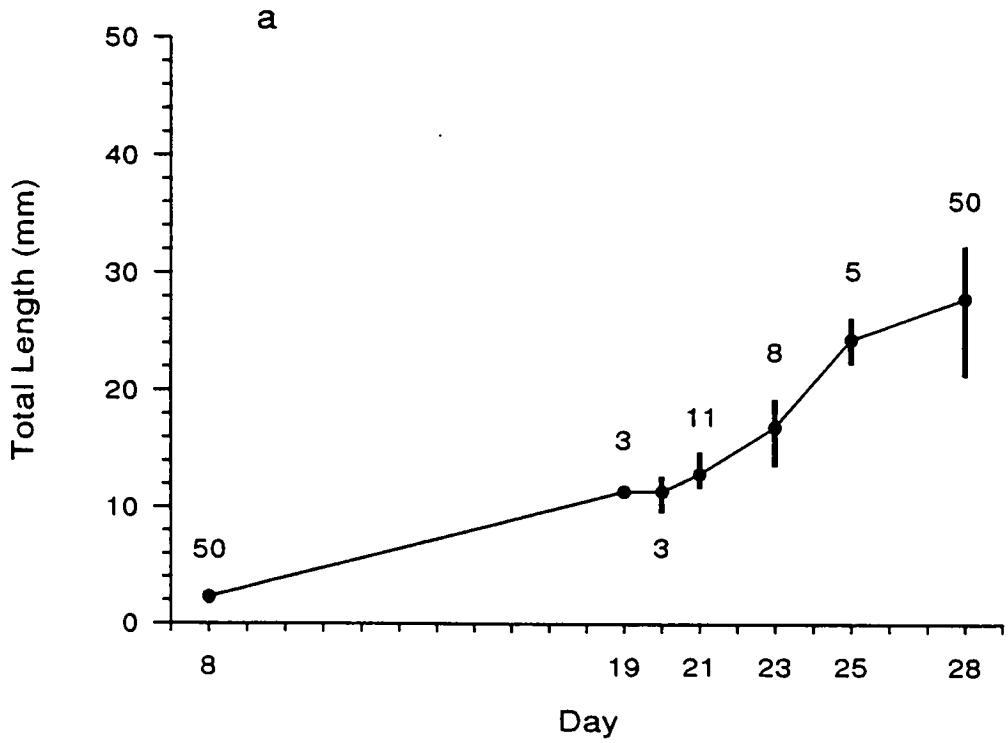
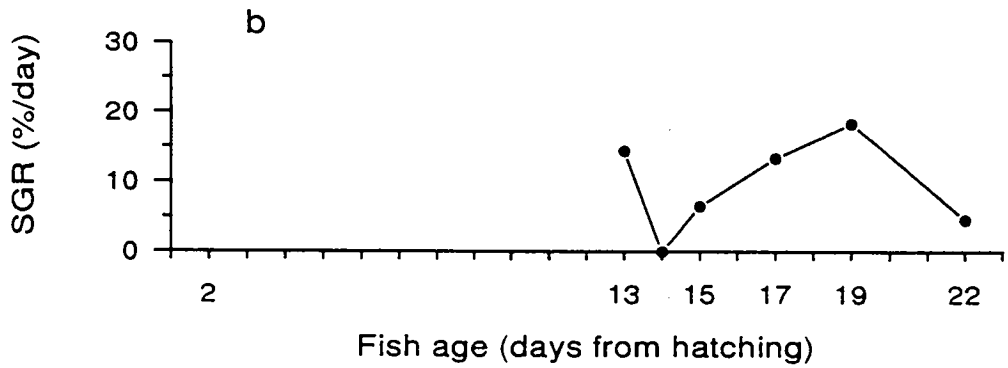
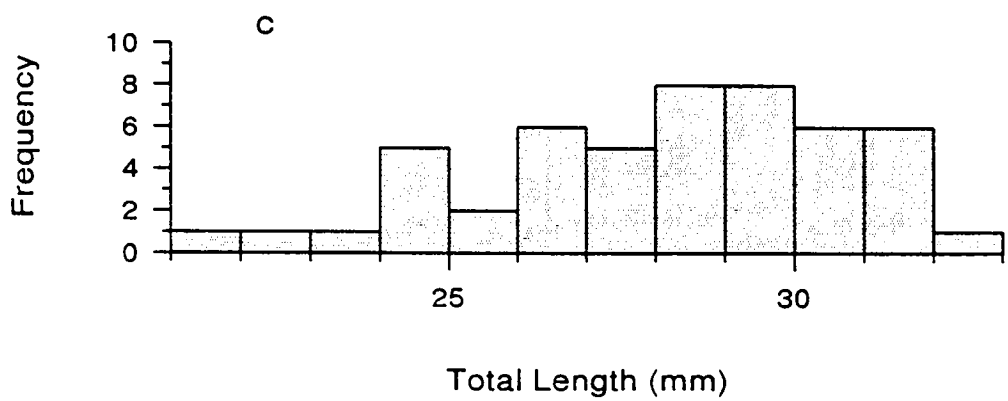

>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
1
5
10
15
20
25
Day
Rotifers
Nauplii
Copepods
Cladocerans
Other

Figure 7.36 (a) Growth of barramundi during pond trial P9 (14 November - 11 December 1991). Mean and range of each sample shown; numbers above or beneath bars represent sample size. (b) Specific growth rate (SGR) for barramundi during pond trial P9. (c) Size frequency of barramundi harvested at the end of pond trial P9 (n=50).


a
Total Length (mm)
50
40
30
20
10
0
50
3
3
11
8
5
50
8
19
21
23
25
28
Day


b
SGR (%/day)
30
20
10
0
2
13
15
17
19
22
Fish age (days from hatching)


c
Frequency
10
8
6
4
2
0
25
30
Total Length (mm)

Trial P10: 12 December 1991 - 7 January 1992

This trial commenced immediately after the termination of the previous trial, and again the pond substrate was not dried between trials.

Mean daily water temperature in this trial ranged from 31.2 to 32.8°C and salinity increased gradually from 23.2 to 27.3 ppt during the course of the trial (Fig. 7.37); the brief drop in salinity recorded on day 8 is attributable to the addition of low salinity river water to top up the pond (Fig. 7.37). Cloud cover was generally light and little rain fell during this trial (Fig. 7.38); total rainfall for the trial was 22 mm (Table 7.3). Dissolved oxygen data were not available due to malfunction of the SDL. pH increased slowly from 7.4 during the first two weeks of the trial to a peak of 9.2 and then decreased slowly until the end of the trial (Fig. 7.37).

Densities of zooplankton were low for the first two weeks of this trial, including the time immediately following stocking (Fig. 7.39). Zooplankton density at stocking was only 143 organisms/litre ($>37\mu$m) and 259 organisms/litre ($>100\mu$m) (Table 7.5). The peaks in density of zooplankton in the $>37\mu$m and $>100\mu$m size classes (Fig. 7.39) are attributable to rotifers which predominated in both these size classes at this time (Fig. 7.40). Copepodites and copepod adults generally predominated in the $>250\mu$m size class throughout the trial (Fig. 7.40). Very low densities of the $>250\mu$m size class were found throughout the trial, and densities were increasing when the trial was terminated on day 27 (Fig. 7.39). All size classes of zooplankton appeared to be increasing in density just prior to the end of the trial on day 28 (Fig. 7.39).

70,000 barramundi larvae were stocked into the pond on day 8. No barramundi larvae were sampled from the pond throughout the trial. When the pond was drained, 50 barramundi ranging in size from 54.8 to 119.0 mm TL (mean 95.7 mm TL) were found. These fish were presumably survivors of the previous trial which had escaped detection during the previous harvest. Since the pond had been refilled immediately following the completion of the previous trial, these fish could have survived hidden in inaccessible parts of the concrete raceway or in other damp areas.

Poor survival of barramundi larvae in this trial is at least partly attributable to both the low plankton densities at stocking and the presence of the larger barramundi which would have preyed on the smaller fish which were stocked in this trial.

Figure 7.37 Water quality parameters for pond trial P10 (12 December 1991 - 7 January 1992): daily mean temperature sampled near the surface at two-hourly intervals; salinity sampled opportunistically near the surface; and pH of the water sample used for plankton density estimates. Missing data are the result of equipment malfunction. The day of stocking is marked with an arrow.

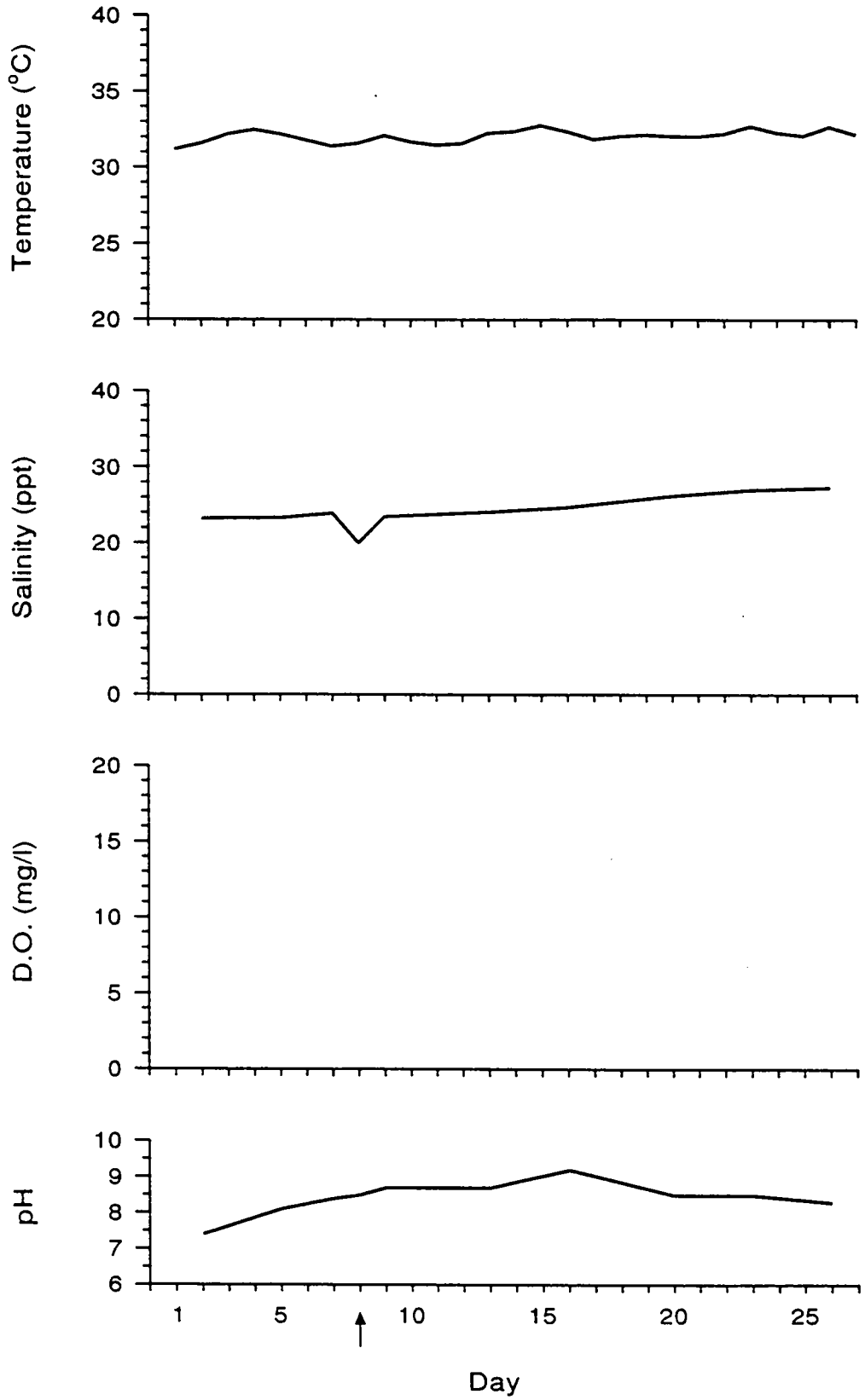

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
Day

Figure 7.38 Air temperature at 0300 and 1500, cloud cover at 1500 and daily rainfall for Innisfail during pond trial P10 (12 December 1991 - 7 January 1992). The day of stocking is marked with an arrow.

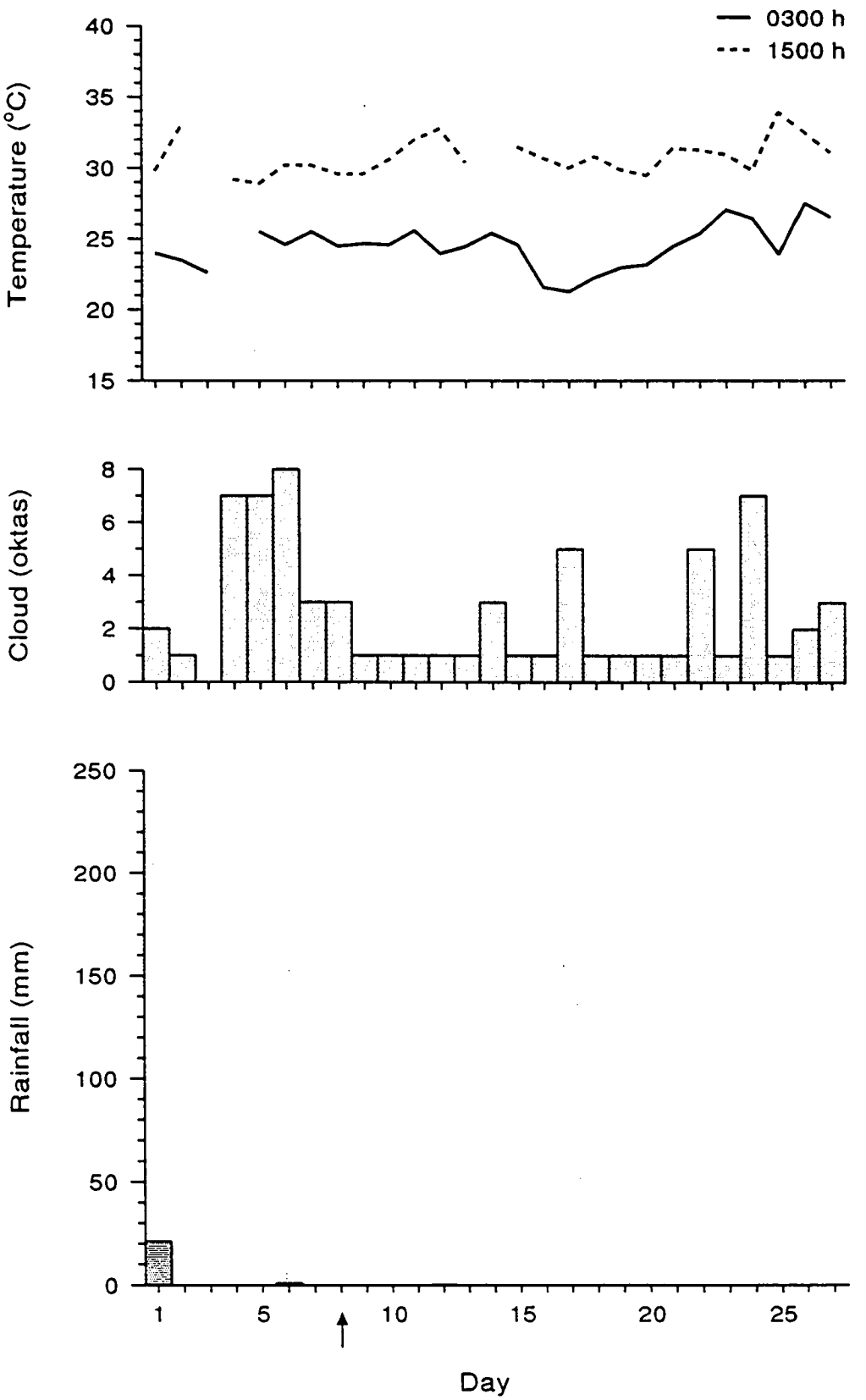

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
Day

Figure 7.39 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P10 (12 December 1991 - 7 January 1992). The day of stocking is marked with an arrow.

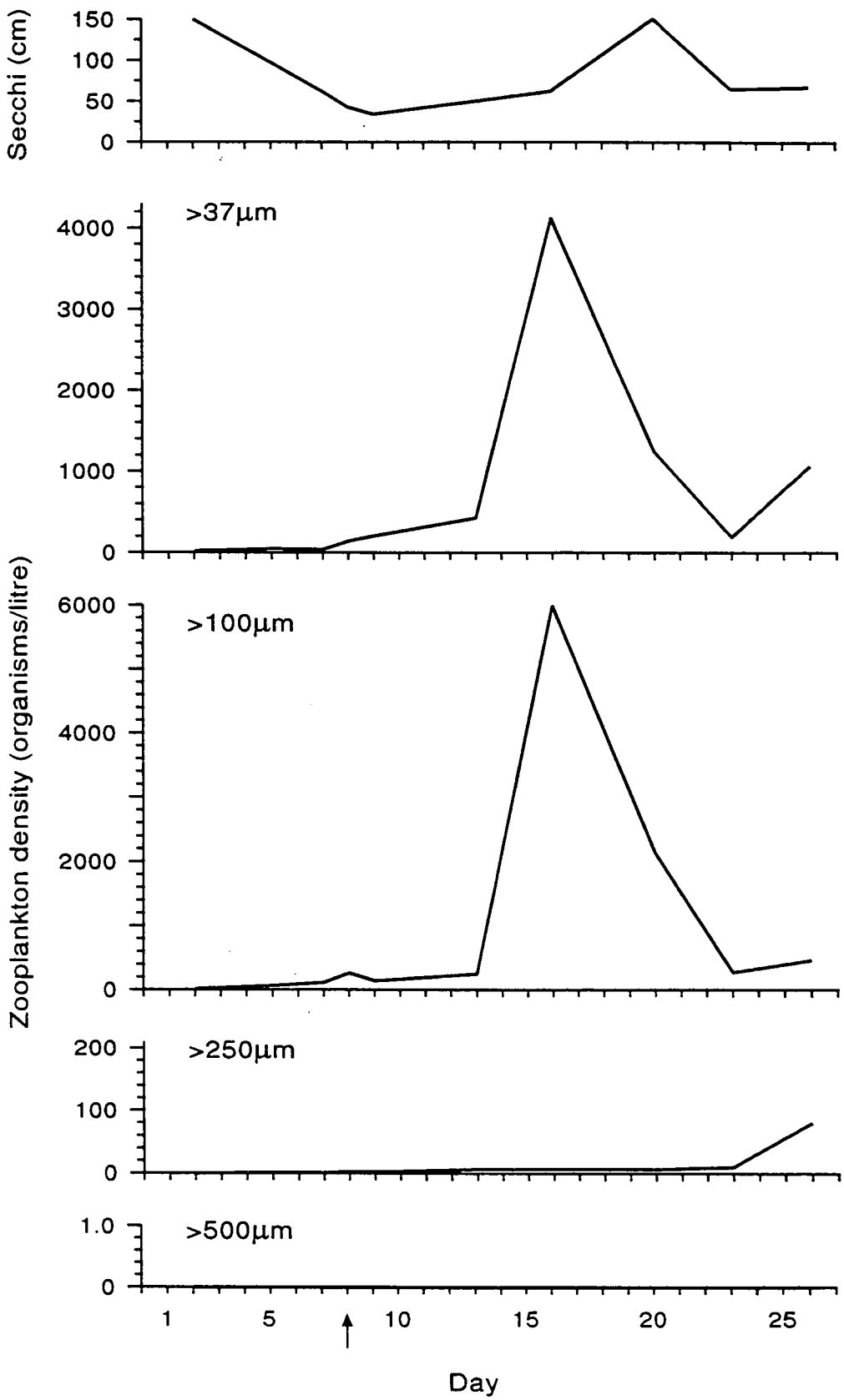
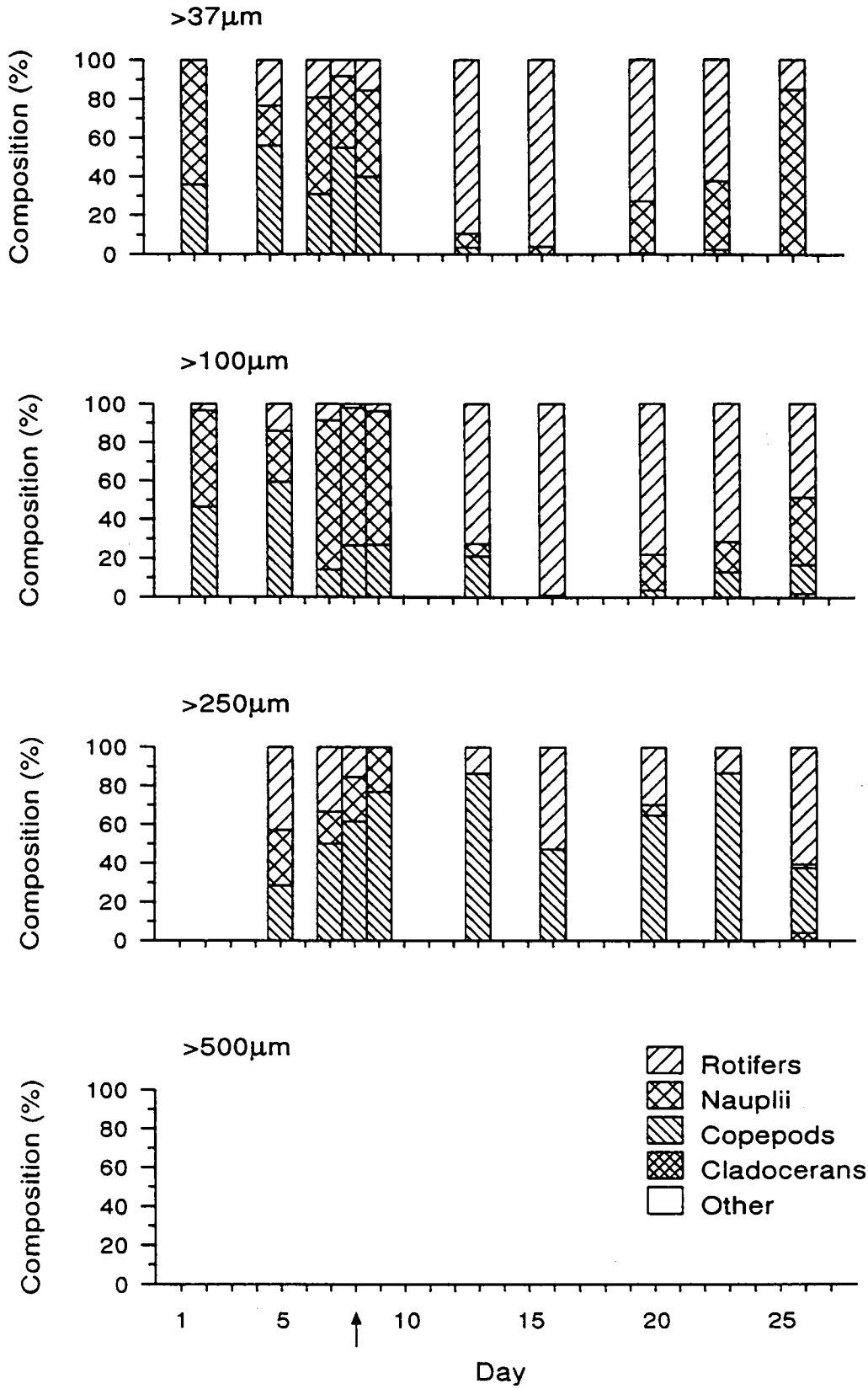

Secchi (cm)
150
100
50
0
>37µm
4000
3000
2000
1000
0
Zooplankton density (organisms/litre)
>100µm
6000
4000
2000
0
>250µm
200
100
0
>500µm
1.0
0
1
5
10
15
20
25
Day

Figure 7.40 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other organisms in sieved samples (Fig. 7.39) from pond trial P10 (12 December 1991 - 7 January 1992). The day of stocking is marked with an arrow.


>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
1
5
10
15
20
25
Day
Rotifers
Nauplii
Copepods
Cladocerans
Other

Trial P11: 14 January 1992 - 14 February 1992

The pond was left empty for about one week following pond trial P10 in order to thoroughly dry the substrate prior to the commencement of pond trial P11.

Mean daily water temperature in this trial ranged from 30.4 to 35.1°C and salinity remained relatively stable at about 28-30 ppt for the first week of the trial before decreasing to 15.0 ppt on day 28, and then increasing to 20.1 ppt prior to harvest (Fig. 7.41). The decrease in salinity during the latter part of the trial is attributable to moderate rain which fell during that time (Fig. 7.42); total rainfall for the trial was 224 mm (Table 7.3). Cloud cover was generally light during the first half of the trial, but increased during the last two weeks (Fig. 7.42). Limited dissolved oxygen data for this trial indicate that dissolved oxygen ranged from 4.4 to 8.9 mg/l. pH was relatively stable, ranging from 7.5 to 8.6 (Fig. 7.41).

Densities of zooplankton were exceptionally high during this trial. Densities of zooplankton in both the $>37\mu m$ and $>100\mu m$ size classes increased rapidly after the pond was filled and initially peaked on day 9 (Fig. 7.43). Densities then declined rapidly before increasing to peak again on day 21 ($>37\mu m$) and day 18 ($>100\mu m$). The first peaks in both these size classes are attributable to rotifers and copepod nauplii, while the second peak was attributable largely to rotifers (Fig. 7.44). Copepodites and copepod adults generally predominated in the $>250\mu m$ size class throughout the first three weeks of this trial (Fig. 7.44). Zooplankton densities in the $>250\mu m$ size class were low and this size class did

not reach peak density until day 25 (Fig. 7.43) when cladocerans predominated in this size class (Fig. 7.44).

70,000 barramundi larvae were stocked into the pond on day 10. Densities of zooplankton in the $>37\mu m$ and $>100\mu m$ size classes were extremely high at stocking: 4,283 and 2,673 organisms/litre respectively (Table 7.5). Larvae were first captured on day 14 (day 6 from hatching) in the light trap. The crude growth rate generally increased as the fish grew (Fig. 7.45a); overall growth for this trial was 1.7 mm/day over the duration of the trial (Table 7.2). Specific growth rate increased from 6.3 %/day for the period from day 10 to day 14 (days 2-6 from hatching) to 20.6 %/day to day 21, then decreased gradually to harvest (Fig. 7.45b); overall SGR for this trial was 12.8 %/day (Table 7.2).

The density of barramundi found on the lift nets averaged 32.3/m^2 during the course of the trial, giving a predicted population of 43,000 fish in the pond. 14,000 juvenile barramundi were harvested at the end of this trial, a survival rate of 20%. Harvested fish averaged 40.6 mm TL (range 33.2 - 47.1 mm TL) (Fig. 7.45c) at day 24 from hatching (677.5 degree-days).

Figure 7.41 Water quality parameters for pond trial P11 (14 January - 14 February 1992): daily mean temperature sampled near the surface at two-hourly intervals; salinity sampled opportunistically near the surface; daily minimum dissolved oxygen sampled hourly from near the pond bottom; and pH of the water sample used for plankton density estimates. Missing data are the result of equipment malfunction. The day of stocking is marked with an arrow.

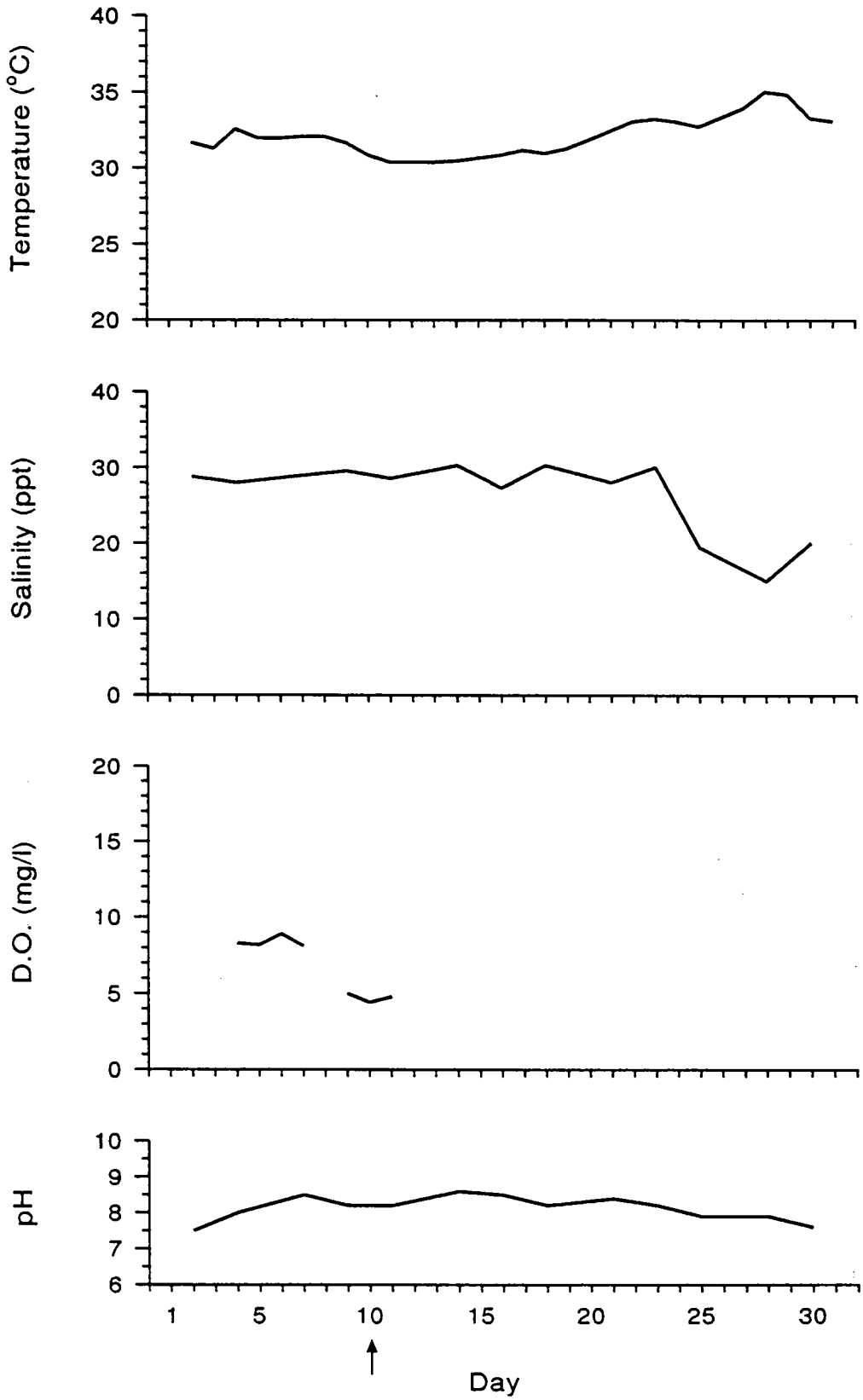

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
30
Day

Figure 7.42 Air temperature at 0300 and 1500, cloud cover at 1500 and daily rainfall for Innisfail during pond trial P11 (14 January - 14 February 1992). The day of stocking is marked with an arrow.

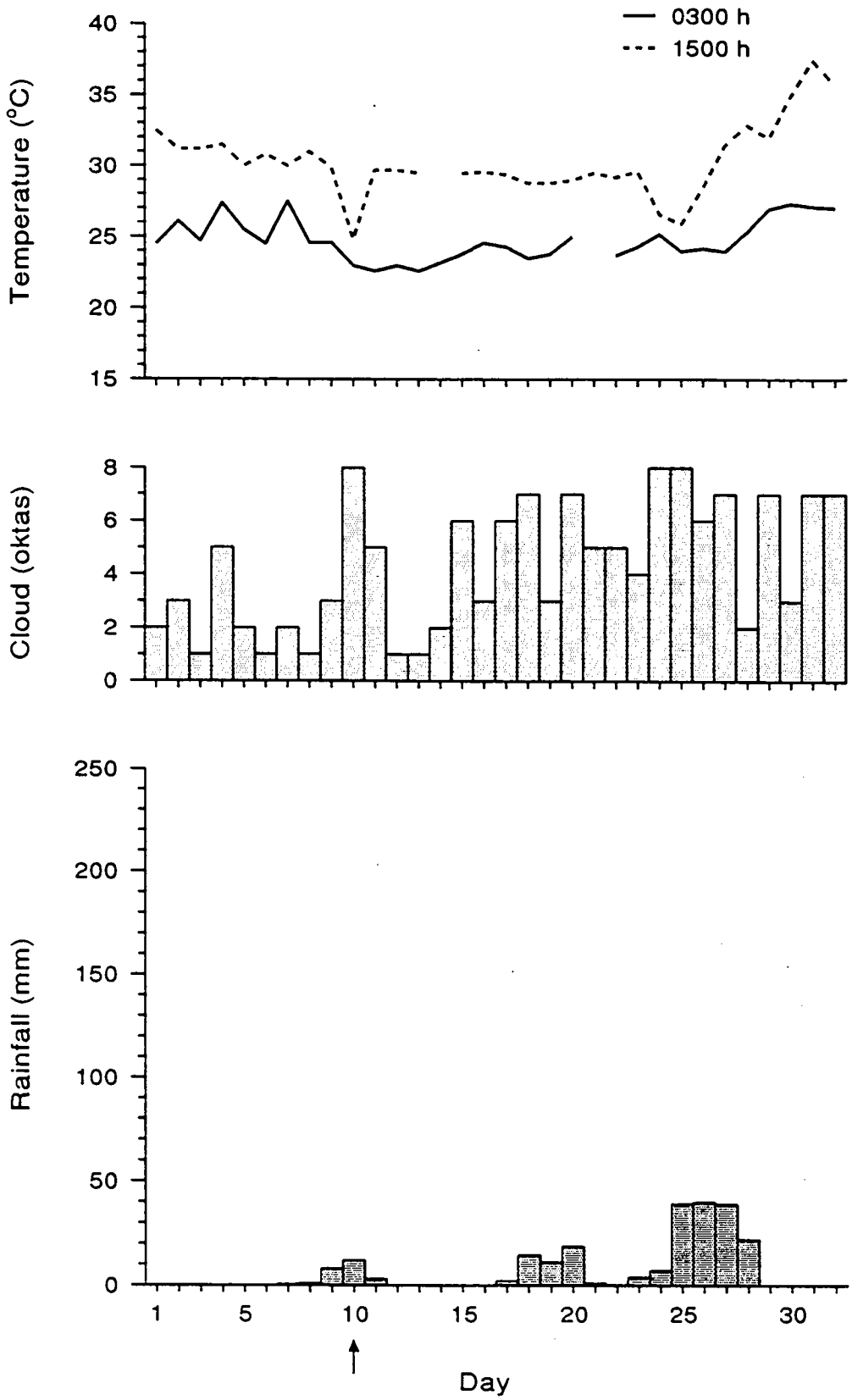

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
30
Day

Figure 7.43 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P11 (14 January - 14 February 1992). Note that the y-axis range for the $>37\mu$m and $>100\mu$m size classes has been increased in this figure due to the high densities of zooplankton during this trial. The day of stocking is marked with an arrow.

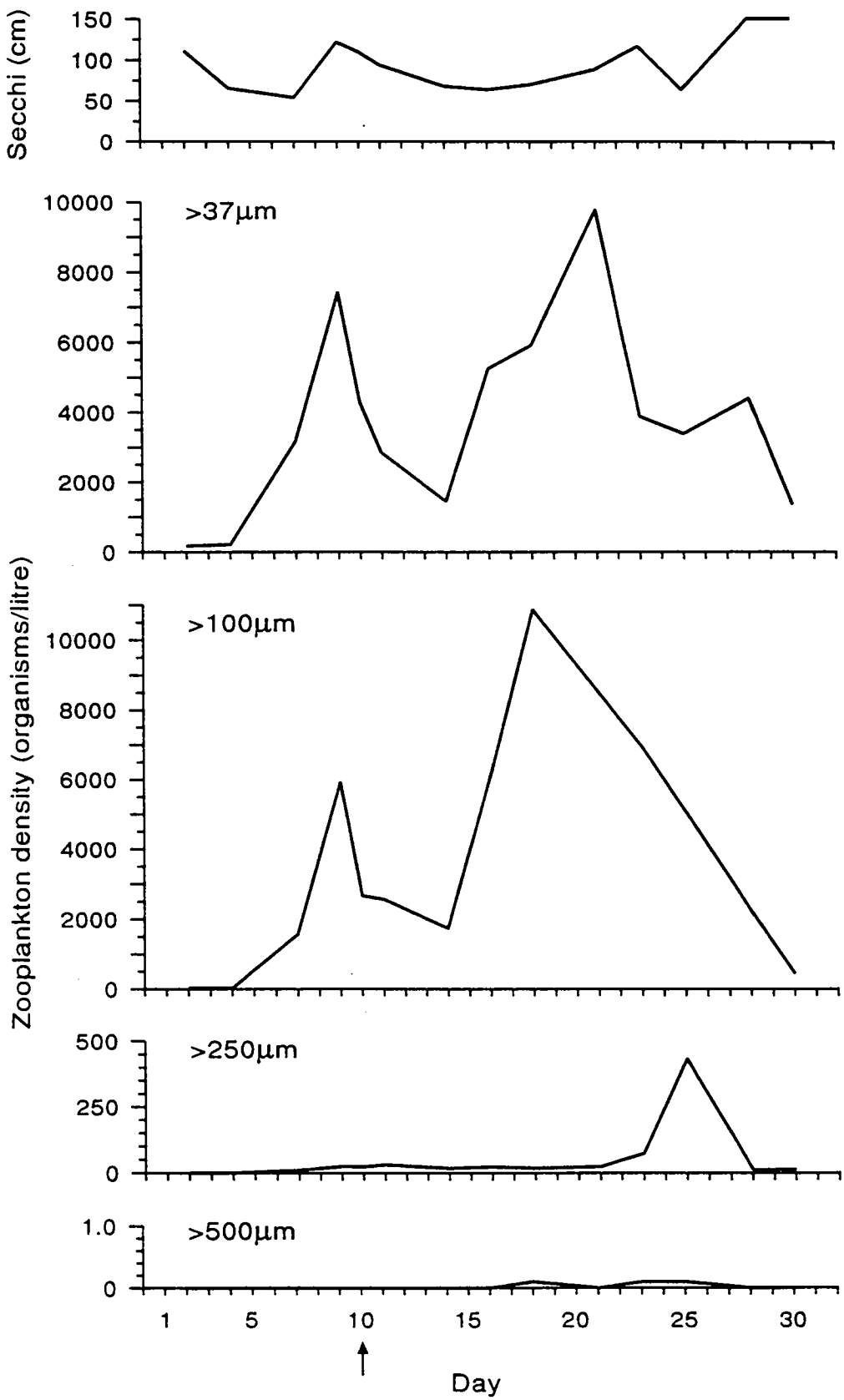

Secchi (cm)
150
100
50
0
>37µm
10000
8000
6000
4000
2000
0
Zooplankton density (organisms/litre)
>100µm
10000
8000
6000
4000
2000
0
>250µm
500
250
0
>500µm
1.0
0
1
5
10
15
20
25
30
Day

Figure 7.44 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other organisms in sieved samples (Fig. 7.43) from pond trial P11 (14 January - 14 February 1992). The day of stocking is marked with an arrow.

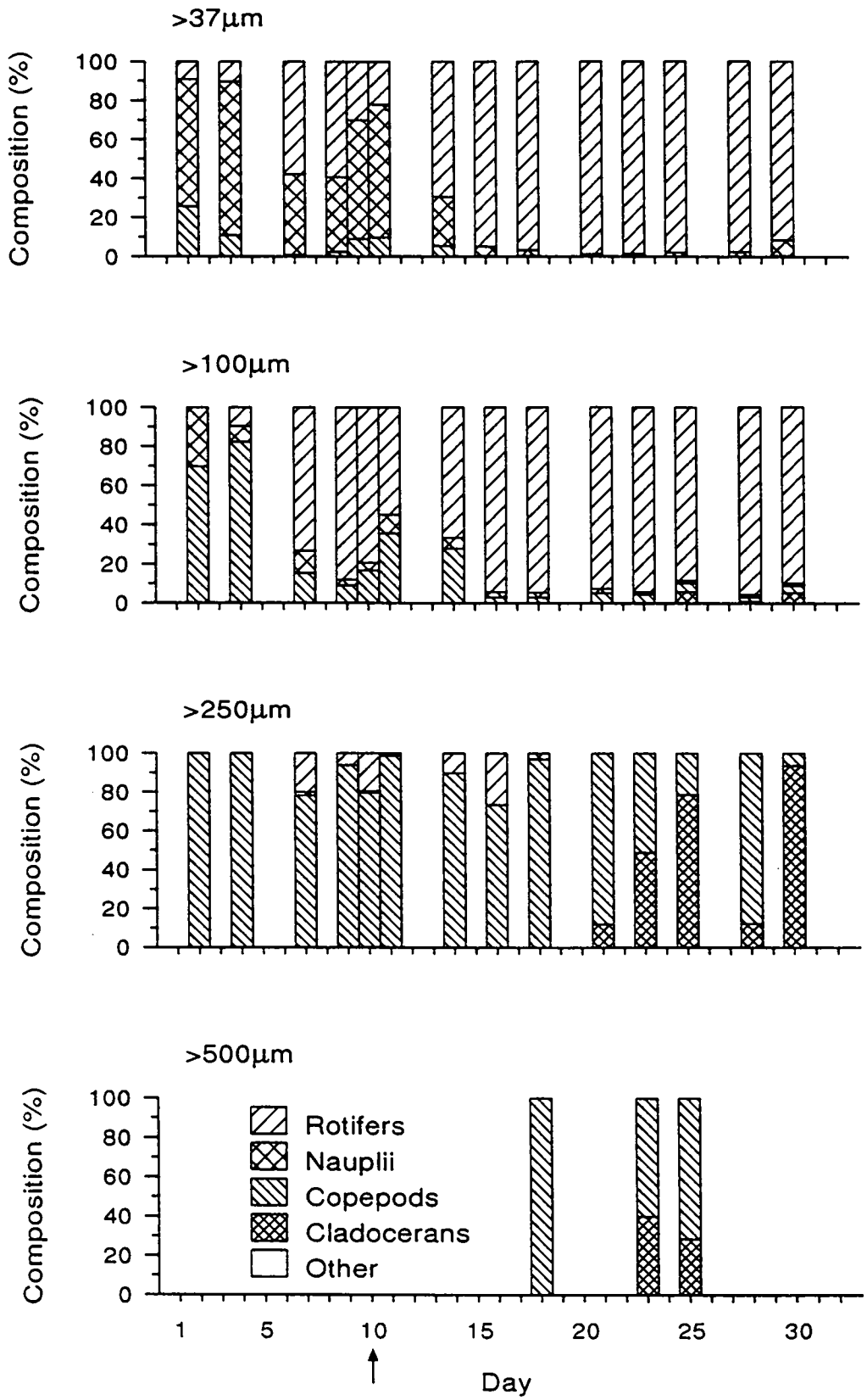
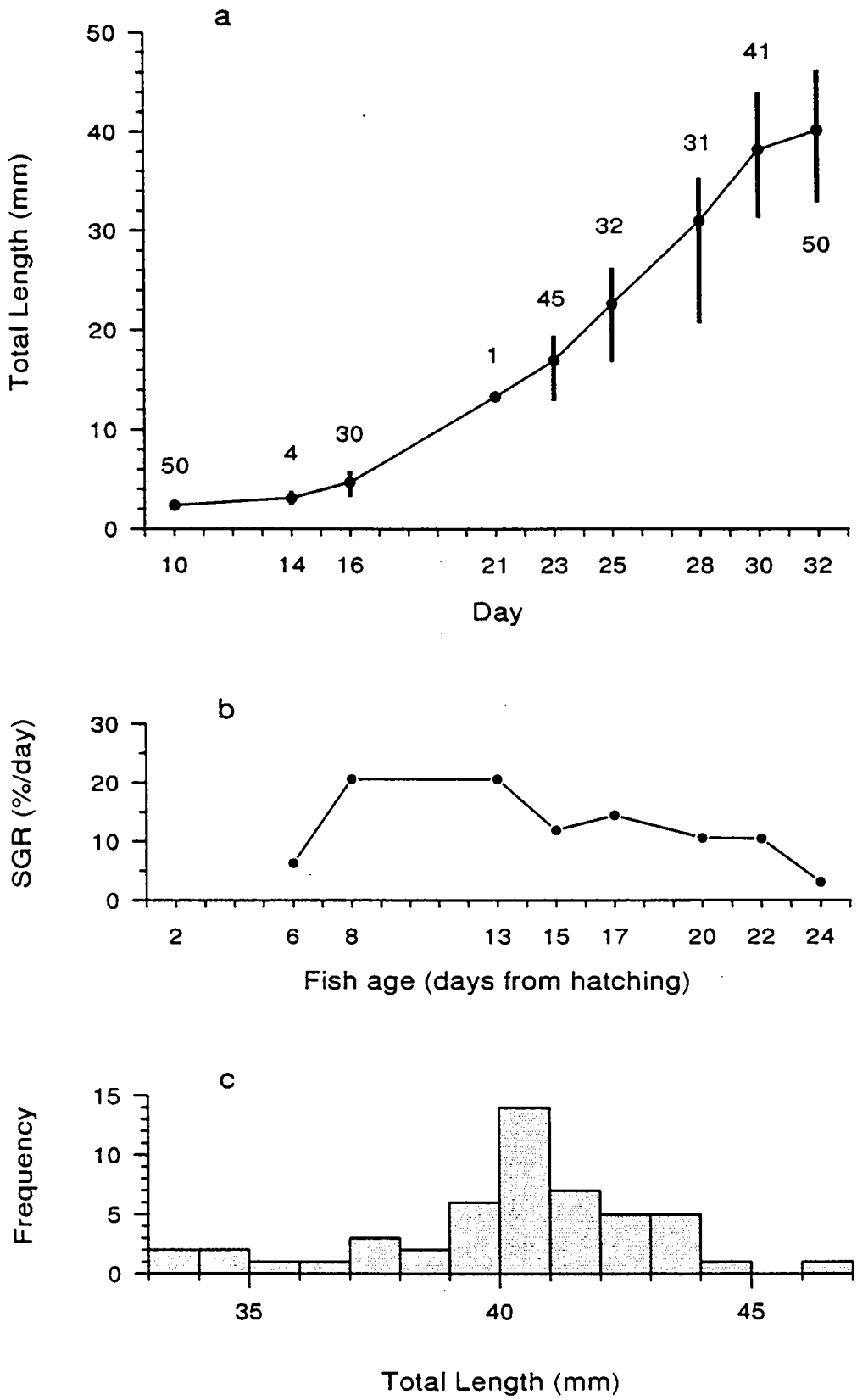

>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Other
1
5
10
15
20
25
30
Day

Figure 7.45 (a) Growth of barramundi during pond trial P11 (14 January - 14 February 1992). Mean and range of each sample shown; numbers above or beneath bars represent sample size. (b) Specific growth rate (SGR) for barramundi during pond trial P11. (c) Size frequency of barramundi harvested from pond trial P11 (n=50).


a
Total Length (mm)
50
40
30
20
10
0
50
4
30
1
45
32
31
41
50
10
14
16
21
23
25
28
30
32
Day
b
SGR (%/day)
30
20
10
0
2
6
8
13
15
17
20
22
24
Fish age (days from hatching)
c
Frequency
15
10
5
0
35
40
45
Total Length (mm)

Trial P12: 18 February - 20 March 1992

Aeration was employed throughout this trial to examine the effects on water quality, and survival and growth of larvae. Mean daily water temperature during this trial ranged from 29.5 to 33.0°C. Salinity decreased from around 29 ppt at the beginning of the trial to a low of 16.1 ppt on day 23, then increased to 20.5 ppt prior to harvest (Fig. 7.46). This decrease in salinity is attributable to moderate rainfall from day 8 to day 15 (Fig. 7.47); total rainfall for the trial was 268 mm (Table 7.3). Cloud cover was heavy during the second week, but was generally light during the rest of the trial (Fig. 7.47). Dissolved oxygen ranged from 3.7 to 7.1 mg/l during the trial, and pH remained relatively stable, ranging from 7.6 to 8.7 (Fig. 7.46).

Densities of zooplankton in both the $>37\mu m$ and $>100\mu m$ size classes increased rapidly after the pond was filled and both size classes peaked on day 7 (Fig. 7.48). Rotifers and copepod nauplii predominated in the $>37\mu m$ samples at this time, while rotifers predominated in the $>100\mu m$ samples (Fig. 7.49). After peaking, densities of zooplankton in both the $>37\mu m$ and $>100\mu m$ size classes decreased steadily. Zooplankton in the $>37\mu m$ size class began to increase rapidly in density just prior to harvesting (Fig. 7.48). Copepodites and copepod adults initially predominated in the $>250\mu m$ size class, followed by cladocerans (Fig. 7.49). Zooplankton densities in the $>250\mu m$ size class peaked at day 16-18 (Fig. 7.48). Zooplankton in the $>500\mu m$ size class were generally at low density, but showed two peaks in density on days 14 and 18 (Fig. 7.48).

One hundred thousand barramundi larvae were stocked into the pond; 50,000 on day 10 and another 50,000 on day 11. (For convenience, and because it was impossible to separate the different batches of fish, all measurements have been taken from the day 10 stocking). Zooplankton densities at stocking were very high: 1,981 and 1,769 organisms/litre for the $>37\mu m$ and $>100\mu m$ size classes respectively (Table 7.5). Barramundi larvae were first captured on day 14 (day 6 from hatching) in the $300\mu m$ mesh size zooplankton net. Growth rate averaged 1.4 mm/day over the duration of the trial, with fastest growth in the period from day 21 to day 25 and day 28 to day 32 (Fig. 7.50a). Specific growth rate increased from 3.6 %/day on day 14 (day 6 from hatching) to a maximum of 28.2 %/day (the highest SGR recorded during these trials) on day 16, before decreasing again to 8.0 %/day on day 32 (Fig. 7.50b); overall SGR for this trial was 11.7 %/day (Table 7.2).

The density of barramundi found on the lift nets averaged 32.2/m^2 during the course of the trial, giving a predicted population of 43,000 fish in the pond. 86,000 juvenile barramundi were harvested at the end of this trial, a survival rate of 86%. Harvested fish averaged 33.2 mm TL (range 25.9 - 39.7 mm TL) (Fig. 7.50c) at day 24 from hatching (659.7 degree-days). Despite the two stockings of larvae in this trial, the frequency distribution of fish harvested was effectively unimodal (Fig. 7.50c).

Figure 7.46 Water quality parameters for pond trial P12 (18 February - 20 March 1992): daily mean temperature, daily mean salinity, and daily minimum dissolved oxygen, sampled four-hourly from near the pond bottom; and pH of the water sample used for plankton density estimates. Missing data are the result of equipment malfunction. The days of stocking are marked with arrows.

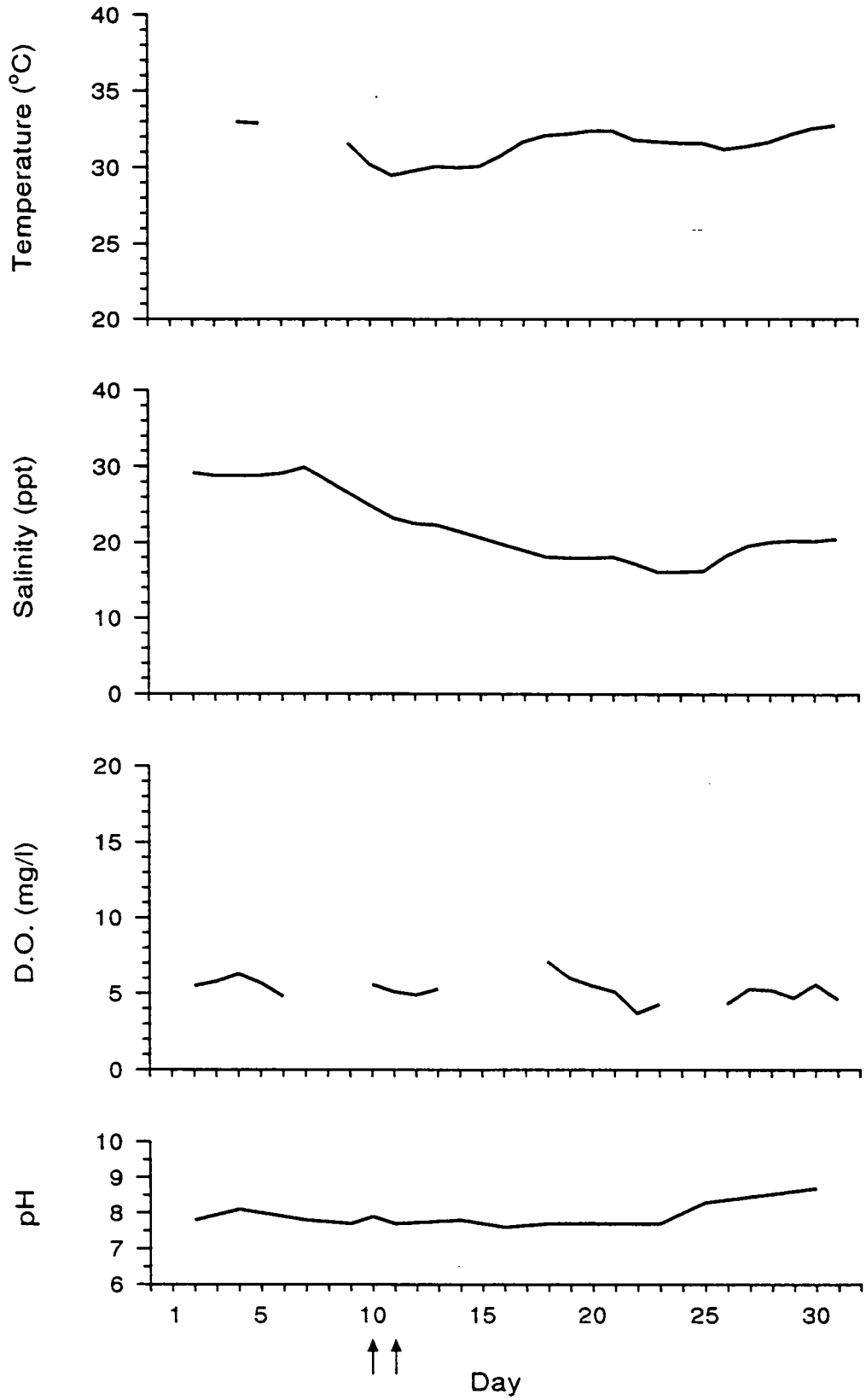

Temperature (°C)
40
35
30
25
20
Salinity (ppt)
40
30
20
10
0
D.O. (mg/l)
20
15
10
5
0
pH
10
9
8
7
6
1
5
10
15
20
25
30
Day

Figure 7.47 Air temperature at 0300 and 1500, cloud cover at 1500 and daily rainfall for Innisfail during pond trial P12 (18 February - 20 March 1992). The days of stocking are marked with arrows.

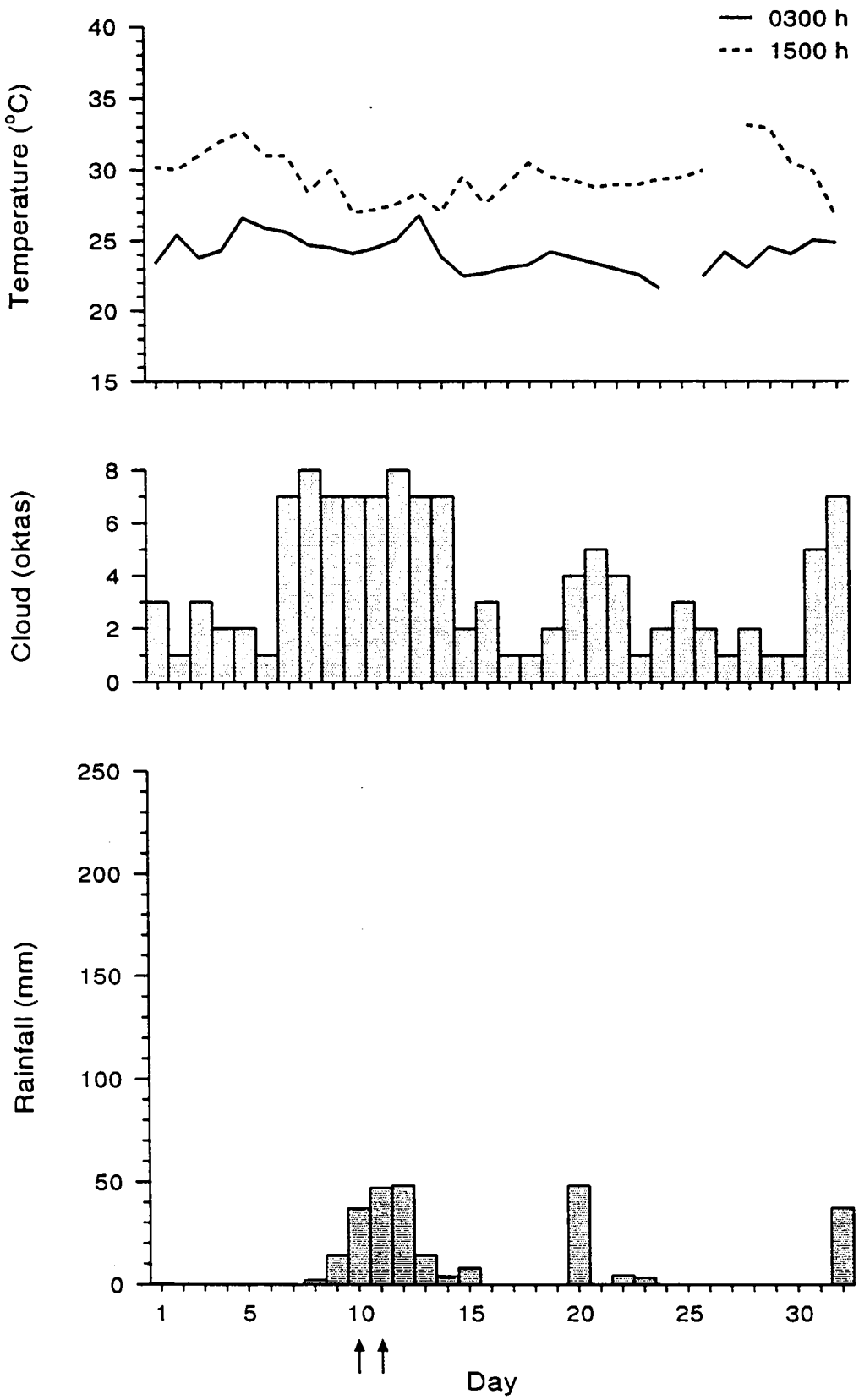

0300 h
1500 h
Temperature (°C)
40
35
30
25
20
15
Cloud (oktas)
8
6
4
2
0
Rainfall (mm)
250
200
150
100
50
0
1
5
10
15
20
25
30
Day

Figure 7.48 Secchi disk visibility and densities of zooplankton sampled using a vertical tube sampler and sieved into various size classes during pond trial P12 (18 February - 20 March 1992). The days of stocking are marked with arrows.

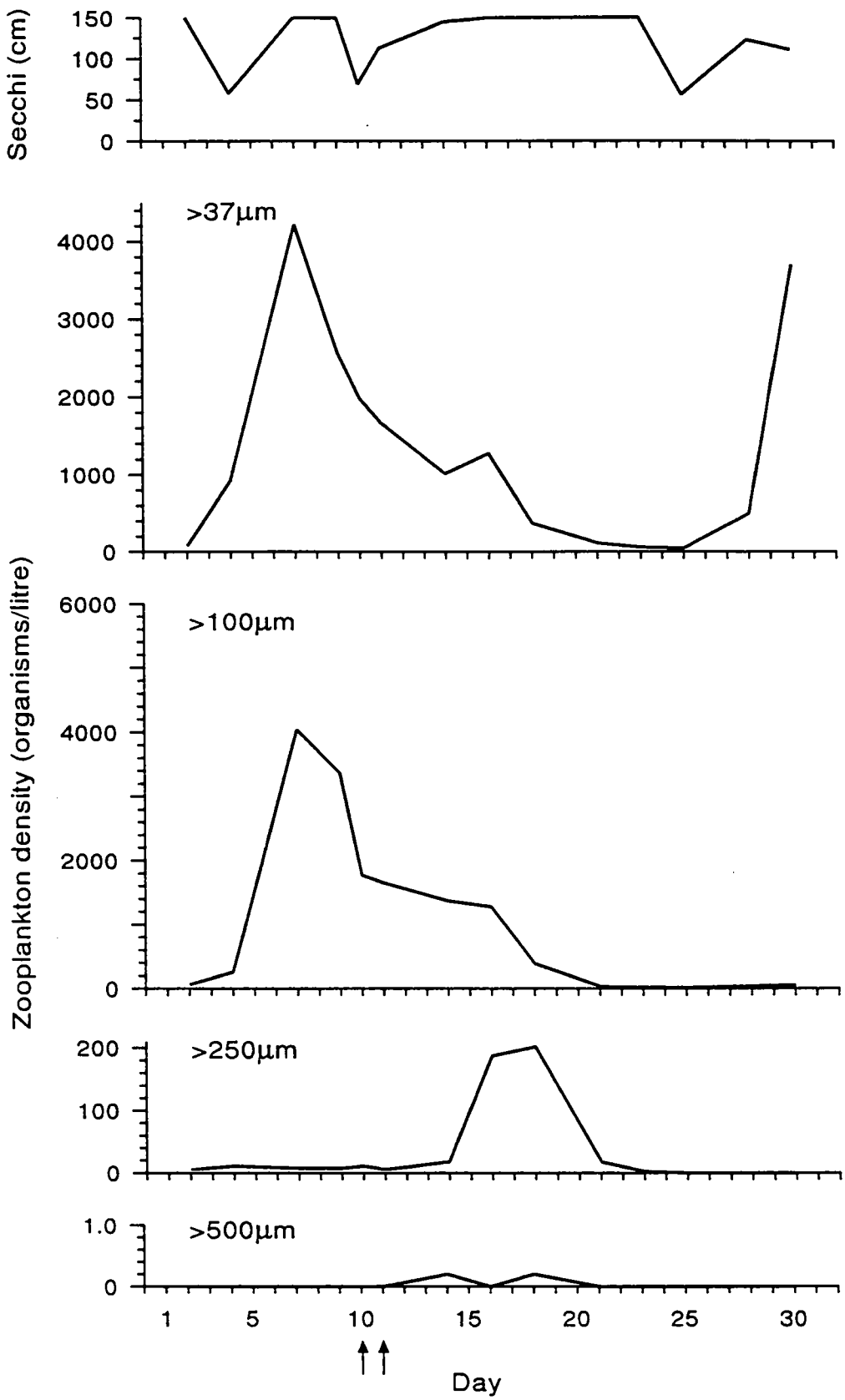

Secchi (cm)
150
100
50
0
>37µm
4000
3000
2000
1000
0
Zooplankton density (organisms/litre)
6000
>100µm
4000
2000
0
200
>250µm
100
0
1.0
>500µm
0
1
5
10
15
20
25
30
Day

Figure 7.49 Relative proportions of rotifers, copepod nauplii, copepods (copepodites and copepod adults), cladocerans and other organisms in sieved samples (Fig. 7.48) from pond trial P12 (18 February -20 March 1992). The days of stocking are marked with arrows.

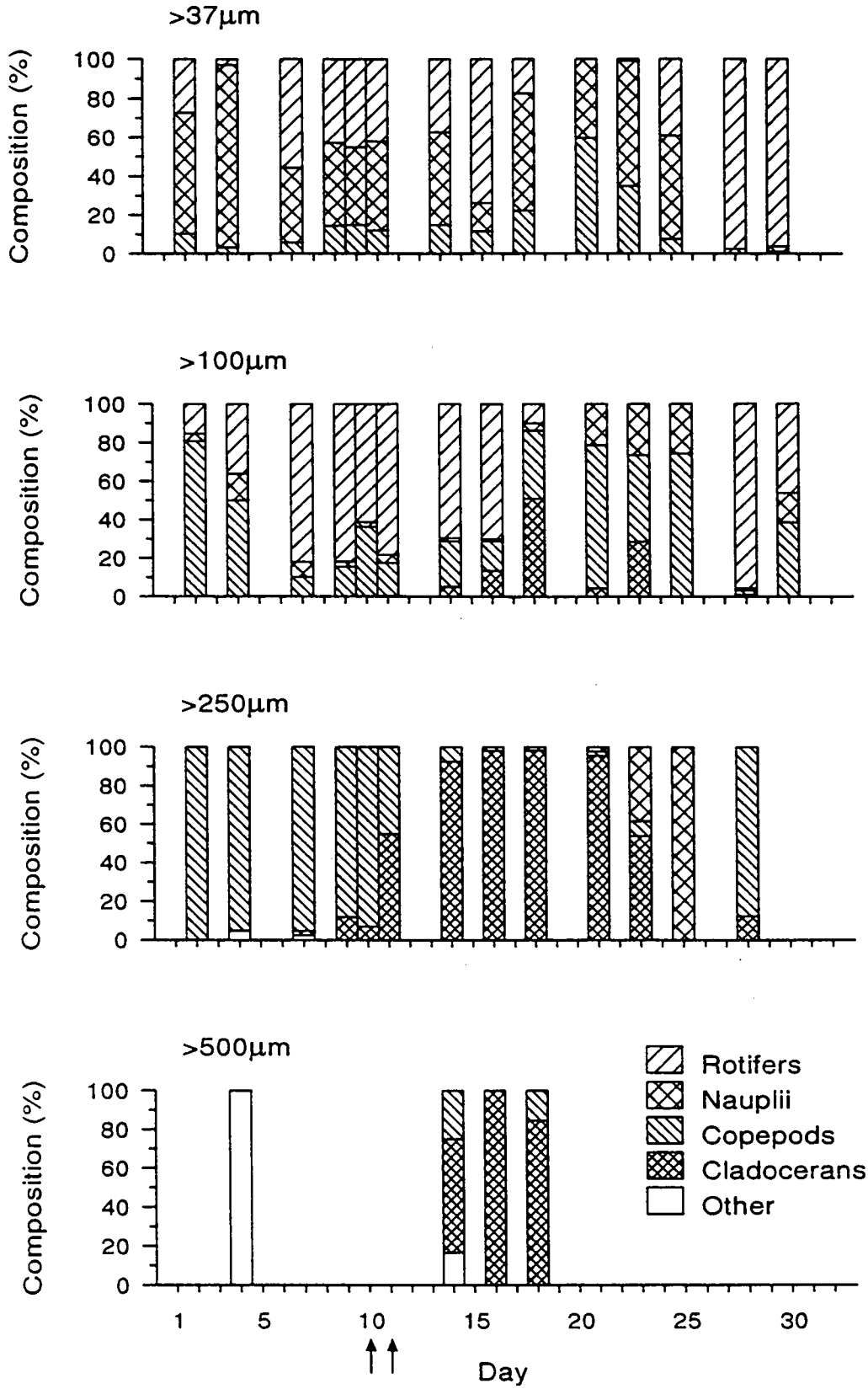
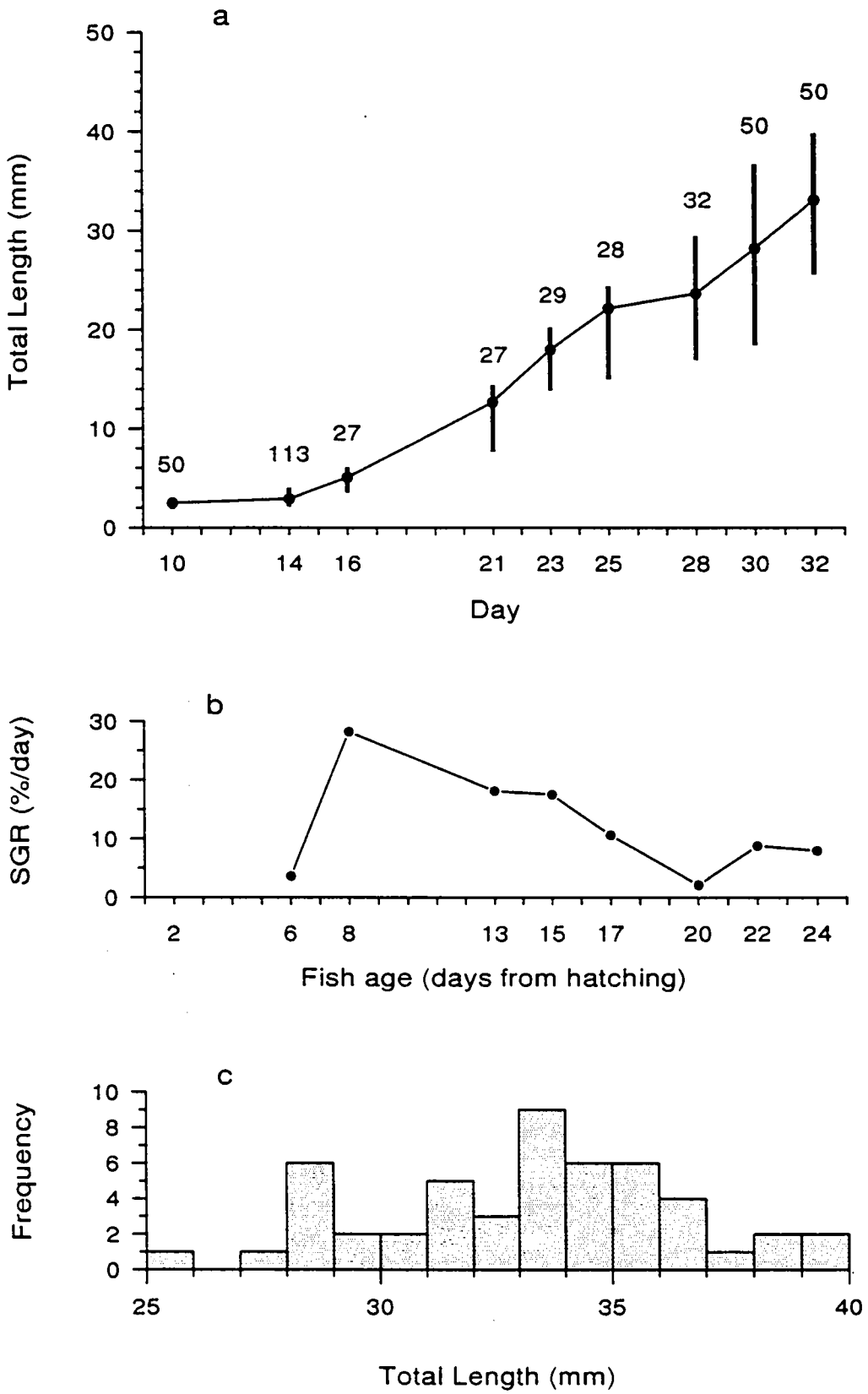

>37µm
>100µm
>250µm
>500µm
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Other
1
5
10
15
20
25
30
Day

Figure 7.50 (a) Growth of barramundi during pond trial P12 (18 February - 20 March 1992). Mean and range of each sample shown; numbers above or beneath bars represent sample size. (b) Specific growth rate (SGR) for barramundi during pond trial P12. (c) Size frequency of barramundi harvested at the end of pond trial P12 (n=50).


a
Total Length (mm)
50
40
30
20
10
0
50
113
27
27
29
28
32
50
50
10
14
16
21
23
25
28
30
32
Day
b
SGR (%/day)
30
20
10
0
2
6
8
13
15
17
20
22
24
Fish age (days from hatching)
c
Frequency
10
8
6
4
2
0
25
30
35
40
Total Length (mm)

Technique Evaluation

As noted previously, several sampling techniques were evaluated to determine whether less time-intensive sampling procedures could be adopted by commercial aquaculturists with minimal decrease in accuracy.

Phytoplankton Abundance

Chlorophyll *a* concentration was found to be negatively correlated with Secchi disk visibility, with the untransformed data indicating a curvilinear relationship (Fig. 7.51a). The linear correlation model which most closely fitted the data was a correlation of the Secchi disk visibility and natural logarithm (ln) of chlorophyll *a* (Fig. 7.51b) which gave an adjusted r^2 value of 0.58 ($F=32.7$, $P<0.01$)

Measurement of Secchi disk visibility is a technique that is readily available to commercial aquaculturists who generally do not have the expertise or equipment to perform analyses of chlorophyll *a* concentration. The correlation between chlorophyll *a* and Secchi disk visibility indicates that Secchi disk visibility is an accurate indicator of phytoplankton density in ponds where most turbidity results from phytoplankton blooms, such as larval rearing ponds. Although suspended clay colloids can also affect turbidity, the presence of high concentrations of suspended particles in ponds can be readily identified by farmers, and Secchi readings can be discontinued when this problem arises (e.g. during periods of heavy rainfall that cause erosion of pond banks). Almazan and Boyd (1978) found a similar relationship between chlorophyll *a* and Secchi disk visibility in

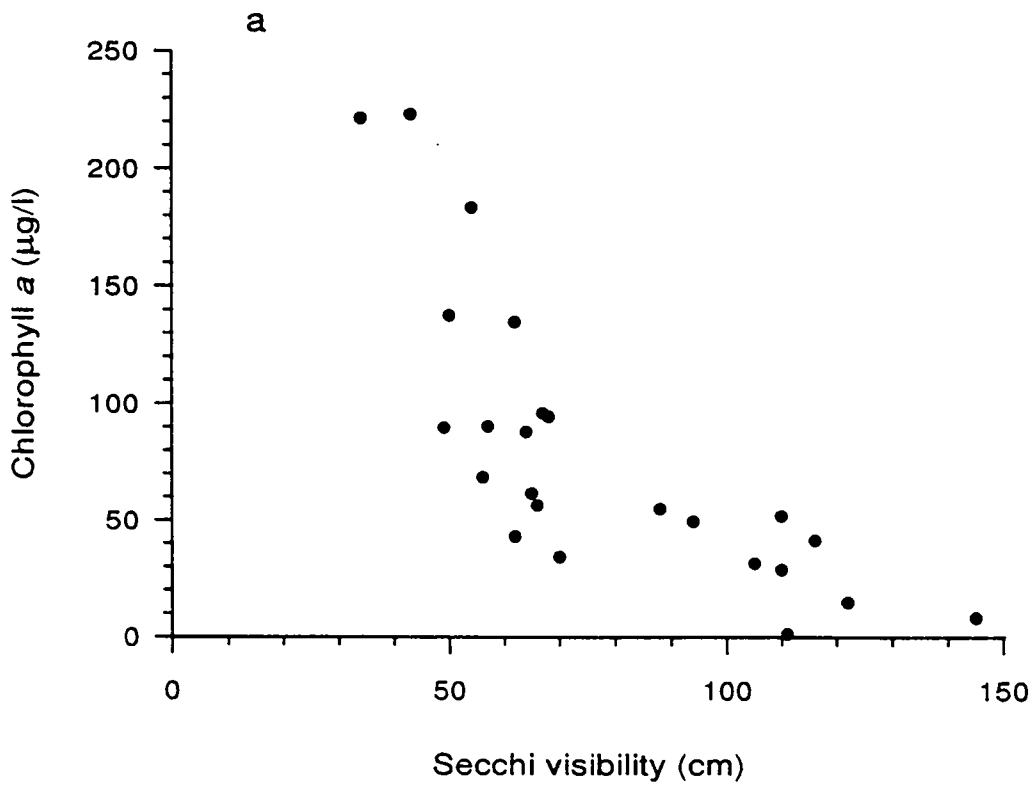
freshwater ponds, although a slightly less accurate relationship between Secchi disk visibility and numbers of phytoplankters in the pond.

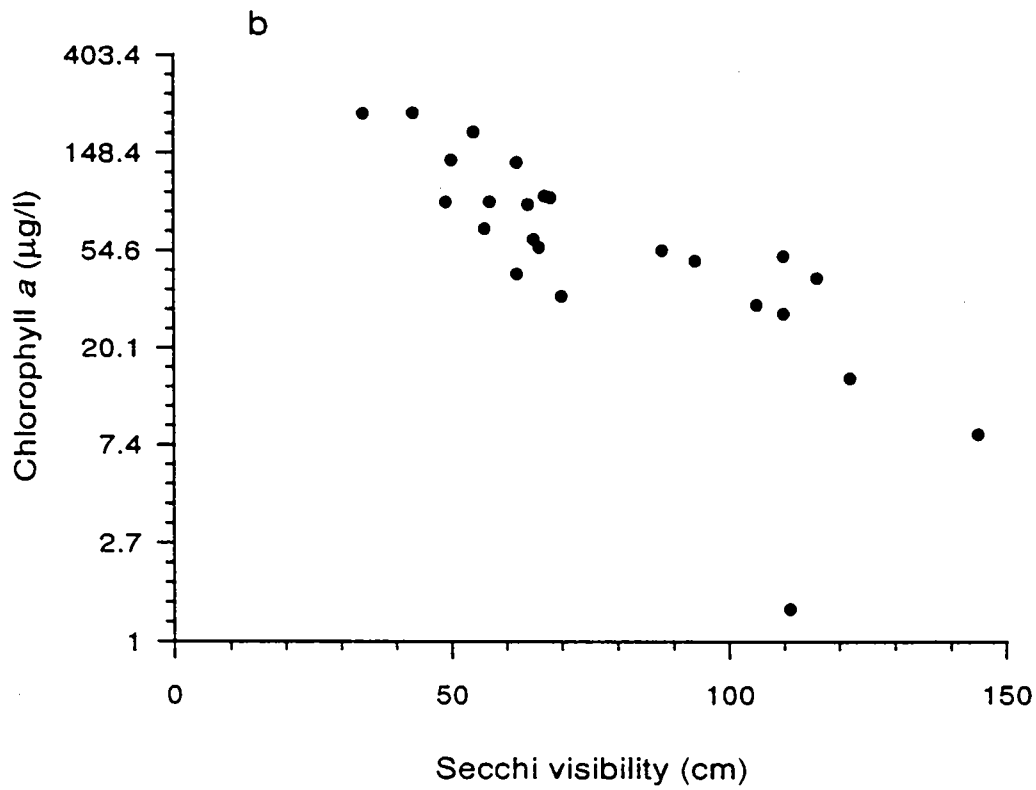
The logarithmic relationship between chlorophyll *a* concentration and Secchi disk visibility has important implications for pond management. Phytoplankton density, as estimated by chlorophyll *a* concentration, increases at an exponential rate with respect to decreasing Secchi disk visibility. Thus at high phytoplankton densities, even small changes in Secchi disk visibility indicate major changes in phytoplankton density. Very high densities of phytoplankton can result in low dissolved oxygen concentrations due to overnight respiration and can thus directly affect survival of the fish in the pond (Boyd 1990). High densities of phytoplankton may also cause elevated pH due to the use of carbon dioxide during photosynthesis (Boyd 1990).

Boyd (1990) recommends Secchi disk visibility of 40 to 60 cm in ponds, whereas Secchi disk visibility recommended for the extensive larval rearing of red drum is about 30 cm (W.P. Rutledge, pers. comm.). Secchi disk visibility in this study was rarely less than 50 cm and commonly ranged from 50 to 100 cm. A disadvantage of low phytoplankton densities is that this allows light to reach the pond bottom which usually results in the growth of filamentous green algae and other benthic algae. These algae can form dense 'mats' which trap fish during harvesting. However, this problem was not experienced during the present study, despite the frequent occurrence of lower phytoplankton densities than are recommended for larval rearing of red drum.

Although cyanobacteria may proliferate in the eutrophic environment of larval rearing ponds (as discussed in Chapter 6), only a single outbreak of cyanobacteria was recorded during this study. This occurred during pond trial P9 and, as noted above, was managed by overflowing the pond to remove the floating cyanobacterial cells. No adverse effects were recorded during the cyanobacterial bloom, presumably because it was relatively small in extent and was successfully controlled by overflowing the pond.

Figure 7.51 Relationship between chlorophyll *a* and Secchi disk visibility plotted using (a) linear axes, and (b) natural log - linear axes. Correlation coefficient for (b): adjusted $r^2 = 0.58$.


a
250
200
150
100
50
0
Chlorophyll a (µg/l)
0
50
100
150
Secchi visibility (cm)


b
403.4
148.4
54.6
20.1
7.4
2.7
1
Chlorophyll a (µg/l)
0
50
100
150
Secchi visibility (cm)

Zooplankton Abundance

The zooplankton populations which developed in the pond used for this study exhibited a relatively low diversity. Both the rotifers and the cladocerans were represented only by a single species (Table 7.6). Because of resource limitations, only a limited number of zooplankton samples were examined, and these samples contained only 4 copepod species (Table 7.6). It is expected that further sampling may yield additional copepod species.

Table 7.6 Dominant members of the zooplankton fauna from the pond used in this study. Rotifer identified by R. Shiel, Murray-Darling Freshwater Research Centre, Albury-Wodonga, NSW. Copepods and cladoceran identified by D. MacKinnon, Australian Institute of Marine Science, Townsville, Qld.

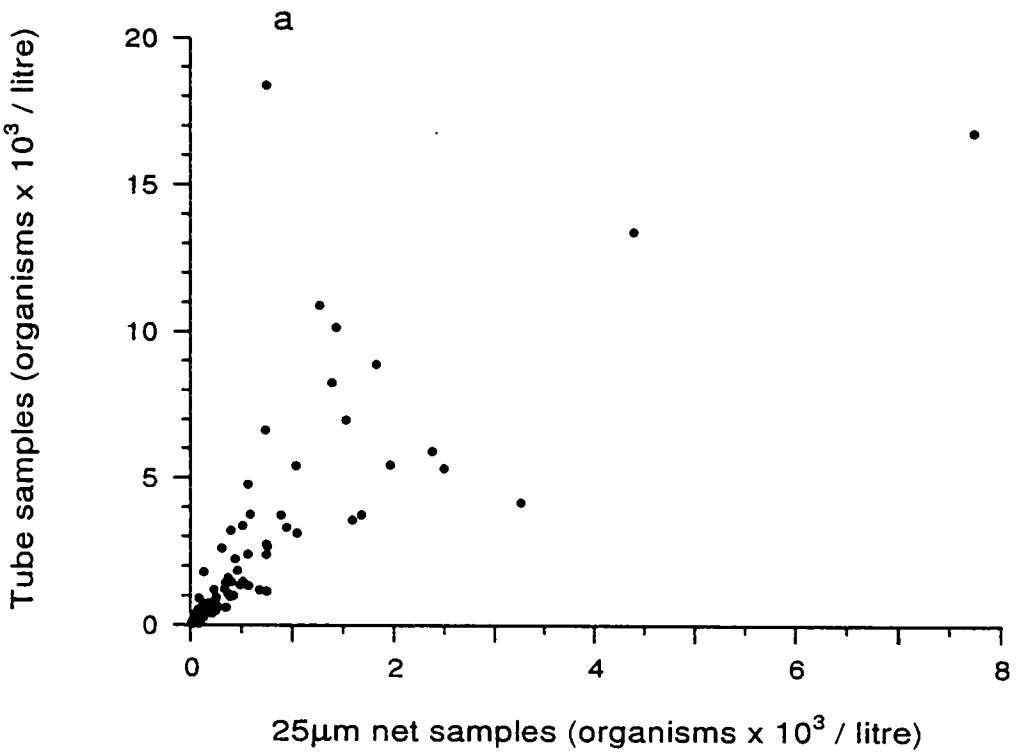
Phylum	Class	Species
Rotifera		*Brachionus plicatilis*
Crustacea	Copepoda	*Acartia* sp.
		Oithona sp.
		Pseudodiaptomus sp.
		Parvocalanus crassirostris
	Cladocera	*Diaphanosoma* sp.

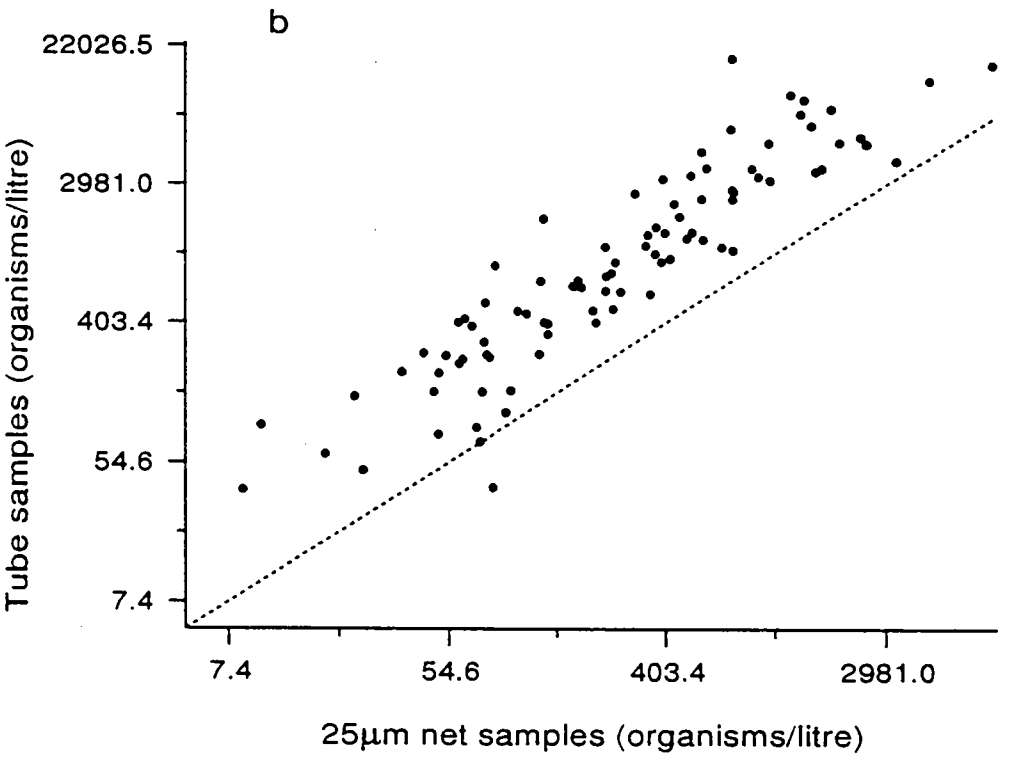
Total densities of zooplankton (i.e. with different zooplankton groups and size classes pooled) sampled using a 25μm plankton net and a vertical tube sampler are compared in Figure 7.52. There was a very poor correlation between the two zooplankton sampling methods using untransformed data (Fig. 7.52a). However, transformation of both variables using natural logarithms (ln) resulted in a significant correlation (adjusted r^2= 0.83; F= 450.5, P<0.01) (Fig. 7.52b). Total densities of zooplankton sampled using the vertical tube method were consistently greater than those obtained using the 25μm plankton net (Fig. 7.52). Similar results have been recorded by other researchers comparing tube samplers and plankton nets for estimating zooplankton abundance (Graves and Morrow 1988, DeVries and Stein 1991).

These results suggest that the plankton net consistently underestimated zooplankton density. A common problem with the use of fine-mesh nets for sampling zooplankton is that the meshes rapidly clog with small zooplankters or large phytoplankters (Graves and Morrow 1988, DeVries and Stein 1991), which causes the net to stop filtering and push planktonic organisms in front of the net on its 'bow wave'. Plankton nets become less accurate in estimating zooplankton density when zooplankton densities are high because clogging occurs more rapidly. The logarithmic relationship between net samples and vertical tube samples obtained in this study suggests that this was the case with the 25μm plankton net, since the variance of estimates increased with increasing zooplankton density (Fig. 7.52a).

Despite these drawbacks, plankton nets are suitable for use in commercial applications where accuracy is less critical and relative, rather than absolute, estimations of zooplankton density can be used. However, increasing the mesh size of the zooplankton net would help prevent rapid clogging and increase the accuracy of the estimates. Analysis of the diet of extensively reared barramundi larvae (Chapter 8) indicates that the minimum size of zooplankton ingested by barramundi is about 60μm, indicating that a mesh size of 50μm width (rather than the 25μm width used in this study) would be suitable to obtain density estimates of the zooplankton prey of barramundi larvae. An ideal compromise between these two techniques is the apparatus described by Farquhar and Geiger (1984) which utilises a truck-mounted vertical tube sampler and pump to obtain a known volume of pond water which is then filtered through a plankton net.

Figure 7.52 Correlation of total zooplankton sampled using a 25μm net and a vertical tube sampler plotted on (a) untransformed axes and (b) natural logarithmic (ln) axes. The broken line indicates the 'line of equivalence', i.e. the relationship which would be observed if the total zooplankton densities estimated using these two techniques were equivalent. Correlation coefficient for (b): adjusted $r^2 =$ 0.83.


a
Tube samples (organisms x 10^3 / litre)
20
15
10
5
0
0
2
4
6
8
25µm net samples (organisms x 10^3 / litre)


b
Tube samples (organisms/litre)
22026.5
2981.0
403.4
54.6
7.4
7.4
54.6
403.4
2981.0
25µm net samples (organisms/litre)

Fish Abundance

Because survival of fish in extensive rearing ponds is generally determined by early larval survival (Rowland 1986, Rutledge 1988, Turner 1988, McCarty and Gregg 1992), estimation of early larval survival is essential to avoid wasting resources on pond trials which have low or negligible survival. Stocking success (i.e. short-term survival after stocking) in red drum and striped bass larval rearing ponds is measured two days after stocking using a standardised plankton net sampling technique; if fewer than a set number of larvae are sampled, poor survival is indicated and the pond is restocked with larvae (Rutledge 1988, Turner 1988, McCarty and Gregg 1992).

Numerous attempts were made to sample barramundi larvae from the pond during pond trials P3 - P11 using 150μm and 300μm mesh plankton nets. Samples were taken from all depths in the pond, including samples taken just above the substrate and along the raceway. At the stocking densities used in these trials (50,000 to 100,000 larvae) the density of larvae in the pond would have been 25-50 larvae/m^3, assuming the larvae were distributed throughout the water column. The plankton nets used sampled a water volume of approximately 0.5m^3 on each tow, and thus should have captured between 12 and 25 larvae per tow if the larvae were distributed evenly throughout the water column. Most tows resulted in no larval captures (although on one occasion a single larva was captured using this method), even though survival to harvest in these trials was up to 70% (Table 7.2).

Observation of barramundi larvae in the aquarium used to simulate the pond environment indicated that the larvae remained in the upper 20-25 cm of the water column. Larvae were concentrated in the central part of the aquarium where light levels were highest. However, larvae still maintained this distribution when the light was switched off for up to one hour during the day. Based on these observations, intensive sampling of the upper 20-25 cm of the water column in the pond was undertaken, but no barramundi larvae were found.

It is possible that barramundi larvae were confined to the area immediately above the substrate where they could not be sampled effectively. Sampling this layer resulted in considerable amounts of mud and decomposing lucerne pellets filling the net. Sorting barramundi larvae from the mud and lucerne was difficult and larvae could easily have been missed in these samples. Attempts to sample just above the substrate, by swimming the net underwater, were also unsuccessful in obtaining samples of barramundi larvae.

In contrast to the earlier trials, during pond trial P12 larvae were caught at the first attempt (day 6 after hatching) in the 300μm mesh width plankton net and could readily be caught thereafter using the same technique. The turbulence caused by aeration of the pond during this trial may have altered the distribution of larvae in the pond, moving larvae throughout the water column and resulting in increased catchability of larvae using the plankton net.

Turner (1988) found that sampling striped bass and hybrid striped bass - white bass larvae using a 1 mm width mesh net 36-48 hours after stocking could distinguish ponds with total or almost total larval mortality from those which had

moderate or abundant numbers of fish, but could not accurately predict larval survival. McCarty and Gregg (1992) found that the count of red drum larvae in plankton tows collected 48 hours after stocking was an accurate predictor of the number of fingerlings harvested from the pond 30 days later. The small number of samples available from pond trial P12 preclude a detailed analysis of estimates of early larval survival, but the larger number of samples of late larval and juvenile barramundi obtained using lift nets permits examination of the accuracy of predicting survival using density estimates based on these samples.

The smallest juvenile barramundi captured on the lift nets averaged 9.9 mm TL, 11 days from hatching (i.e. 9 days after stocking). Densities of barramundi estimated by counting the number of fish on the 1 m^2 lift nets in pond trials P5, P8, P9, P11 and P12 are listed in Table 7.7. Density estimates varied greatly during each trial and the predicted population of barramundi in the pond (based on the mean density estimate) usually differed substantially from the actual number of barramundi harvested at the end of the pond trial (Table 7.7).

Table 7.7 Accuracy of pre-harvest estimates of fish density. Predicted population sizes were derived from mean density estimated using two 1 m^2 lift nets multiplied by the surface area of the pond (1,340 m^2).

Trial	Density (fish/m^2)		Population	
	Mean	Range	Predicted	Actual
P5	45.1	19.5 - 75.0	60,000	45,000
P8	39.0	21.0 - 56.5	52,000	63,000
P9	2.2	1.0 - 4.0	3,000	1,244
P11	32.3	16.0 - 48.5	43,000	14,000
P12	32.2	20.0 - 37.5	43,000	86,000

There are several possible reasons for the inaccuracy of this technique in estimating survival of barramundi. Although only two nets were used, this should not greatly affect the overall estimate of density in each pond trial because the nets were sampled regularly (2-3 times each week) throughout each trial. Examination of the original data indicated that most variability occurred between samples, and that differences between the nets were relatively minor. The nets may have had some microhabitat effect on juvenile barramundi. The nets may have attracted barramundi, since many barramundi were observed to hide near cover (particularly filamentous algae) when the pond was clear and the nets provided some degree of cover on an essentially featureless substrate. Barramundi may

also have avoided the nets when disturbed during sampling. Juvenile barramundi have a well developed 'escape' response and would flee when disturbed by objects such as the sampling boat, at least when the pond was clear and visibility was good. The lift nets were only useful in estimating crude success rates (i.e. no fish on the nets indicated no survival). However, the lift nets provided an easy and effective method for obtaining samples of barramundi which could be used to measure size, general condition, gut contents and fish health.

Attempts to estimate early larval survival and to predict the number of fish to be harvested were both unsuccessful in the present study. However, aeration of the pond appeared to increase the catchability of barramundi larvae immediately after stocking and future research will concentrate on developing techniques to estimate early larval survival to examine the relationship between early larval survival and the number of barramundi fingerlings harvested.

Pond Management Procedures

Substrate Drying

The rapid refilling of the pond after pond trials P8 and P9 prevented the pond substrate from drying completely. Zooplankton densities in the subsequent trials (P9 and P10) were low during the early part of each trial (Figs 7.34 and 7.39). Peak densities of zooplankton occurred late in each trial: day 26 in trial P9 and day 16 in trial P10. Following a rest period between pond trials P10 and P11, and between P11 and P12, during which the substrate was allowed to dry,

zooplankton densities were dramatically higher throughout pond trial P11 (Fig. 7.43) and were again high in pond trial P12 (Fig. 7.48).

Although based on a limited number of observations, these results support the use of substrate drying as a pond management procedure to recycle nutrients trapped in the bottom mud of ponds (Boyd 1990). A proportion of the nutrients added in pond trials P8, P9 and P10 presumably became 'locked up' in the pond substrate and were subsequently released by drying the pond after pond trial P10. The excess nutrients released by substrate drying may have promoted the extremely high zooplankton densities observed in pond trial P11. However, further examination of the processes of nutrient cycling and the role of the substrate as a nutrient sink are necessary to validate this hypothesis. Further research on this subject requires direct measurement of dissolved nutrient levels in the pond under various management regimes.

Aeration

Because aeration was used throughout only one trial (P12) it is premature to draw conclusions as to the effect of aeration on brackishwater larval rearing ponds. The major advantage of aeration observed in the present study, was the ability to sample barramundi larvae from the pond within a few days of stocking. The effects of aeration on the diet and feeding behaviour of barramundi larvae are discussed in Chapter 8. Because dissolved oxygen levels in the pond were normally high, aeration had no discernable effect on dissolved oxygen levels. Additional benefits of aeration, such as better circulation and utilisation of

nutrients, and increased dissolved oxygen levels, should be investigated as part of future research on the extensive larval rearing of marine fish.

Factors Affecting Survival and Growth

Survival

Survival of barramundi in these pond trials ranged from 0 to 86% (Table 7.2) and averaged 22.3% overall for pond trials P1-P12. To examine the relationship between survival and the measured physicochemical and biological parameters for the pond, a stepwise multiple regression of survival (after arcsine transformation) and the summary data for physicochemical parameters and zooplankton density listed in Tables 7.3 to 7.5 was undertaken. One of each pair of highly correlated variables (e.g. maximum and minimum water temperature, 2-9 days from hatching) was removed from the data set to increase the sensitivity of the analysis. The variable retained from each highly correlated pair was the one most likely to affect survival in these pond trials (e.g. maximum, rather than minimum, water temperature, 2-9 days from hatching).

The multiple regression indicated that two of these variables were significantly correlated with survival: maximum water temperature (2-9 days from hatching) and maximum pH (2-9 days from hatching). Together, these two variables accounted for 64% of the variance in this analysis:

$$S = \sin (7.49 - 0.073(T) - 0.542(P))^2$$

where S = (proportional) survival, T = maximum water temperature (°C), 2-9 days from hatching, and P = maximum pH, 2-9 days from hatching;

adjusted $r^2 = 0.54$; $F = 6.3$, $P < 0.05$.

Based on this result, further analyses of these two variables were carried out to determine the relationship between survival and the individual variables. The regression model that best fitted the data for maximum water temperature (2-9 days from hatching) and survival was a logistic regression (Fig. 7.53a):

Maximum water temperature (days 2-9) (Fig. 7.53a):

$S = 1 / (1 + 10^{(-17.63 + 0.578\ T)})$

where S = (proportional) survival and T = maximum water temperature (°C), 2-9 days from hatching;

$t = -362.3$, $P < 0.01$.

A linear regression model best described the relationship between maximum pH (2-9 days from hatching) and survival (Fig. 7.53b), following normalisation of the survival data using an arcsine transformation (Zar 1984).

Maximum pH (days 2-9) (Fig. 7.53b):

$S = \sin (8.30 - 0.92\ P)^2$,

where S = (proportional) survival and P = maximum pH, 2-9 days from hatching;

adjusted $r^2 = 0.44$; $F = 7.96$, $P < 0.05$.

Figure 7.53 Relationship between survival rate of barramundi and water quality parameters recorded during the first week following stocking (i.e. days 2-9 from hatching) in pond trials P3-P12 (Table 7.4):

(a) Maximum water temperature:

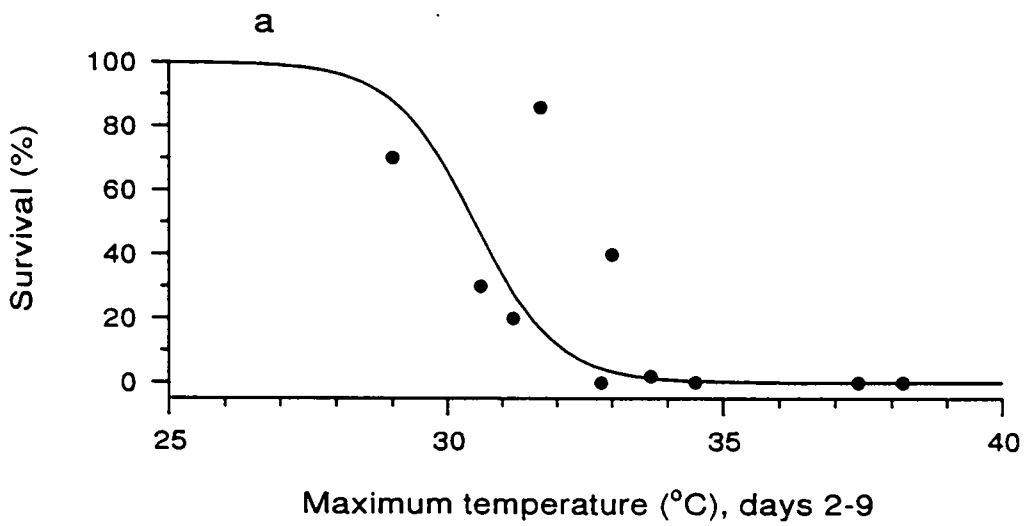
Regression line:

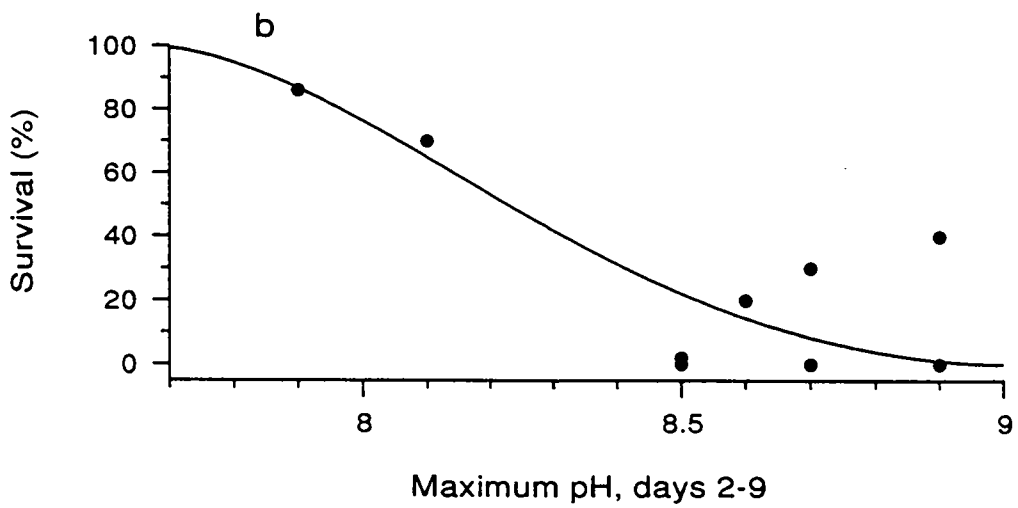
$Y = 1 / (1 + 10^{(-17.63 + 0.578 X)})$;

(b) Maximum pH:

Regression line:

$Y = \sin (8.30 - 0.921 X)^2$.


a
Survival (%)
100
80
60
40
20
0
25
30
35
40
Maximum temperature (°C), days 2-9


b
Survival (%)
100
80
60
40
20
0
8
8.5
9
Maximum pH, days 2-9

The observed effects of temperature and pH on survival only during the first week after stocking, and not during the entire trial, add support to the validity of these results since the tolerances of fish larvae to various water quality parameters are generally least in newly hatched and first-feeding larvae (Brownell 1980a).

The regression curve illustrated in Figure 7.53a suggests that temperature in these trials was consistently close to the upper thermal tolerances of barramundi larvae during the first week after stocking. For this reason, the regression of survival and maximum water temperature for the first week after stocking (Fig. 7.53a) presumably more accurately represents the effects of temperature on survival than would minimum water temperature during the same period, which was excluded from the analysis. Survival decreased dramatically when maximum water temperatures exceeded about 31°C and survival was negligible when maximum temperatures exceeded 33.5°C during the first week after stocking. However, two trials with high survival had maximum water temperatures of 31.7 and 33.0°C during the first week after stocking. These results suggest that, although water temperature during the first week after stocking may directly affect survival of larval barramundi, other factors may also be influencing survival. In particular, the wide range of survival values in the temperature range 32-33°C (0-86% survival) suggests that factors other than temperature may have considerable influence on larval survival. Both temperature and pH together affected the survival of barramundi larvae in these trials, and it is possible that other factors not measured in this study (e.g. ammonia) also affect early larval survival.

Attempts to further examine complex relationships between water quality and biological variables using multivariate analysis techniques were frustrated by the relatively low number of trials compared with the large number of variables examined.

The relationship between survival and maximum pH (Fig. 7.53b), although statistically significant, appears to be greatly influenced by the two points on the left of the graph. If these two points are disregarded, the remaining data points suggest no meaningful relationship between pH and larval survival. However, the relationship illustrated in Figure 7.53b may reflect Brownell's (1980b) results which indicate that pH values from 8.4 to 8.5 reduce first feeding success in marine fish larvae and a 'negligible effect pH' is not likely to exceed pH 8.4. Certainly, the results of the pond trials undertaken to date indicate that pH values greater than 8.4 during the first week after stocking have a negative effect on barramundi larval survival.

As noted in Chapter 6, extensive rearing principles are largely based on Hjort's hypothesis that survival of fish larvae is directly related to the availability of prey organisms at the very earliest stages of fish larval development (Lasker 1981, Geiger 1983a,b, Rowland 1983, Sturmer 1990). Rutledge (1988) found that the best predictor of survival for extensively reared red drum was the density of copepod adults in the pond over the 3 days immediately after the red drum larvae were stocked. However, this correlation may not reflect a direct causal relationship since red drum larvae feed on copepod nauplii, rather than copepod adults, at first feeding (Rutledge 1988). In the pond trials described in this thesis,

there was no correlation between barramundi larval survival and zooplankton densities in any size class. Feeding and prey selection in barramundi larvae are discussed in greater detail in Chapter 8.

Rutledge (1988) also found a significant correlation between the number of red drum larvae stocked into extensive rearing ponds and the number of fish harvested. No such association was seen for barramundi in the present study (Table 7.2).

It is possible that differences between batches of barramundi larvae, and in the handling and tempering process prior to stocking, may have affected survival of barramundi in these trials. As much as possible, these factors were standardised between trials. Most batches of larvae came from a single tank of barramundi broodstock held at NFC, which contained 4 male and 3 female fish. The definition of larval 'quality' remains a contentious issue in larviculture, and to date there is no standardised methodology for assessing the quality of newly hatched marine fish larvae. As noted in the Materials and Methods, the only assessment of larval quality undertaken in this study was based on high fertilisation and hatching rates for each batch of eggs and larvae, and only those larvae that had relatively high fertilisation and hatching rates (exceeding 70% and 80% respectively) were used in these trials. In fact, intensive rearing trials at NFC have shown that even barramundi from batches with poor fertilisation and hatching rates (<50%) can be reared to metamorphosis with good survival (c. 50% survival). This suggests that 'larval quality' is not a major factor affecting survival in intensive rearing, although, as noted previously, extensive rearing

ponds are a less benign environment than hatchery tanks, and thus larval quality may have more influence in extensive rearing. Until further research is undertaken on the assessment of larval 'quality', no assessment of this factor in these rearing trials can be discussed in a meaningful fashion.

Predation was not observed to play a major role in the survival of barramundi in these trials. As noted previously, apparent total mortality of barramundi in an early larval rearing trial (P1, undertaken by W.P. Rutledge) was associated with high densities of comb jellyfish (Phylum Ctenophora) and arrow-worms (Phylum Chaetognatha), and the arrow-worms were observed to attack and kill newly hatched barramundi larvae (Rutledge and Rimmer 1991). This occurrence may be ascribed either to the lengthy period between pond filling and initial fertilisation and larval stocking (c. 1 month, rather than the 8-10 day period used in other trials) or to the lack of a smaller mesh (300 μm) 'sock' filter to remove potential predators, which was introduced in later trials. Odonata (dragonfly) larvae have been found to be important predators of juvenile barramundi (< c. 20 mm TL) in freshwater ponds (Barlow *et al.* 1991). However, dragonfly larvae rarely occurred in the pond used in this study, and when they did occur they were found at low density and relatively late in the rearing cycle. At the time that dragonfly larvae occurred in the pond, the barramundi larvae were at the 'lurking predator' stage of development (i.e. 18-20 mm TL or larger) when they are generally not subject to predation by dragonfly larvae (Barlow *et al.* 1991). There was no evidence of bird predation affecting barramundi survival in these trials.

In conclusion, the results of these trials indicate that both temperature and pH during the week after stocking affect survival of extensively reared barramundi larvae. The exact nature of the relationship between survival and water quality parameters requires further elucidation. In particular, larger data sets (i.e. more trials) are necessary to corroborate or refute the relationships associated with the existing data set and to allow examination of the interactions between the various physicochemical and biological variables.

Growth

Crude growth rates for the period between fish samples in pond trials P5-P12 ($[TL_{sample\ n} - TL_{sample\ n-1}] /$ days) were analysed for any correlation with water temperature, salinity, pH, Secchi transparency, density of zooplankton in the $>37\mu m$, $>100\mu m$ and $>250\mu m$ size classes, fish age at sampling, mean TL of fish sampled, specific growth rate for the interval between samples and degree-days up to the time of sampling. Final samples (i.e. lengths of harvested fish) from each pond trial were excluded from each analysis because corresponding water quality and plankton density data were usually missing from these samples.

Significant correlations were found between growth and five of the measured variables:

TL; $r = 0.90$, $P<0.01$;

Degree-days; $r = 0.80$, $P<0.01$;

Fish age; $r = 0.78$, $P<0.01$;

Water temperature; $r = 0.49$, $P<0.01$.

Air temperature at 1500 h; $r = 0.47$, $P<0.01$.

Individual regressions of TL, degree-days, fish age, water temperature and air temperature at 1500 h with growth rate produced significant regression relationships (Fig's. 7.54 and 7.55):

Total Length (Fig. 7.54a):

$G = 0.177 + 0.0871\ TL$

where G = growth rate (mm/day) and TL = mean total length of fish sampled (mm);

adjusted $r^2 = 0.81$; $F = 110.9$, $P<0.01$.

Degree-days (Fig. 7.54b):

$G = -0.275 + 0.00469\ DD$

where G = growth rate (mm/day) and DD = degree-days;

adjusted $r^2 = 0.63$; $F = 45.1$, $P<0.01$.

Fish age (Fig. 7.54c):

$G = -0.524 + 0.141\ A$

where G = growth rate (mm/day) and A = fish age (days);

adjusted $r^2 = 0.60$; $F = 39.8$, $P<0.01$.

Water temperature (Fig. 7.55a):

$G = -6.71 + 0.268\ WT$

where G = growth rate (mm/day) and WT = water temperature (°C);

adjusted $r^2 = 0.21$; $F = 8.0$, $P < 0.01$.

Air temperature at 1500 h (Fig. 7.55b):

$G = -4.89 + 0.225\ AT$

where G = growth rate (mm/day) and AT = air temperature (°C);

adjusted $r^2 = 0.19$; $F = 7.2$, $P < 0.05$.

A stepwise multiple regression of growth rate and the five variables listed above indicated that 80.9% of the variance was accounted for by TL and no other variables significantly contributed to the regression.

Figure 7.54 Relationship between mean growth rate of barramundi between samples in pond trials P5-P12 and

(a) Mean TL of fish sampled,

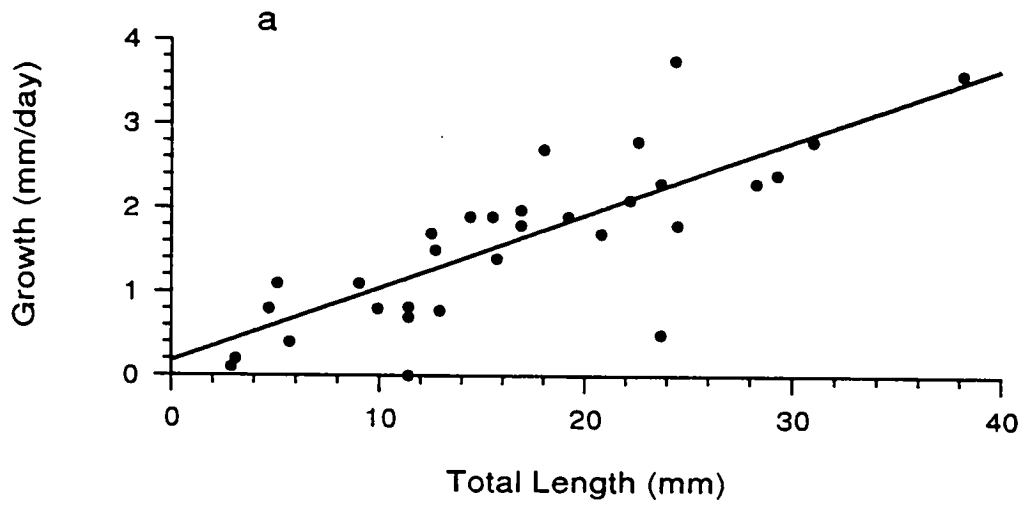
Regression line: $Y = 0.177 + 0.087\ X$;

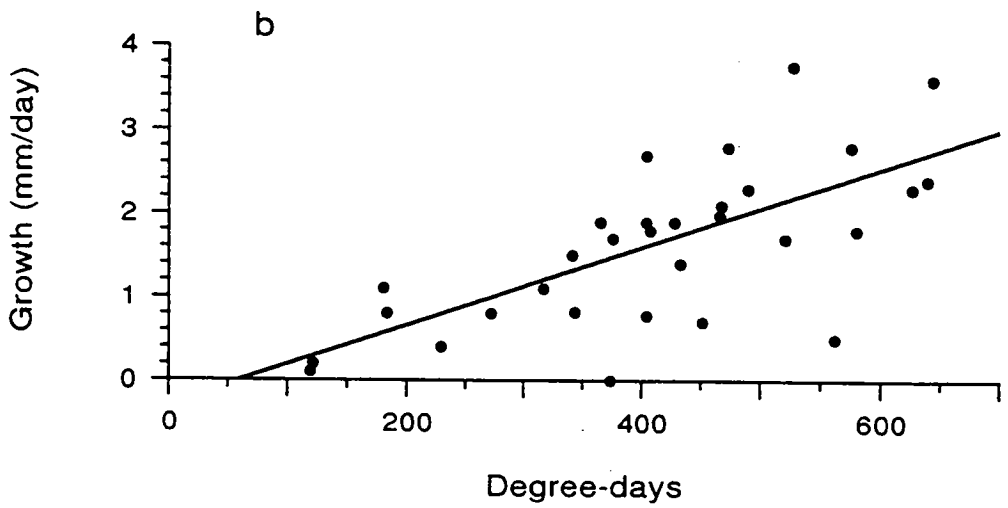
(b) Degree-days at time of sample,

Regression line: $Y = -0.275 + 0.00469\ X$;

(c) Fish age at time of sample,

Regression line: $Y = -0.524 + 0.141\ X$.


a
Growth (mm/day)
0
1
2
3
4
0
10
20
30
40
Total Length (mm)


b
Growth (mm/day)
0
1
2
3
4
0
200
400
600
Degree-days

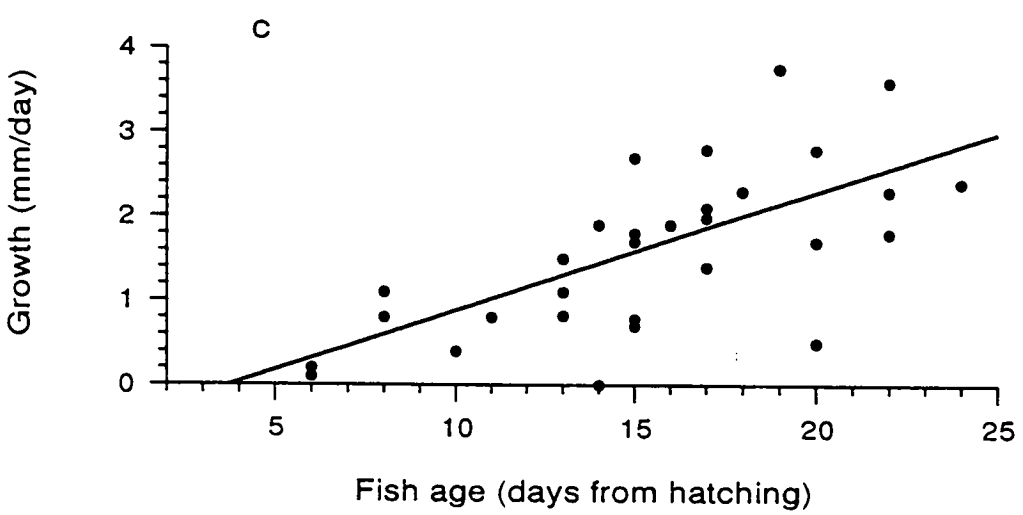

c
Growth (mm/day)
0
1
2
3
4
5
10
15
20
25
Fish age (days from hatching)

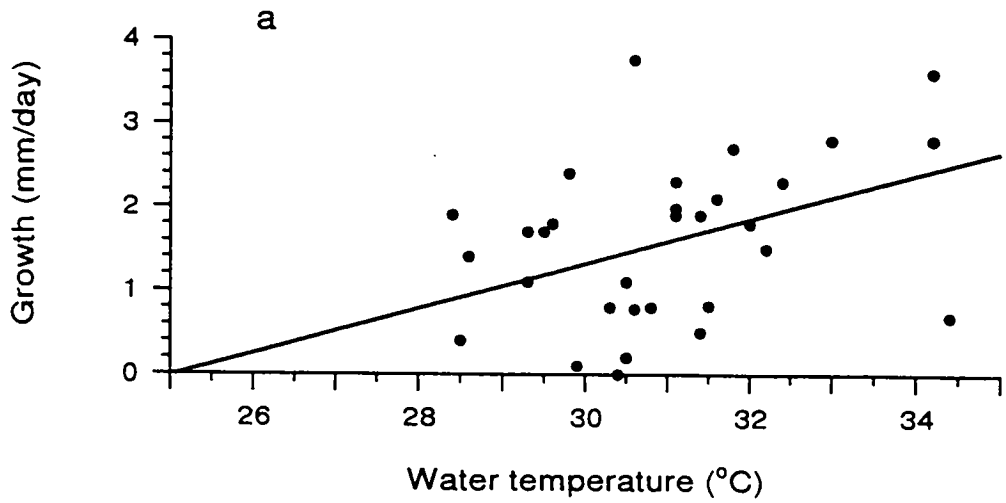
Figure 7.55 Relationship between mean growth rate of barramundi between samples in pond trials P5-P12 and

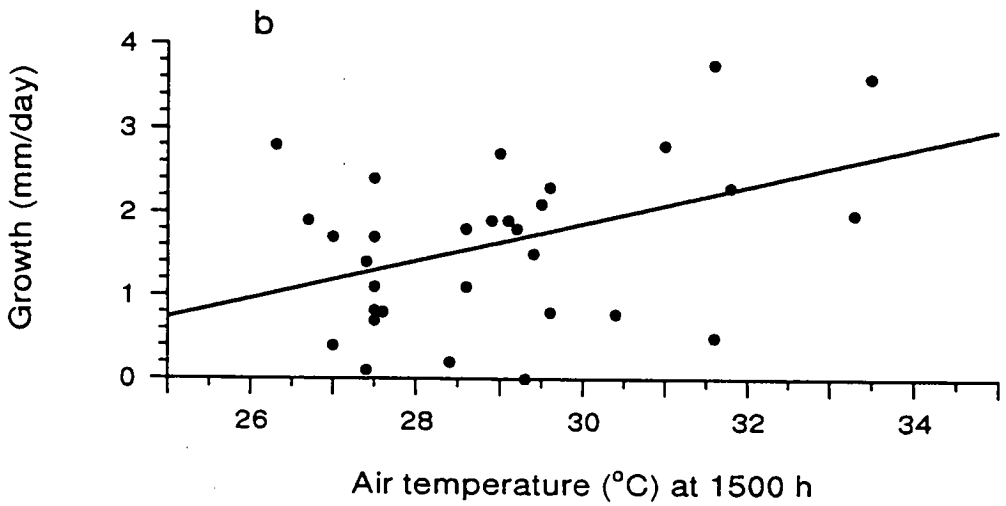
(a) Mean daily water temperature between samples,

Regression line: $Y = -6.71 + 0.268\ X$;

(b) Mean air temperature at 1500 h between samples,

Regression line: $Y = -4.89 + 0.225\ X$.


a
Growth (mm/day)
0
1
2
3
4
26
28
30
32
34
Water temperature (°C)


b
Growth (mm/day)
0
1
2
3
4
26
28
30
32
34
Air temperature (°C) at 1500 h

All five of the variables found to be correlated with growth rate are functions of fish size and age (TL, age) and environmental temperature (water temperature, air temperature at 1500 h) or both (degree-days). That higher water temperatures result in faster growth of fish, up to some critical point, is well established (Boyd 1990). The correlation between growth and TL indicates that crude growth rates of barramundi in these pond trials increased as the fish became larger. Part of this increase in crude growth rate is attributable to the measure used (mm/day) since a 10% increase in length for a small fish (e.g. 0.5 mm for a 5 mm TL fish) is small compared with the same proportional growth for a larger fish (e.g. 3.0 mm for a 30 mm TL fish); thus crude growth rate is higher for the larger fish, although proportional growth is the same. The bias inherent in measuring growth as crude growth rate was overcome by using specific growth rate which measures growth as a relative proportion of size (%/day).

Specific growth rate for the period between fish samples was analysed for any correlation with the variables used for the analyses for crude growth rate. No significant correlations were found for specific growth rate and any of the variables examined. Specific growth rate varied dramatically during each pond trial, generally starting at a low value, rapidly increasing and then gradually declining until harvest (e.g. Fig's. 7.45b, 7.50b). The precise changes in specific growth rate varied between pond trials and may reflect a variety of influences including physicochemical parameters, food availability and prey type (see also Chapter 8).

Mean TL for each sample of fish was analysed for any correlation with the variables used in the previous analyses in order to determine whether fish length could be accurately predicted from physicochemical data. Degree-days was the most accurate predictive variable for mean TL ($r=0.94$; $P<0.01$). The regression model used to fit the data for degree-days and TL was a quadratic regression (Zar 1984) (Fig. 7.56):

$$TL = 0.37 + 0.0149\ DD + 0.0000548\ DD^2;$$

where TL = mean total length (mm) of fish sampled and DD = degree days;

adjusted $r^2 = 0.90$; $F=123.1$, $P< 0.01$.

The equation for predicting total length based on degree-days shows a high degree of correlation with the available data (90% of the variance is explained by the regression equation) which indicates that this equation is a useful management tool for predicting fish length in pond trials. Similar predictive equations using degree-days have been developed for use in the culture of pike (*Esox lucius*) reared in freshwater ponds (Bry *et al.* 1991). Barramundi are generally harvested from ponds when they have reached a size suitable for stocking into the wild or for weaning onto artificial diets, i.e. c. 20-30 mm TL. Hence the ability to predict fish length based on degree-days can be used to determine when the fish will reach a size suitable for harvesting. If mean daily water temperature data are available, this predictive equation could be used to develop production schedules in different locations or at different times of the year. Accurate estimates of the duration of pond rearing cycles could be incorporated into an overall production

strategy, enabling aquaculturists to plan induced spawnings, extensive larval rearing 'runs' and harvests well in advance.

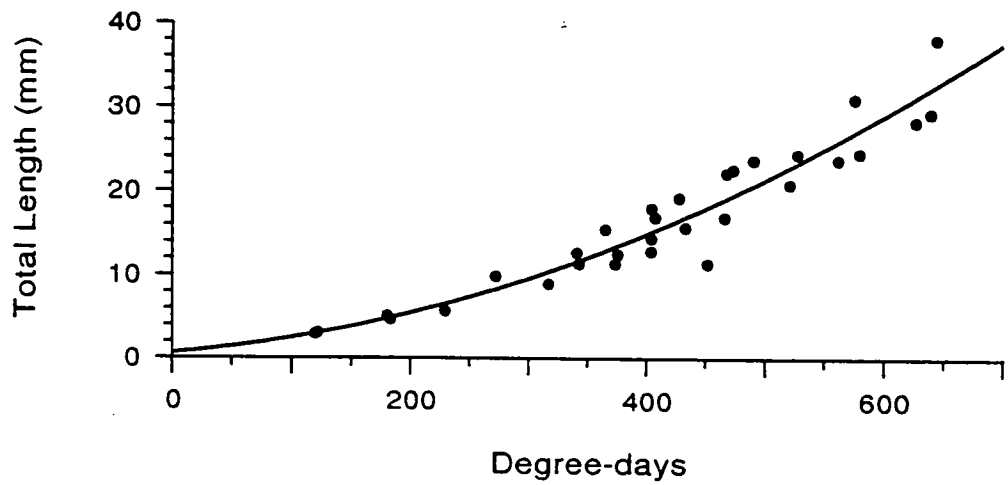


Figure 7.56 Relationship between mean total length of barramundi sampled during pond trials P5-P12 and degree-days to the time of sampling;

Regression line: $Y = 0.37 + 0.0149\ X + 5.48x10^{-5}\ X^2$

CHAPTER 8

Diet and Feeding Patterns of Extensively Reared Barramundi Larvae and Juveniles

Introduction

A knowledge of the diet and feeding behaviour of cultured fish larvae is essential in developing management strategies for extensive rearing. Extensively reared marine fish larvae generally follow a dietary progression directly related to their size and their ability to cope with different prey types. Typically, the larvae begin feeding on smaller zooplankton (rotifers and copepod nauplii), then move to progressively larger zooplankters (copepod adults) and, later, insect larvae, amphipods, polychaetes and small shrimp (Geiger 1983b, Kvenseth and Oiestad 1984, Porter and Maciorowski 1984, Sturmer 1990).

As noted in Chapter 6, pond management strategies for extensive rearing are centred around providing maximal densities of suitable prey organisms at the time of first feed. To successfully develop such a strategy, the diet of the cultured species at first feed must be established. In addition, information on diet throughout the rest of the rearing period assists with pond management, e.g. by indicating whether the invertebrate populations in the pond provide suitable prey for the cultured species.

Barramundi in the wild are opportunistic predators. Larval and juvenile barramundi up to 50 mm TL have been found to feed predominantly on microcrustacea, primarily copepods, although larger invertebrates are also taken by larger fish (De 1971, Patnaik and Jena 1976, Ghosh and Pandit 1979, Davis

1985b, Russell and Garrett 1985). Barlow *et al.* (1993) examined the diet of juvenile barramundi (>*c.* 10 mm TL) reared in freshwater ponds and found that juvenile barramundi undergo a significant change in feeding habit at about 18 mm TL, from 'roving zooplanktivore' to 'lurking predator'. Juvenile barramundi between 10 and 17 mm TL were zooplanktivorous, but between 17 and 50 mm TL the diet changed progressively from zooplankton to insect larvae and small vertebrates (Barlow *et al.* 1993).

The diet and feeding habits of extensively reared barramundi larvae were examined to assist in developing pond management strategies for this species. Specifically, the research described in this chapter investigated:

1. dietary patterns of barramundi larvae in extensive rearing ponds, and particularly the prey utilised at first feed;
2. diel variation in prey consumption and prey selection;
3. prey selection in terms of prey type and prey size;
4. the effects of pond aeration on prey selection and diet.

Materials and Methods

Gut contents of barramundi larvae and juveniles were sampled from the pond during pond trials P8, P9, P12 and P14 (see Chapter 7). (Note that the results of pond trial P14 are not included in this thesis, but a limited sampling of larvae was undertaken to verify the change in prey selection observed during pond trial P12, which was believed to be associated with aeration of the pond). Barramundi larvae and juveniles were captured using the methods described in Chapter 7, i.e.

a plankton net, a light trap, and lift nets. Fish captured using the light trap were not used for gut content analysis because there was frequently a high density of fish in the trap which presumably affected prey availability and feeding behaviour. Captured fish were killed immediately to prevent regurgitation or continued digestion of gut contents and were preserved in 10% formalin.

Enclosures

Because of the difficulty of capturing barramundi larvae during the first week of each trial, a set of enclosures was constructed and installed in the pond. Each enclosure was formed by four replaceable screens and a solid bottom piece arranged around a central pivot which carried the air supply and distribution system (Fig. 8.1). 200μm mesh width screens were used at the beginning of each trial to retain newly hatched larvae; these screens were replaced with larger mesh sizes (400μm and 1 mm mesh widths) as the fish grew. Each enclosure was aerated by four air stones placed in the corners, and each was supplied with two airlifts which pumped pond water from 1 m depth and from 10 cm above the pond bottom (c. 1.4 m depth) to supply zooplankton from the pond to each enclosure.

Larval and juvenile barramundi were sampled from the enclosures using hand nets. Zooplankton samples were taken from each enclosure with a vertical tube sampler (25 mm diameter, 40 cm length) and fitted with a non-return valve to retain the entire sample after removal from the enclosure. Plankton samples were preserved in FAA and three 1 ml subsamples were examined to enumerate the

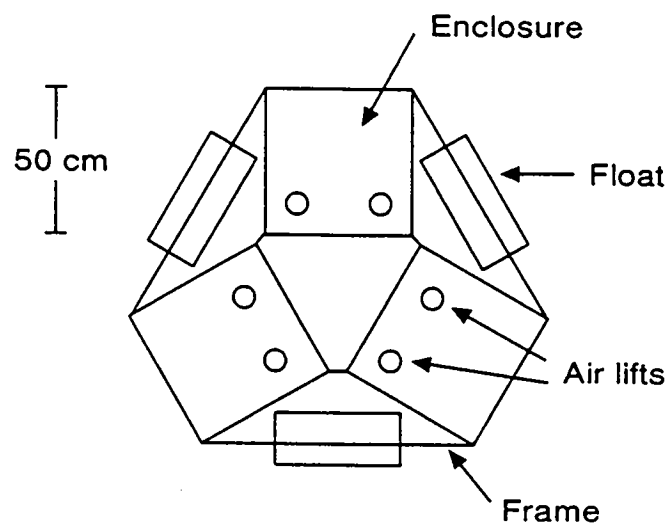
density of major zooplankton groups. Samples from all three enclosures were pooled for analysis.

Diel Variation Sampling

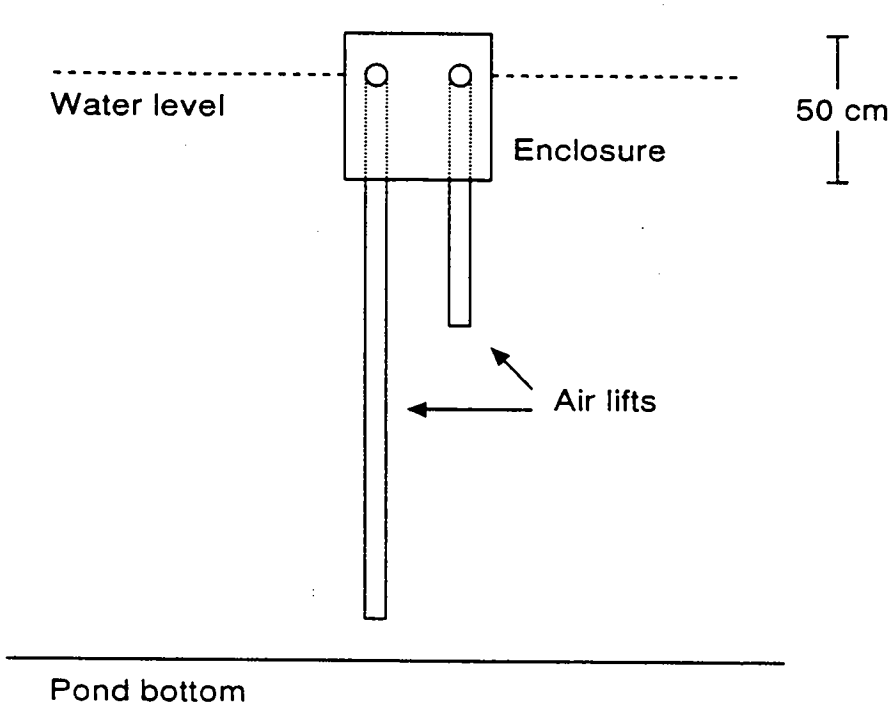
Barramundi larvae were sampled from the pond at four times during the day: 0715-0730 (i.e. soon after daybreak), 1100, 1500 and 1830 (i.e. about sunset) eastern daylight saving time. Sampling was restricted to daylight hours since barramundi larvae feed only in daylight (Barlow *et al.* 1993). Samples for examination of diel variation were taken 7, 13 and 20 days from hatching during a single pond trial (P12). Fish were sampled on day 7 using a 300μm plankton net, and on days 13 and 20 using the lift nets (see Chapter 7 for details of sampling apparatus). Zooplankton samples were taken at the same time to determine the availability of different zooplankton groups. Zooplankton were sampled with a vertical tube sampler and sieved into different size classes ($>37\mu$m, $>100\mu$m, $>250\mu$m and $>500\mu$m) as described in Chapter 7.

Figure 8.1 (a) Plan and (b) lateral views of the experimental enclosures used to retain early larval stages of barramundi used for gut content analyses.

a


Enclosure
50 cm
Float
Air lifts
Frame

b


Water level
50 cm
Enclosure
Air lifts
Pond bottom

Gut Contents

Up to 20 barramundi larvae and juveniles from each sample were measured either using a binocular microscope equipped with an eyepiece micrometer (fish < 7 mm TL) or with vernier calipers (fish ≥ 7 mm TL). The intestinal tract of each fish was dissected and examined under a binocular microscope, and the number of prey organisms from each prey group (rotifers, nauplii, copepods, cladocerans, ostracods, chironomids, other organisms) was counted.

The widths of up to 20 organisms from each prey group (except rotifers) were measured, since prey width is the critical dimension for the ingestion of zooplankton prey by fish larvae (Hunter 1981). Rotifers (*Brachionus plicatilis*) ingested by barramundi larvae were found to be laterally compressed. This compression appeared to 'collapse' the rotifer, rather than elongate it further. Consequently, rotifers were measured along the long axis and this measurement was converted to width using a linear regression equation derived from 200 length and width measurements of *B. plicatilis* (M. Pearce, unpublished data). This estimate provides a more accurate measure of rotifer width prior to ingestion, and hence is more relevant to prey availability, than the measurement of ingested rotifers. Chironomid larvae were measured across the cephalic region. Accurate estimation of body width based on measurement of cephalic width was not possible, since body width in these organisms is largely independent of cephalic width. Although the body width of chironomid larvae frequently exceeds the cephalic width, the cephalic width is a more accurate representation of the limiting

width of the organisms since the body is soft and could readily be compressed during ingestion, while the chitinous cephalic region is not compressible.

Zooplankton Dry Weight

The dry weight of zooplankton sampled from the pond was determined to provide an estimate of the mass of prey eaten by extensively reared barramundi larvae. Direct weight measurement of the gut contents of barramundi larvae was not possible because of the very small amounts of material involved (particularly in small larvae) and the difficulty in handling gut contents to ensure that no prey organisms were lost. Consequently, dry weight of larval gut contents was estimated from the numerical and size analysis of gut contents of individual fish, and the mean dry weight of zooplankton sampled from the pond.

Cellulose nitrate filter papers (WCN type, 25 mm diameter, 0.2 μm pore size) were individually numbered and dried at 40°C for 20 hours, then weighed on a Sartorius 1700 series balance (to the nearest 0.1 μg) Zooplankters of different types (rotifers, nauplii, copepods, cladocerans) sieved into various size classes ($>37\mu$m, $>100\mu$m, $>250\mu$m; see Chapter 7 for details) and chironomids were isolated onto the pre-weighed filter papers using a vacuum flask apparatus to ensure retention of the zooplankters on the filter papers. Up to 3000 organisms were isolated onto one filter paper to provide enough material to obtain a meaningful weight. The filter papers and zooplankton were dried at 40°C for 20 hours and reweighed (to the nearest 0.1 μg). Three replicates of each group

were weighed, and the results averaged to provide a mean dry weight for each type and size class of zooplankton (Table 8.1).

Table 8.1 Dry weight of zooplankton sieved into different size classes, and chironomids.

		Dry weight (μg)	
Size class	Zooplankton	Mean	Standard error
>37μm	Nauplii	0.27	0.07
	Copepods	0.63	0.17
>100μm	Rotifers	0.23	0.08
	Nauplii	0.67	0.10
	Copepods	0.40	0.06
>250μm	Copepods	3.86	0.83
	Cladocerans	2.29	0.53
Chironomids		112.6	9.0

Although there appear to be some inconsistencies in the zooplankton dry weight data (in particular, copepods >100μm weigh less than copepods >37μm), it is not apparent whether these are real or were caused by the technical problems inherent in weighing such small organisms. Because copepods >100μm may represent different taxa to those in the >37μm group, there could be substantial differences in weight because of different body shapes.

Dry weight of the gut contents of barramundi larvae was estimated by multiplying the number of prey organisms of that particular type and size class by the mean weight for that group as listed in Table 8.1. This estimate ignores the contribution of prey items not listed in Table 8.1. In practice, this affects very few fish, since only a small proportion of samples had substantial quantities of 'other' prey organisms in the gut contents.

Zooplankton Prey Selection

Prey selection in larval barramundi was examined using the Bray-Curtis dissimilarity index (Nogrady 1982). This index differs from other indices commonly used in fisheries biology, such as Ivlev's electivity index, in that it provides a single measure of dissimilarity for the sample, rather than a series of individual indices for each taxon. This single index is more easily interpretated over a time series than the multiple indices provided by other methods.

As used in this study, the Bray-Curtis dissimilarity index measures the sum of the differences between the proportion of different prey types in the gut and the proportion of those prey types available to the fish. The resulting index represents the level of dissimilarity, rather than similarity, between the diet of the fish and the availability of prey, and ranges from 0 (identical) to 1 (completely dissimilar).

Prey selection was measured only for samples in which zooplankton prey predominated in the gut contents. Because no estimates were made of the availability of benthic organisms, and because any such estimates would not have

been directly comparable with estimates of zooplankton availability, no attempt was made to assess prey selection when barramundi larvae were feeding primarily on benthic organisms such as chironomid larvae.

Mouth Size

Jaw width and gape (at 45° and 90°) were derived from measurements of total length for each fish using the regression equations listed in Appendix 4. Jaw width is generally preferred to jaw gape as a measurement of mouth size since it can be measured more accurately (Hunter 1981). Jaw gape could not be measured directly in barramundi larvae because of the small size and delicacy of the fish and because of the difficulty in obtaining a consistent opening angle of the jaws for measurement. Instead jaw gape was estimated using the jaw length measurement; following of the procedure given in Appendix 4. Estimates of jaw width and gape derived from these equations were used to examine the relationship between mouth size and prey size in extensively reared barramundi. Barramundi larvae less than 2.4 mm TL sampled from the pond (a total of 10 fish) were excluded from these analyses because these fish were below the lower limit for the predictive equations listed in Appendix 4.

Statistical Analyses

All statistical analyses were carried out using the statistical packages Statistix and GenStat. Various transformations were carried out to ensure that the data met the assumptions of homogeneous variances and normal distribution required by

ANOVA (Underwood 1981); these transformations are noted in the results where applicable. Fish with empty guts were excluded from the analyses of prey size and zooplankton prey selection. In a few cases, single extreme outliers were excluded from the analyses in order to ensure homoscedasticity. If more than one outlier influenced the analysis, all data were used for the ANOVA. Although this technically invalidates the analysis, ANOVA is relatively robust to heteroscedasticity (Zar 1984) and mild heteroscedasticity is preferable to the sampling bias introduced by omitting outliers where these may form a substantial proportion of the data set.

Results

Enclosures

Numerous problems were encountered with the enclosures designed to allow sampling of barramundi larvae prior to about day 10 from hatching. The 200μm mesh screens clogged rapidly with microalgae and small zooplankton, so that the enclosures overflowed. These overflows presumably resulted in the loss of barramundi larvae from the enclosures. Consequently, retention (or survival) of barramundi larvae in the individual enclosures was highly variable, but generally low. Because of these difficulties, the number of fish sampled from the enclosures was severely limited. In the following analyses, enclosure data have been used only where samples of barramundi larvae taken directly from the pond were not available; when barramundi larvae were sampled from the pond,

enclosure data were not used. All data derived from enclosure samples are noted in the relevant figure and table legends.

As noted in Chapter 7, the difficulty in sampling barramundi prior to about day 10 was overcome by aerating the pond for pond trials P12 and P14. This allowed sampling of barramundi larvae directly from the pond for subsequent analysis of gut contents, so the enclosures were not used in these trials.

Diel Variation

Zooplankton Availability

Density data for the four zooplankton samples taken on day 7 and on day 13 from hatching were examined to determine whether the availability of the four zooplankton prey groups (rotifers, copepod nauplii, copepod adults and cladocerans) varied during the day. Densities of zooplankton from the sieved size class corresponding to the mean size of zooplankton in the gut contents of barramundi sampled at the same time were used (i.e. >100 μm on day 7 and >250 μm on day 13). This analysis was not undertaken for the data from day 20 from hatching because zooplankton densities at this time were too low for any meaningful analysis. All zooplankton density data were transformed to a normal distribution using the transformation $\sqrt{(x+1)}$ (Underwood 1981).

Densities of zooplankton (all groups combined) did not differ significantly between the four samples taken at different times on day 7 (ANOVA, $F=1.1$, $P>0.05$), nor did they differ significantly on day 13 (ANOVA, $F=1.0$, $P>0.05$)

from hatching. The overall mean density of zooplankton was 344.9 organisms per litre (>100 μm) on day 7 and 3.2 organisms per litre (>250 μm) on day 13.

Gut Contents

The number and dry weight of prey in the guts of barramundi sampled 4 times daily at 7, 13 and 20 days from hatching during pond trial P12 are shown in Figures 8.2 and 8.3 respectively.

On day 7 from hatching, the mean number of prey in the guts of barramundi larvae was significantly higher in the evening (1830 sample) than for the other three sample times (ANOVA, $F=16.3$, $P<0.01$) (Table 8.2). Dry weight data showed an identical pattern, with prey consumption being highest at the 1830 sample (ANOVA, $F=18.2$, $P<0.01$) (Table 8.2). Although rotifers predominated in >100 μm zooplankton samples throughout day 7, very few rotifers were eaten by the barramundi larvae (Fig. 8.4). Small copepodites and adult copepods made up 71-92% (by number) of the gut contents of the barramundi larvae, although copepods comprised only 20-25% of the zooplankton (Fig. 8.4). Selection for nauplii was also high, particularly in the morning samples when nauplii comprised 23-29% of the diet, despite making up only 2% of the zooplankton (Fig. 8.4). This selection for copepods and nauplii by the barramundi larvae is reflected in the Bray-Curtis dissimilarity index for these samples, which was consistently high throughout day 7, ranging from 0.70 to 0.78 (Fig. 8.4).

On day 13 from hatching, the mean number of prey in the guts of barramundi larvae was lowest early in the morning, increased during the day and was significantly higher in the evening (1830 sample) than at any other time during the day (ANOVA using ln(x+1) transformed data, $F=29.8$, $P<0.01$) (Table 8.2). The mean dry weight of prey was also lowest early in the morning, increasing to a maximum at the 1830 sample (ANOVA with one outlier from 1830 sample omitted, $F=16.7$, $P<0.01$) (Table 8.2). Barramundi larvae sampled on day 13 exhibited a lower level of prey selection than those sampled on day 7 from hatching. Cladocerans generally predominated in the >250 μm zooplankton samples and the gut contents of the barramundi larvae comprised mainly copepods and cladocerans (Fig. 8.5). The Bray-Curtis dissimilarity index indicated that prey selection was high early in the day (0.52 for the 0730 sample), primarily due to selection for copepods (Fig. 8.4), and substantially lower (0.20-0.30) for the rest of the day (Fig. 8.5).

On day 20 from hatching, the mean number of prey in the guts of barramundi larvae did not vary significantly throughout the day (ANOVA, $F=1.0$, $P>0.05$) (Table 8.2). However, the mean dry weight of prey did vary significantly throughout day 20 (ANOVA, $F=21.2$, $P<0.01$). Prey consumption, measured as dry weight of prey consumed, was highest in the morning, then decreased during the day before increasing again in the late afternoon (Table 8.2). The discrepancy between prey consumption patterns measured as number of prey and as dry weight of prey in the day 20 samples is the result of the large proportion of chironomid larvae in the diet at this time (Figs 8.6, 8.7); because of the relatively heavy

weight of individual chironomids (Table 8.1), differences of even a few chironomids can substantially affect the mass of prey consumed. An assessment of prey selection patterns for day 20 was not possible because of the low densities of zooplankton in the relevant size class (i.e. >250 μm) on day 20.

Table 8.2 Mean number and dry weight (DW) of prey in gut contents of barramundi larvae sampled at 0715, 1100, 1500 and 1830 on days 7, 13, and 20 from hatching (pond trial P12). Similar superscript letters indicate means that are not significantly different from each other (Tukey's HSD, $P>0.05$).

Sample	Day 7		Day 13		Day 20	
Time	Number	DW (μg)	Number	DW (μg)	Number	DW (μg)
0715	11.6^{a}	4.3^{a}	26.0^{a}	86.3^{a}	27.5^{a}	1941.1^{a}
1100	10.6^{a}	3.8^{a}	57.8^{b}	188.1^{b}	35.5^{a}	816.3^{b}
1500	13.6^{a}	5.2^{a}	72.0^{b}	171.5^{b}	29.3^{a}	$1105.7^{b,c}$
1830	20.6^{b}	8.0^{b}	180.8^{c}	428.2^{c}	17.8^{a}	1471.3^{c}

Figure 8.2 Number of prey organisms in the guts of barramundi sampled 4 times daily at 7, 13, and 20 days from hatching during pond trial P12. (An explanation of box and whisker plots is provided in Appendix 3).

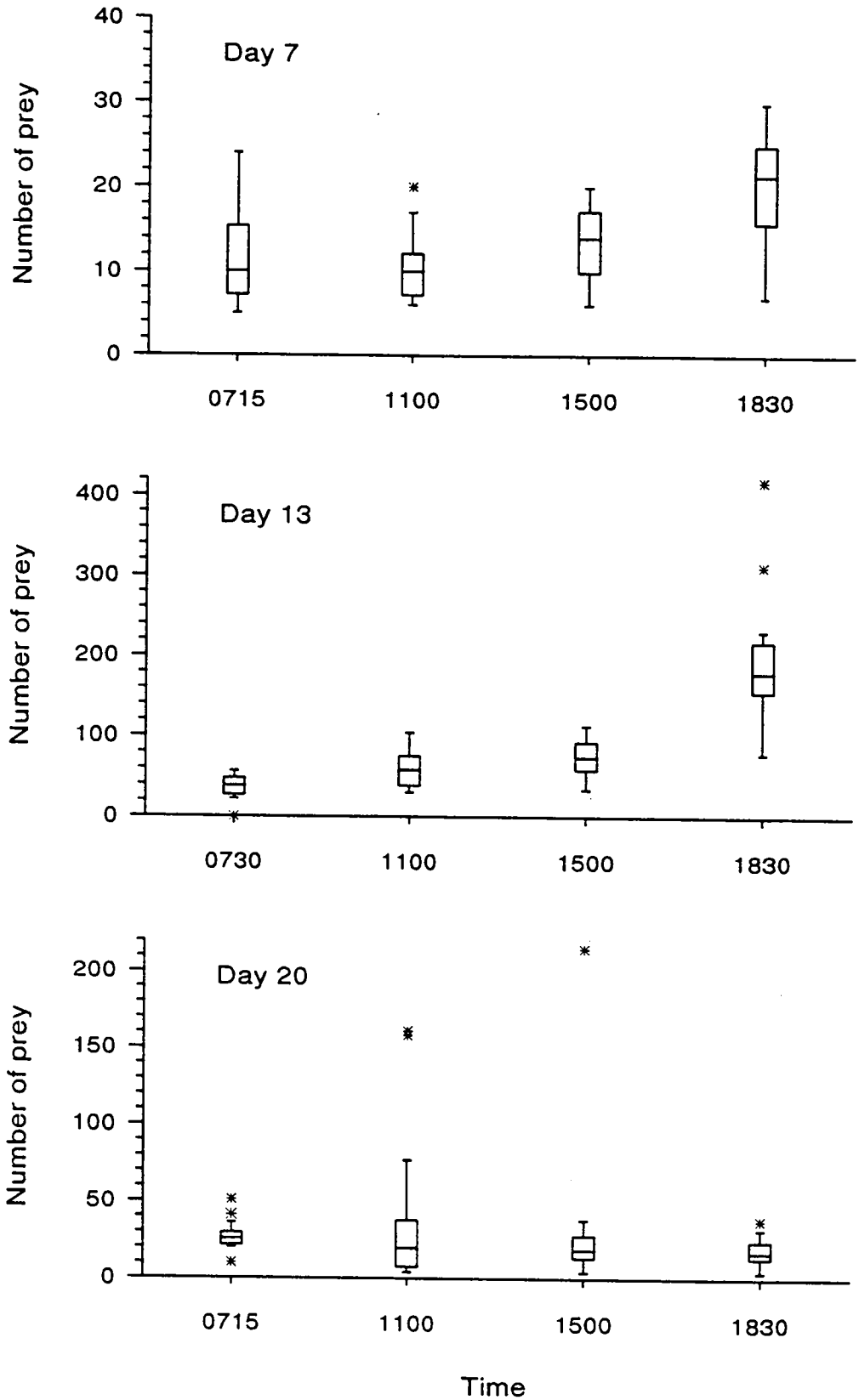

Day 7
Number of prey
40
30
20
10
0
0715
1100
1500
1830
Day 13
Number of prey
400
300
200
100
0
0730
1100
1500
1830
Day 20
Number of prey
200
150
100
50
0
0715
1100
1500
1830
Time

Figure 8.3 Dry weight of prey in guts of barramundi sampled 4 times daily at 7, 13, and 20 days from hatching during pond trial P12. (An explanation of box and whisker plots is provided in Appendix 3).

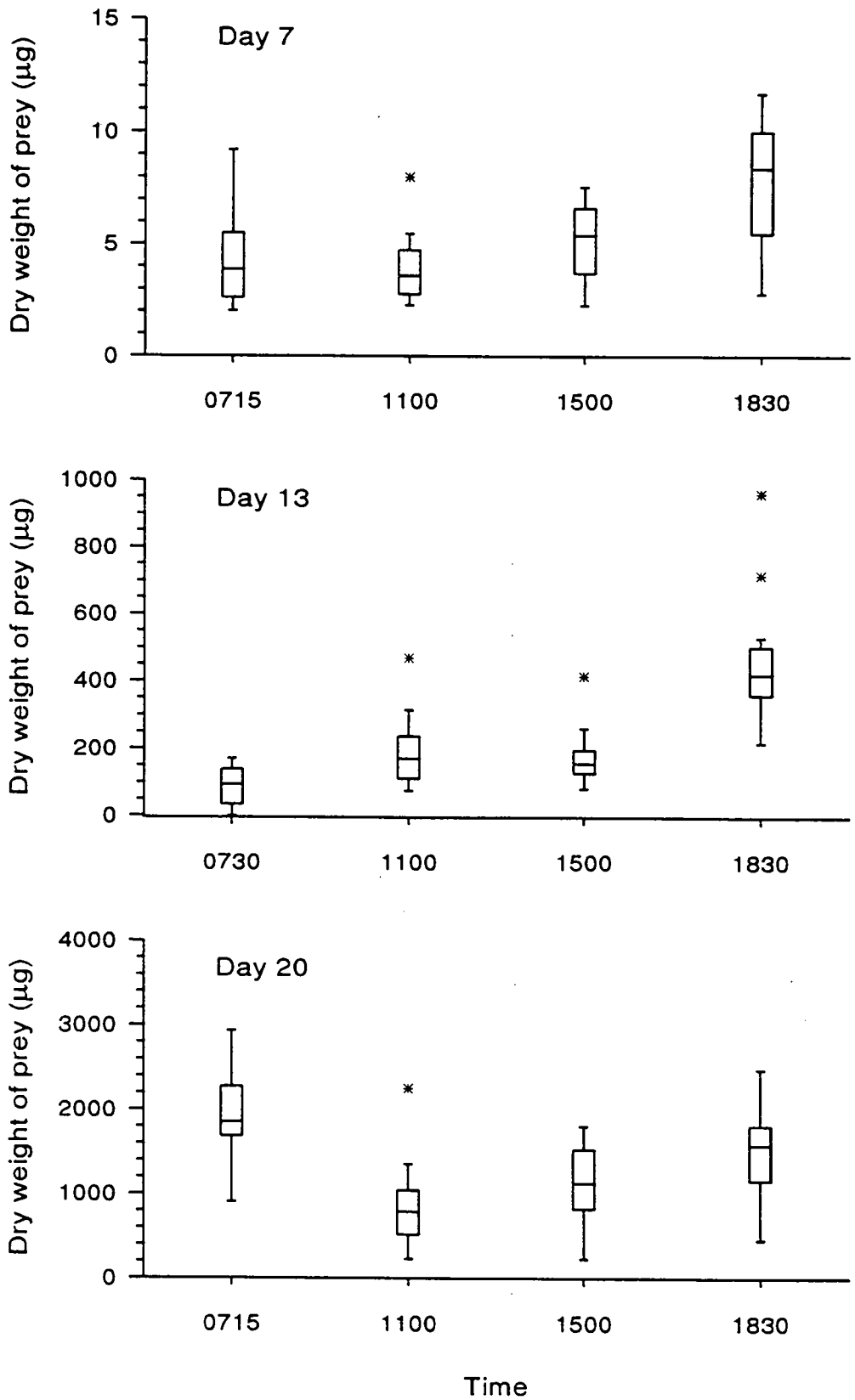

Day 7
Dry weight of prey (µg)
15
10
5
0
0715
1100
1500
1830
Day 13
Dry weight of prey (µg)
1000
800
600
400
200
0
0730
1100
1500
1830
Day 20
Dry weight of prey (µg)
4000
3000
2000
1000
0
0715
1100
1500
1830
Time

Figure 8.4 Zooplankton prey selection by barramundi larvae sampled at 4 times during day 7 from hatching (pond trial P12). All fish were sampled from the pond using a 300μm plankton net.

(a) Proportion of different zooplankton prey types in the gut contents of larvae.

(b) Proportion of different zooplankton prey types in plankton samples (>100 μm size class).

(c) Bray-Curtis dissimilarity index for gut contents and prey availability data.

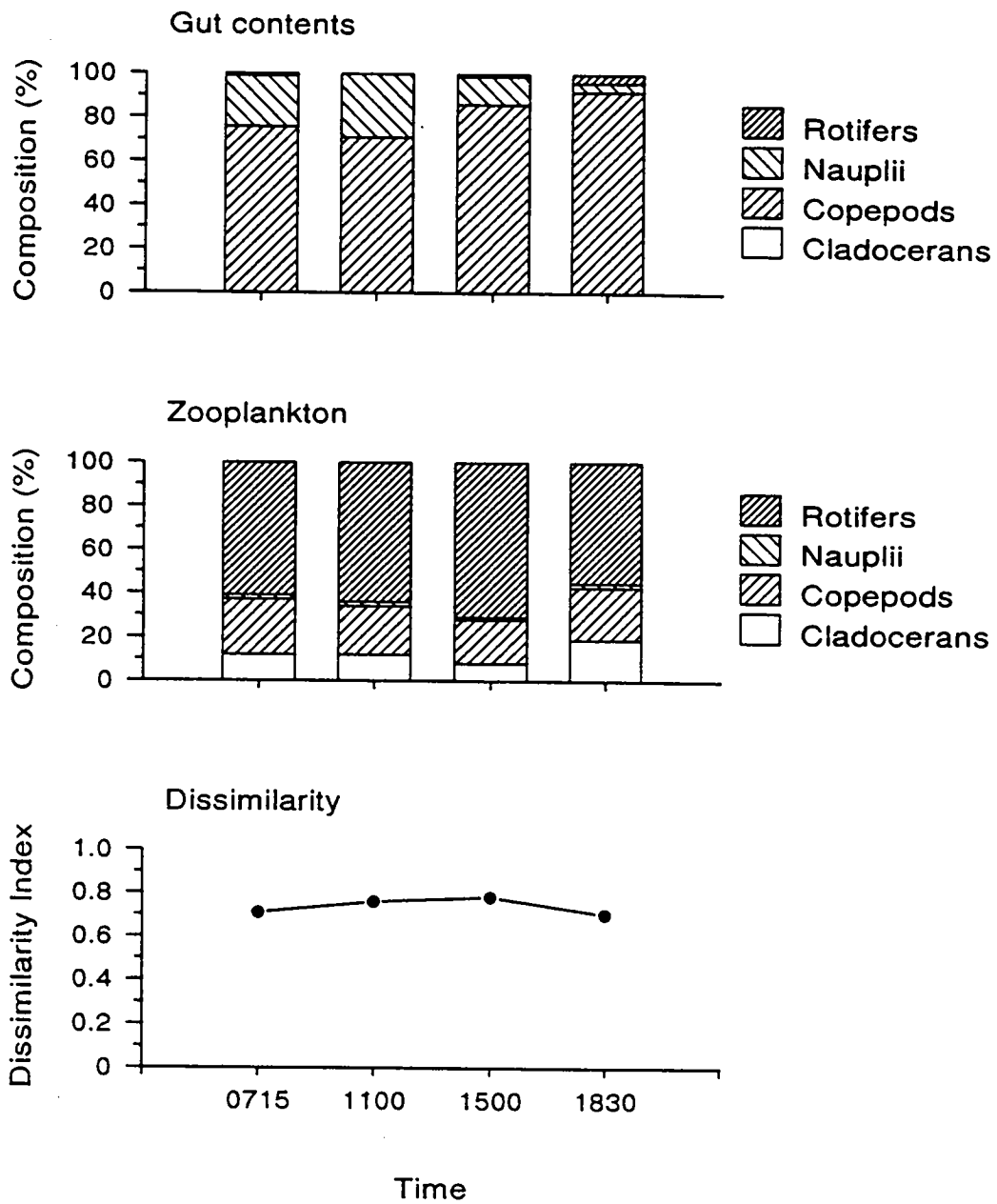
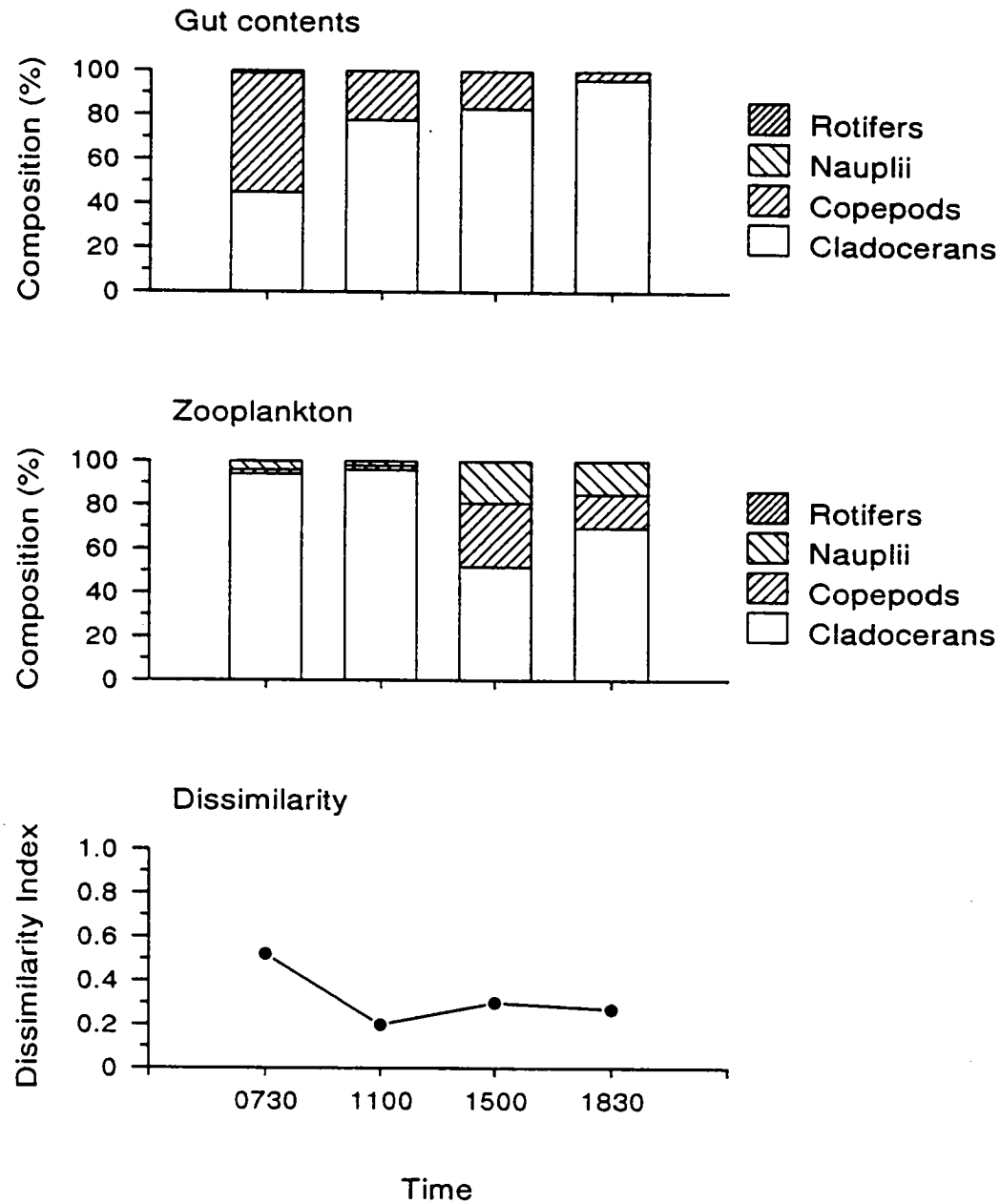

Gut contents
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Zooplankton
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Dissimilarity
Dissimilarity Index
1.0
0.8
0.6
0.4
0.2
0
0715
1100
1500
1830
Time

Figure 8.5 Zooplankton prey selection by barramundi larvae sampled at 4 times during day 13 from hatching (pond trial P12). All fish were sampled from the pond using lift nets.

(a) Proportion of different zooplankton prey types in the gut contents of larvae.

(b) Proportion of different zooplankton prey types in plankton samples (>250 μm size class).

(c) Bray-Curtis dissimilarity index for gut contents and prey availability data.


Gut contents
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Zooplankton
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Dissimilarity
Dissimilarity Index
1.0
0.8
0.6
0.4
0.2
0
0730
1100
1500
1830
Time

Feeding Patterns

In pond trials P8 and P9, rotifers predominated as the initial prey type for barramundi larvae (Figs 8.6, 8.7). In P8, rotifers formed 69-77% of the diet by number, and 55-64% of the diet by dry weight, on days 10-13 (Table 8.3). In P9, rotifers formed 29-88% of the diet by number, and 21-80% of the diet by dry weight, on days 5-9 (Table 8.5). Rotifers were consistently found in the gut contents of barramundi larvae during these two trials: 70-100% of sampled fish had rotifers in the gut contents (Tables 8.4, 8.6). Initially, selection for prey type was low in these two trials, with Bray-Curtis dissimilarity indices ranging from 0.09 to 0.25 in samples where rotifers predominated in the gut contents (Figs 8.8, 8.9).

In contrast, during pond trials P12 and P14, barramundi larvae commenced feeding mainly on copepod nauplii and small copepods (including copepodite stages) (Figs 8.6, 8.7). In pond trial P12, rotifers comprised only 0-2% of gut contents by number and 0-1% by dry weight on days 7-10 (Table 8.7). In pond trial P14, rotifers again comprised $<1\%$ of gut contents by number. Only 1-13% of larvae sampled in these trials contained rotifers in the gut contents (Tables 8.8, 8.10). Prey selection early in P12 was high, with Bray-Curtis dissimilarity measures ranging from 0.59 to 0.73 on days 7-10 (Fig. 8.10). From day 15 (P8) and day 9 (P9), copepods predominated in the gut contents of barramundi larvae (Fig. 8.6). In pond trial P12, cladocerans were the predominant prey type from days 13-15, but copepods predominated in the gut contents from day 17-22

(Fig. 8.6). Copepods were also the predominant prey in pond trial P14, although nauplii predominated from day 20 (Fig. 8.6).

Selection for copepods was variable, but was often extremely high (Figs 8.8-8.10). For example, copepods comprised 97% (by number) of the zooplankton prey of barramundi larvae sampled on day 20 during pond trial P8 (Fig. 8.8a), despite comprising only 28% of the zooplankton in the relevant size class (Fig. 8.8b). The Bray-Curtis dissimilarity index for this sample was 0.69 (Fig. 8.8c). Even higher dissimilarity indices were recorded for pond trials P9 and P12 on days 13 (dissimilarity index = 0.82) and 20 (dissimilarity index = 0.97) respectively when copepods comprised 100% (P9) and 99% (P12) (by number) of the diet but formed only 18% (P9) and 2% (P12) of the zooplankton in the relevant size class (Figs 8.9, 8.10).

Towards the end of pond trials P8, P12, and P14, the barramundi switched from zooplankton prey sources to benthic prey, principally chironomid larvae (Figs 8.6, 8.7; Tables 8.3-8.10). Only one species of chironomid was identified from the pond used in this study (from samples of larvae supplied to B. Ingram, Snobs Creek Freshwater Fisheries Research Station and Hatchery, Victoria): *Kiefferulus longilobus*. Chironomid larvae began appearing in the diet on day 17 (P8), day 13 (P12) and day 20 (P14), at which times the mean size of barramundi sampled ranged from 11.4 to 27.0 mm TL. In trials P8, P12 and P14, chironomids formed a major part of the diet, and were frequently the predominant or sole dietary item during the later stages of these trials (Tables 8.3, 8.7, 8.9).

In contrast, during pond trial P9 only a single chironomid larva was recorded from the guts of barramundi larvae sampled on day 13 (Table 8.5).

Figure 8.6 Number of prey of different types in guts of barramundi sampled during pond trials P8, P9, P12 and P14. Fish sampled on days 3, 5, 7 and 9 during pond trial P9 were sampled from enclosures; all other fish were sampled from the pond using either a 300μm plankton net or lift nets.

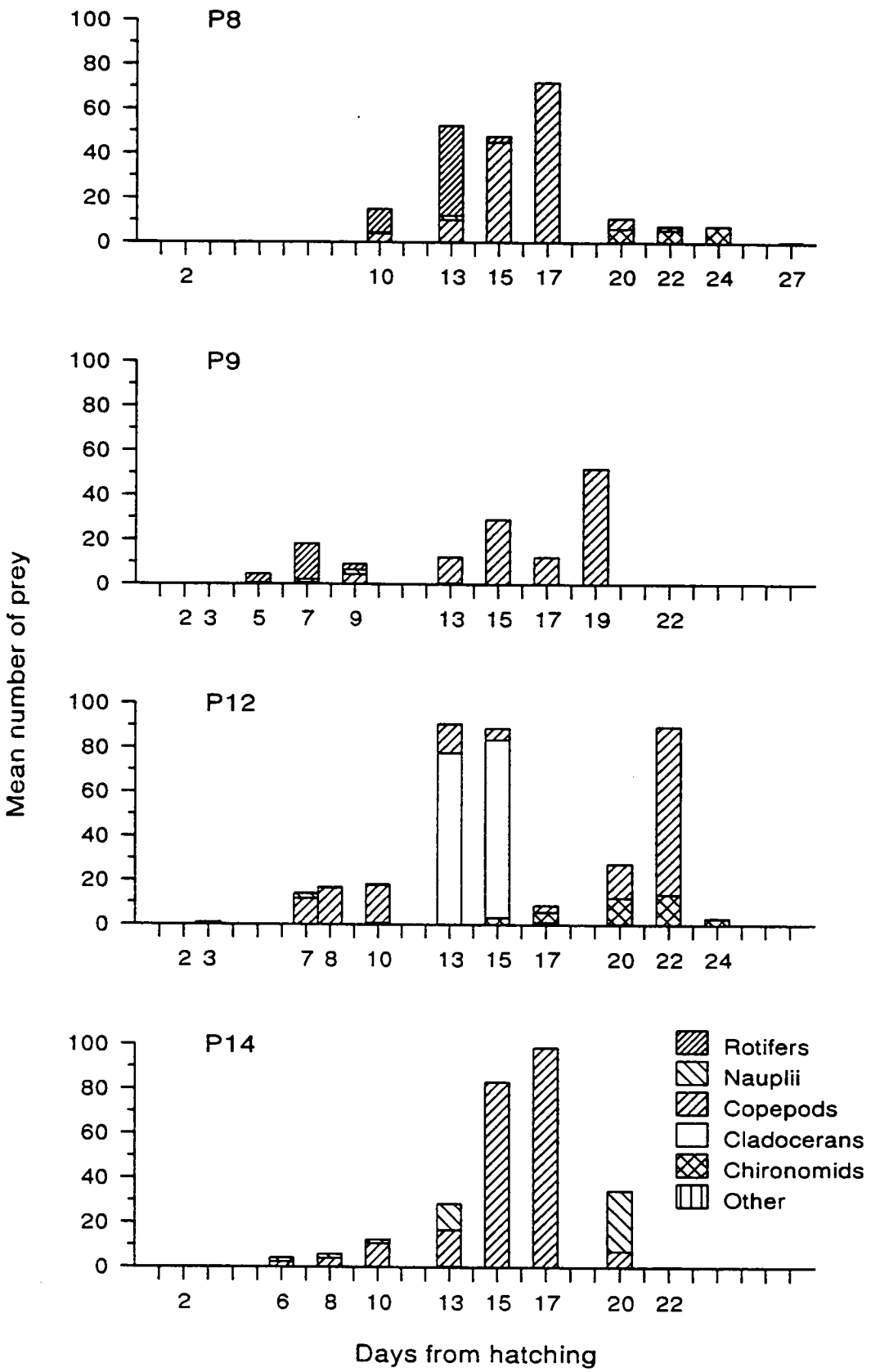
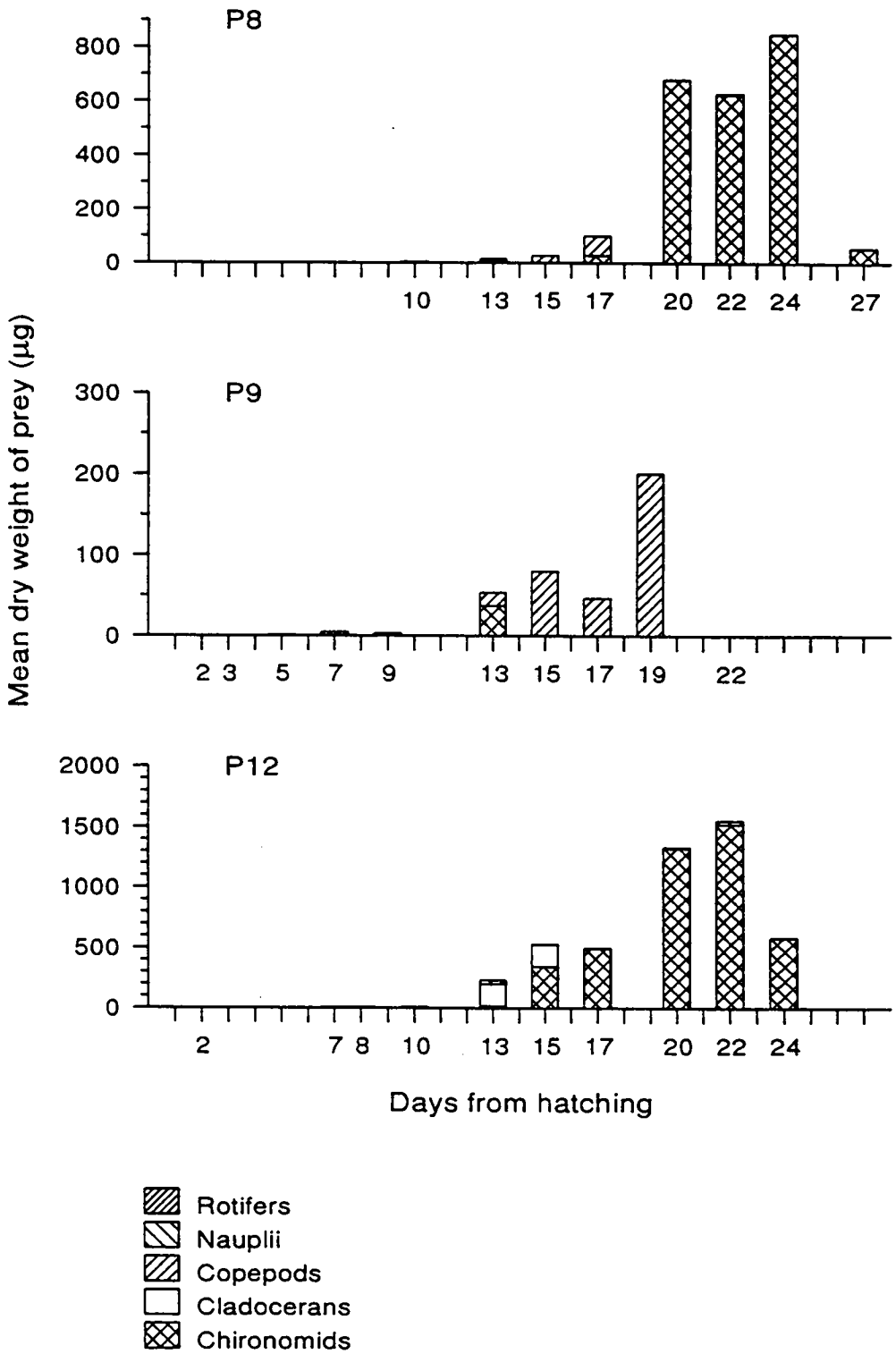

P8
P9
P12
P14
100
80
60
40
20
0
2
10
13
15
17
20
22
24
27
2 3
5
7
9
19
2 3
7 8
6
8
Mean number of prey
Days from hatching
Rotifers
Nauplii
Copepods
Cladocerans
Chironomids
Other

Figure 8.7 Dry weight of prey of different types in guts of barramundi sampled during pond trials P8, P9 and P12. Fish sampled on days 3, 5, 7 and 9 during pond trial P9 were sampled from enclosures; all other fish were sampled from the pond using either a 300μm plankton net or lift nets.


P8
800
600
400
200
0
10 13 15 17 20 22 24 27
P9
300
200
100
0
2 3 5 7 9 13 15 17 19 22
P12
2000
1500
1000
500
0
2 7 8 10 13 15 17 20 22 24
Mean dry weight of prey (µg)
Days from hatching
Rotifers
Nauplii
Copepods
Cladocerans
Chironomids

Key to abbreviations used in Tables 8.3 - 8.10:

Rot.: rotifers

Naup.: copepod nauplii

Cop.: copepodites and copepod adults

Ost.: ostracods

Clad.: cladocerans

Chir.: chironomids

Other: other organisms

Empty: proportion of fish with empty guts.

Notes for Tables 8.3 - 8.10:

1. The data shown in Tables 8.3 - 8.10 are rounded to the nearest 1%. Non-zero data that are less than 0.5% are shown as <1%, rather than being rounded to 0.
2. In many samples the relative proportions of prey type measured by number and by dry weight varies substantially. This can usually be attributed to the presence of chironomids in the diet, since the dry weight of individual chironomids dramatically exceeds that of zooplankters (Table 8.1).

Table 8.3 Percentage frequency by number and by dry weight of different prey types in gut contents of barramundi larvae sampled during pond trial P8.

Day	Prey type (%) by number [and by dry weight]					
	Rot.	Naup.	Cop.	Ost.	Chir.	Other
10	69 [55]	5 [10]	26 [35]	0	0 [0]	0
13	77 [64]	4 [4]	19 [32]	<1	0 [0]	0
15	5 [2]	1 [1]	94 [97]	<1	0 [0]	0
17	0 [0]	0 [0]	100 [72]	0	<1 [28]	0
20	0 [0]	1 [<1]	42 [<1]	0	55 [100]	3
22	0 [0]	8 [<1]	17 [<1]	2	71 [100]	2
24	0 [0]	0 [0]	0 [0]	0	99 [100]	1
27	0 [0]	0 [0]	0 [0]	0	83 [100]	17

Table 8.4 Percentage frequency of occurrence (the proportion of fish in each sample with prey type in gut contents) for barramundi larvae sampled during pond trial P8.

Day	Prey type (%)						Empty
	Rot.	Naup.	Cop.	Ost.	Chir.	Other	(%)
10	77	54	92	0	0	0	0
13	100	70	100	20	0	0	0
15	73	15	100	13	0	0	0
17	0	0	100	0	25	0	0
20	0	10	60	0	90	15	0
22	0	12	33	12	95	17	2
24	0	0	0	0	95	10	0
27	0	0	0	0	20	10	70

Table 8.5 Percentage frequency by number and by dry weight of different prey types in gut contents of barramundi larvae sampled during pond trial P9. Samples from enclosures are marked with an asterisk.

Day	Prey type (%) by number [and by dry weight]				
	Rot.	Naup.	Cop.	Chir.	Other
3*	0 [0]	0 [0]	0 [0]	0 [0]	0
5*	88 [78]	12 [22]	0 [0]	0 [0]	0
7*	88 [80]	7 [11]	5 [9]	0 [0]	0
9*	29 [21]	23 [19]	48 [60]	0 [0]	0
13	0 [0]	0 [0]	97 [30]	3 [70]	0
15	0 [0]	0 [0]	100 [100]	0 [0]	0
17	0 [0]	0 [0]	99 [100]	0 [0]	1
19	0 [0]	0 [0]	100 [100]	0 [0]	0
22	0 [0]	0 [0]	0 [0]	0 [0]	100

Table 8.6 Percentage frequency of occurrence (the proportion of fish in each sample with prey type in gut contents) for barramundi larvae sampled during pond trial P9. Samples from enclosures are marked with an asterisk.

Day	Prey type (%)					Empty
	Rot.	Naup.	Cop.	Chir.	Other	(%)
3*	0	0	0	0	0	100
5*	91	41	0	0	0	9
7*	100	65	50	0	0	0
9*	75	75	100	0	0	0
13	0	0	100	33	0	0
15	0	0	100	0	0	0
17	0	0	100	0	17	0
19	0	0	100	0	0	0
22	0	0	0	0	5	95

Table 8.7 Percentage frequency by number and by dry weight of different prey types in gut contents of barramundi larvae sampled during pond trial P12.

Day	Prey type (%) by number [and by dry weight]					
	Rot.	Naup.	Cop.	Clad.	Chir.	Other
3	0 [0]	100 [100]	0 [0]	0 [0]	0 [0]	0
7	2 [1]	15 [11]	83 [88]	0 [0]	0 [0]	0
8	0 [0]	3 [2]	96 [93]	1 [5]	0 [0]	0
10	0 [0]	2 [1]	93 [78]	5 [21]	0 [0]	0
13	<1 [<1]	<1 [<1]	15 [13]	85 [79]	<1 [8]	<1
15	0 [0]	<1 [<1]	6 [1]	90 [35]	4 [64]	<1
17	0 [0]	1 [<1]	34 [<1]	1 [<1]	51 [100]	13
20	<1 [<1]	<1 [<1]	56 [1]	0 [0]	43 [99]	1
22	0 [0]	0 [0]	85 [2]	0 [0]	15 [98]	<1
24	0 [0]	0 [0]	8 [0]	0 [0]	92 [100]	0

Table 8.8 Percentage frequency of occurrence (the proportion of fish in each sample with prey type in gut contents) for barramundi larvae sampled during pond trial P12.

Day	Prey type (%)						Empty
	Rot.	Naup.	Cop.	Clad.	Chir.	Other	(%)
3	0	100	0	0	0	0	0
7	13	64	100	0	0	0	0
8	0	43	100	14	0	0	0
10	0	40	100	60	0	0	0
13	1	11	88	98	11	1	3
15	0	5	100	100	90	10	0
17	0	5	70	5	45	75	0
20	8	4	84	0	100	14	0
22	0	0	95	0	100	5	0
24	0	0	10	0	50	0	45

Table 8.9 Percentage frequency by number of different prey types in gut contents of barramundi larvae sampled during pond trial P14.

Day	Prey type (%) by number					
	Rot.	Naup.	Cop.	Clad.	Chir.	Other
2	0	0	0	0	0	0
6	0	43	57	0	0	0
8	1	30	69	0	0	0
10	1	13	84	2	0	0
13	0	41	57	2	0	<1
15	0	0	100	0	0	0
17	0	0	100	0	0	0
20	0	79	21	0	<1	<1
22	0	0	57	0	0	43

Table 8.10 Percentage frequency of occurrence (the proportion of fish in each sample with prey type in gut contents) for barramundi larvae sampled during pond trial P14.

Day	Prey type (%)						Empty
	Rot.	Naup.	Cop.	Clad.	Chir.	Other	(%)
2	0	0	0	0	0	0	100
6	0	90	95	0	0	0	0
8	7	79	100	0	0	0	0
10	5	65	100	20	0	0	0
13	0	81	100	6	0	6	0
15	0	0	100	0	0	0	0
17	0	0	100	0	0	0	0
20	0	80	80	0	10	5	10
22	0	0	5	0	0	10	85

Figure 8.8 Zooplankton prey selection by barramundi larvae in pond trial P8. All fish were sampled from the pond using either a 300μm plankton net or lift nets.

(a) Proportion of different zooplankton prey types in the gut contents of larvae.

(b) Proportion of different zooplankton prey types in plankton samples.

(c) Bray-Curtis dissimilarity index for gut contents and prey availability data.

(d) Total lengths of sampled barramundi. Means (horizontal bars) and ranges (vertical bars) shown; numbers above bars represent sample sizes.

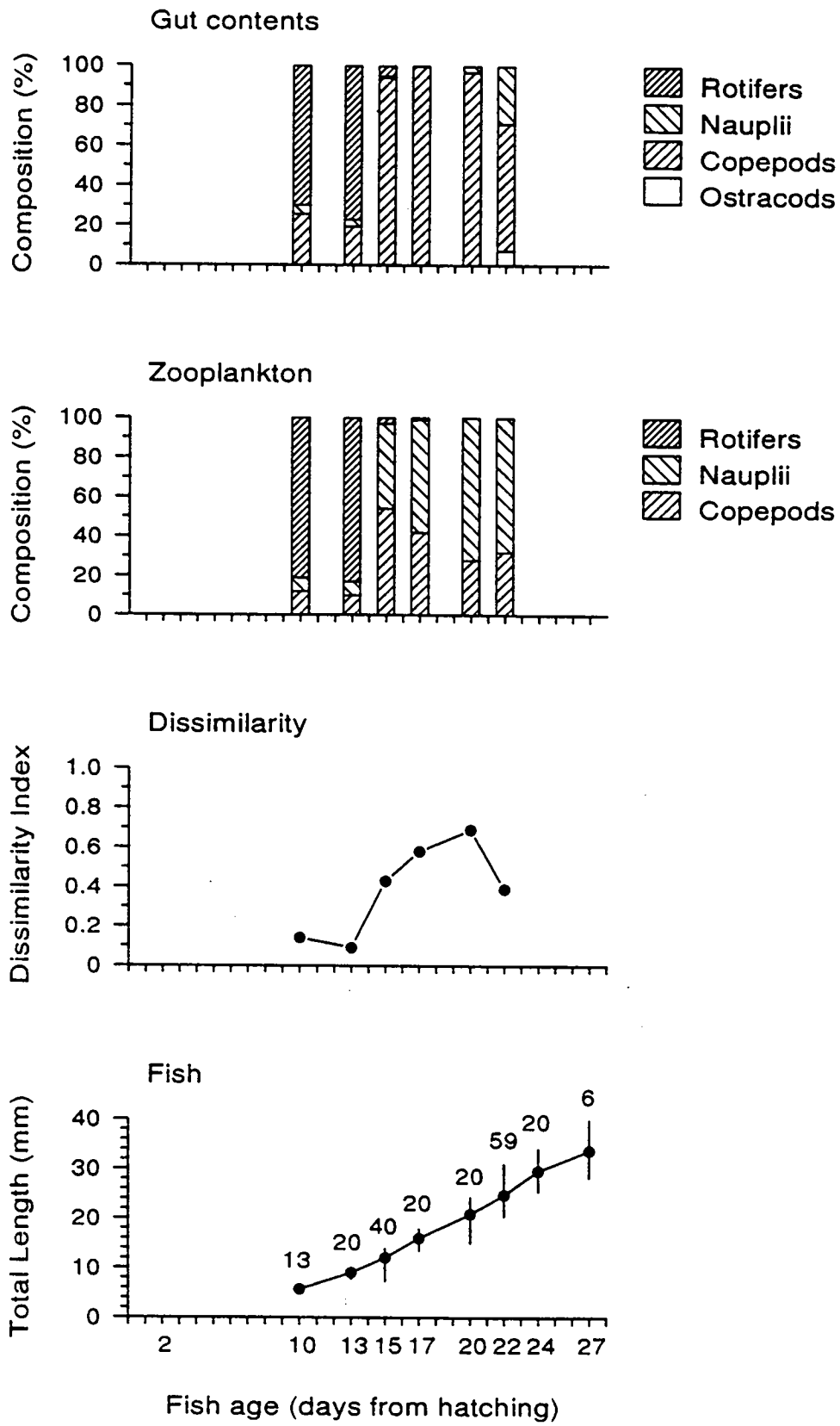

Gut contents
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Ostracods
Zooplankton
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Dissimilarity
Dissimilarity Index
1.0
0.8
0.6
0.4
0.2
0
Fish
Total Length (mm)
40
30
20
10
0
13
20
40
20
20
59
20
6
2
10
13
15
17
20
22
24
27
Fish age (days from hatching)

Figure 8.9 Zooplankton prey selection by barramundi larvae in pond trial P9. Fish sampled on days 5, 7 and 9 were sampled from enclosures; all other fish were sampled from the pond using either a 300μm plankton net or lift nets.

(a) Proportion of different zooplankton prey types in the gut contents of larvae.

(b) Proportion of different zooplankton prey types in plankton samples.

(c) Bray-Curtis dissimilarity index for gut contents and prey availability data.

(d) Total lengths of sampled barramundi. Means (horizontal bars) and ranges (vertical bars) shown; numbers above bars represent sample sizes.

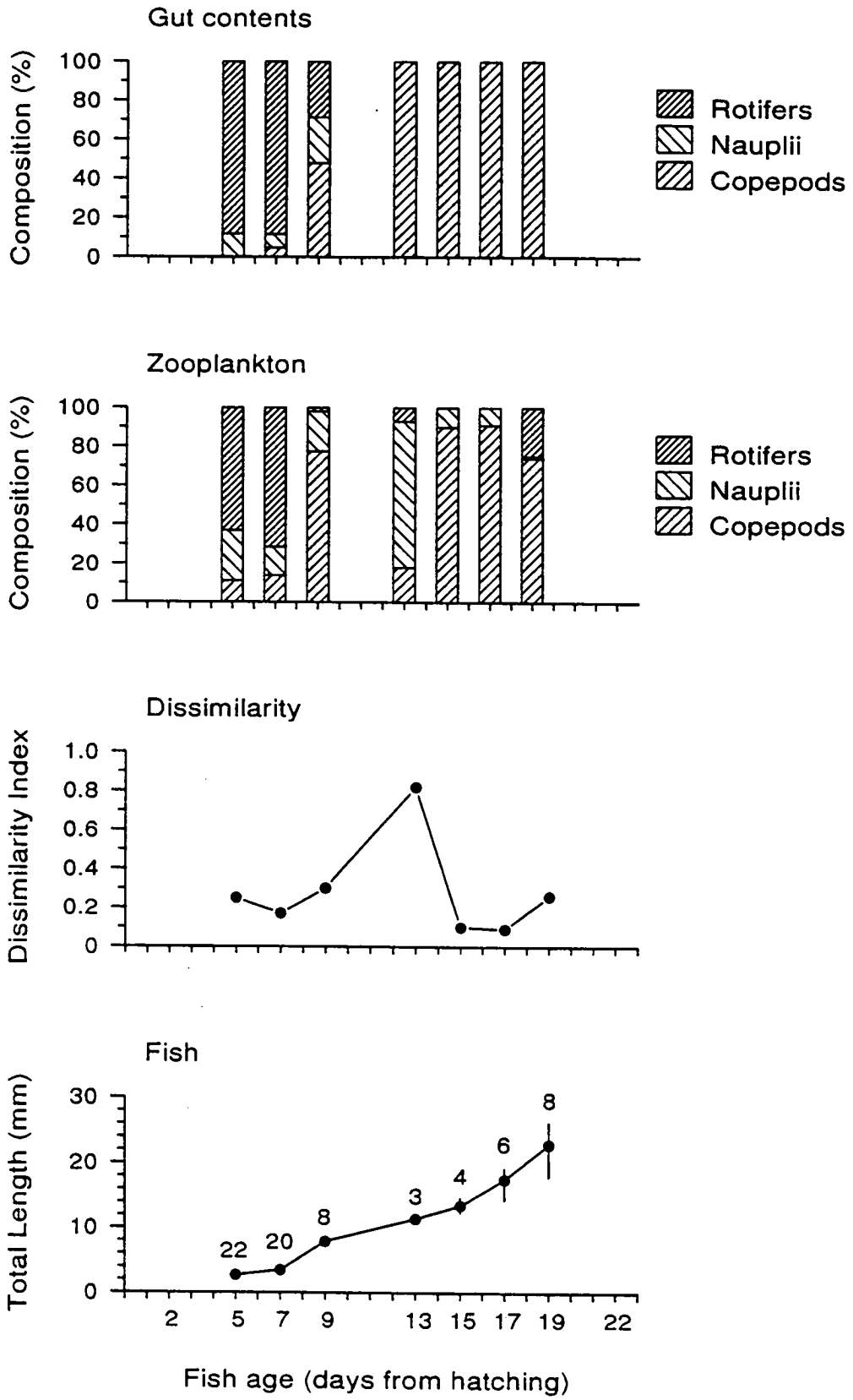

Gut contents
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Zooplankton
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Dissimilarity
Dissimilarity Index
1.0
0.8
0.6
0.4
0.2
0
Fish
Total Length (mm)
30
20
10
0
22
20
8
3
4
6
8
2
5
7
9
13
15
17
19
22
Fish age (days from hatching)

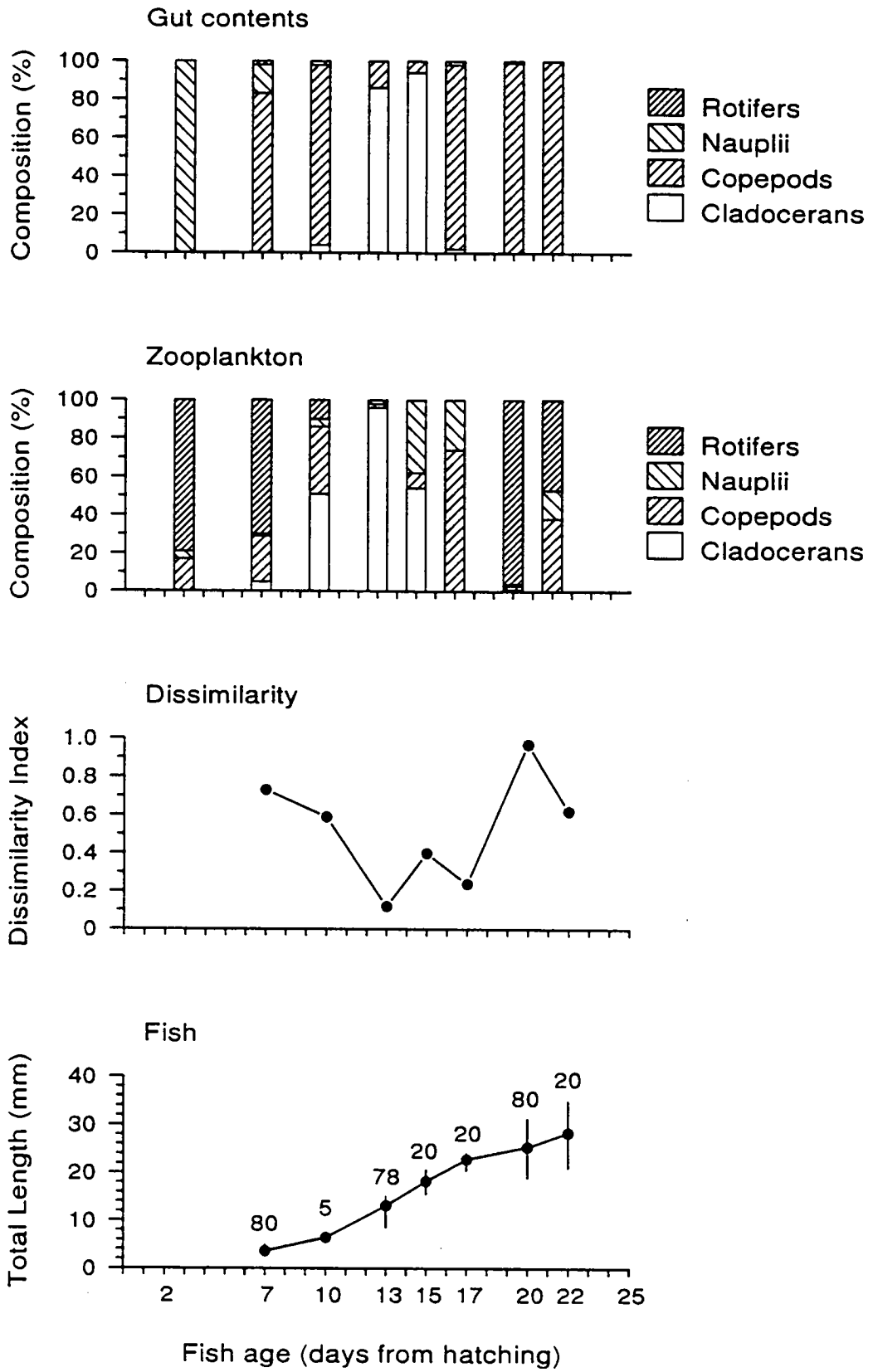
Figure 8.10 Zooplankton prey selection by barramundi larvae in pond trial P12. Fish were sampled from the pond using either a 300μm plankton net or lift nets.

(a) Proportion of different zooplankton prey types in the gut contents of larvae.

(b) Proportion of different zooplankton prey types in plankton samples.

(c) Bray-Curtis dissimilarity index for gut contents and prey availability data.

(d) Total lengths of sampled barramundi. Means (horizontal bars) and ranges (vertical bars) shown; numbers above bars represent sample sizes.


Gut contents
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Zooplankton
Composition (%)
100
80
60
40
20
0
Rotifers
Nauplii
Copepods
Cladocerans
Dissimilarity
Dissimilarity Index
1.0
0.8
0.6
0.4
0.2
0
Fish
Total Length (mm)
40
30
20
10
0
80
5
78
20
20
80
20
2
7
10
13
15
17
20
22
25
Fish age (days from hatching)

Prey Size Selection

The relationship between fish age and mean size of prey ingested is shown in Figure 8.11a. Generally, increasing age and size of extensively reared barramundi larvae was associated with an increase in the mean size of prey ingested. In addition, older (and larger) fish ingested a larger size range of prey than did younger fish (Fig. 8.11a).

The relationship between fish size and prey size (Fig. 8.11b) varied between trials as a result of the differing availability of different sizes of prey in each trial (Chapter 7). However, all three trials showed a similar trend of increasing prey size with increasing fish size; hence data for pond trials P8, P9, and P12 were combined. The regression model which best fitted the combined data was a semi-logarithmic relationship:

regression equation: $PS = e^{(4.772 + 0.042\ TL)}$,

where PS is mean prey size (μm) and TL is fish total length (mm);

adjusted $r^2 = 0.49$, $F = 564.2$, $P < 0.001$.

The regression was strongly influenced by the data for day 17 for pond trial P12 (Fig. 8.11) which contained a number of extremely high values for mean prey size which resulted from the fish devouring small prawns at this time.

Figure 8.12 shows the relationships between the maximum size of prey (with individual prey items averaged for each prey type) in the gut contents of each fish, and jaw gape at 45° and jaw gape at 90°, together with a 'line of

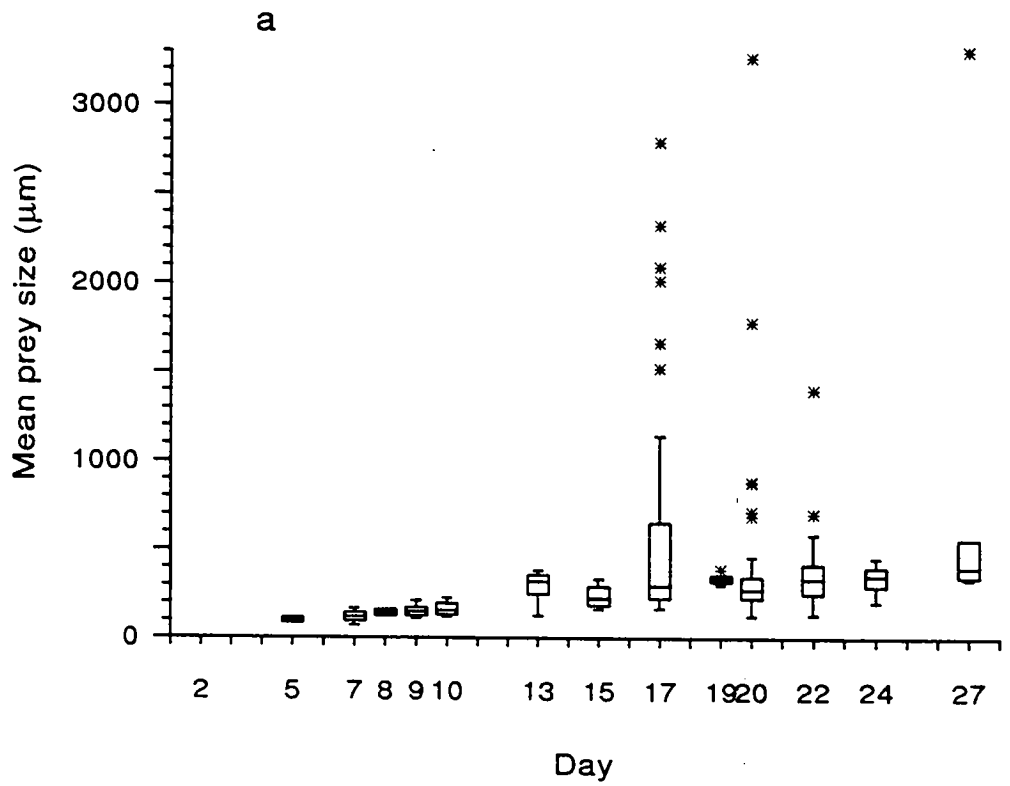
equivalence' which indicates the relationship when prey size is equal to jaw gape. Only 3 fish had ingested prey larger than the estimated jaw gape with the jaw opening angle at 45°. These were all relatively large fish, ranging from 24.1 to 29.5 mm TL. No fish had ingested prey larger than the estimated jaw gape with the jaw opening angle at 90°.

Figure 8.11 Relationship between prey size and fish age and size for extensively reared barramundi.

(a) Mean size of prey in the guts of barramundi sampled during pond trials P8, P9, and P12.

(b) Relationship between fish size and prey size;

Regression line: $Y = e^{(4.772 + 0.042\ X)}$.


a
Mean prey size (µm)
3000
2000
1000
0
2
5
7
8
9
10
13
15
17
19
20
22
24
27
Day

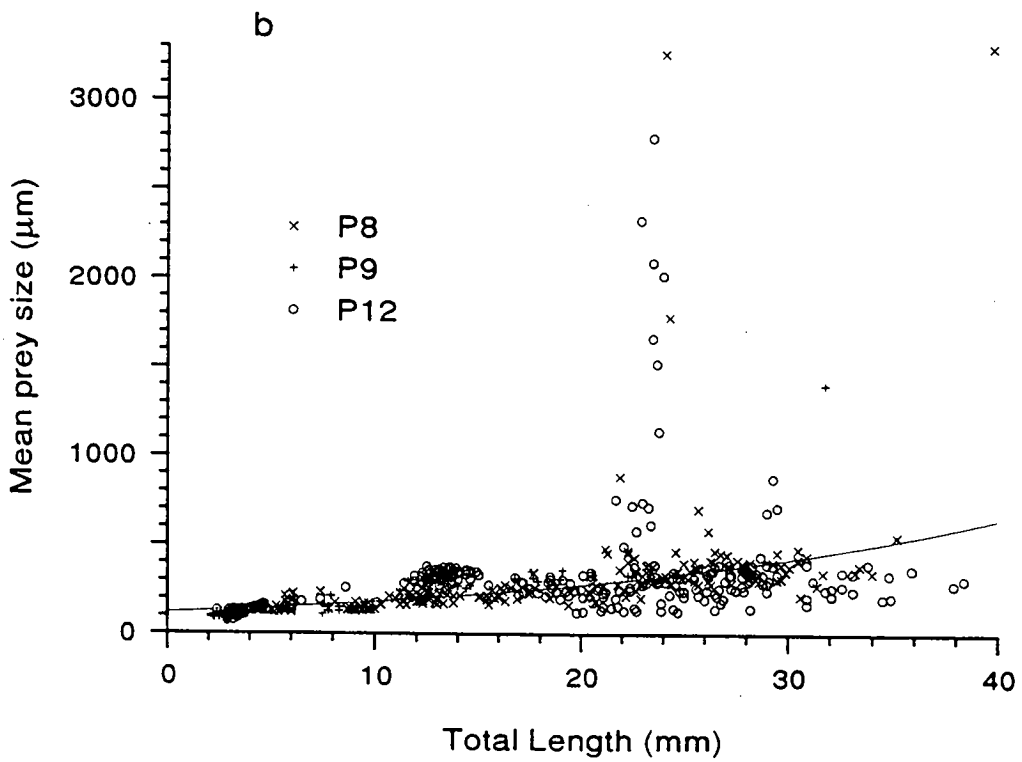
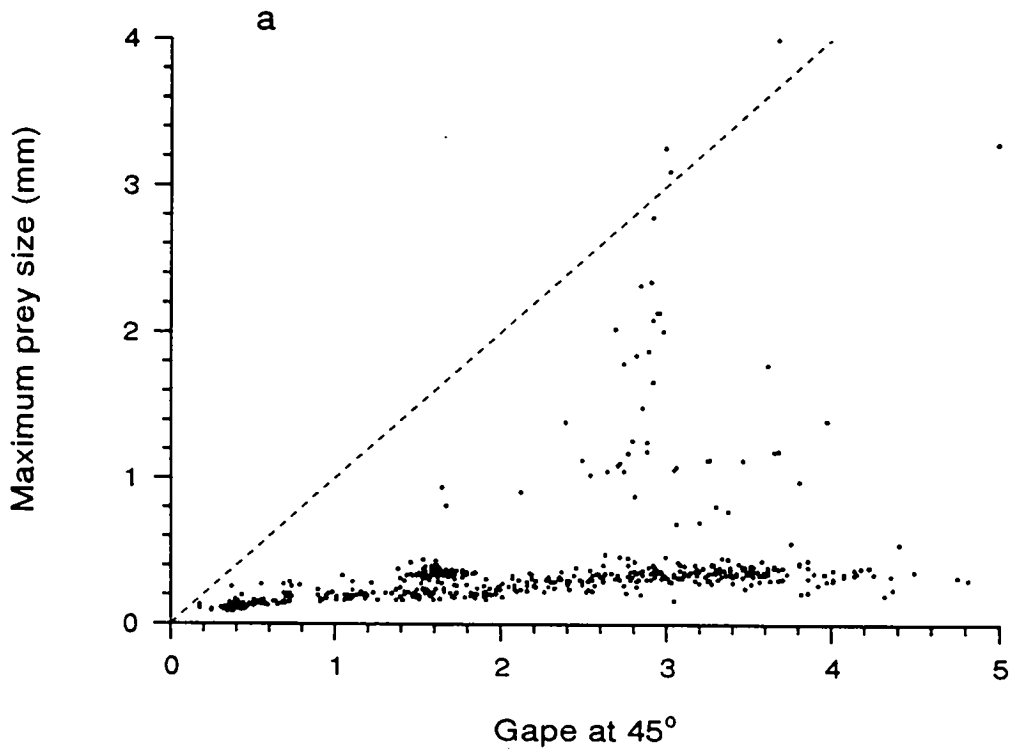
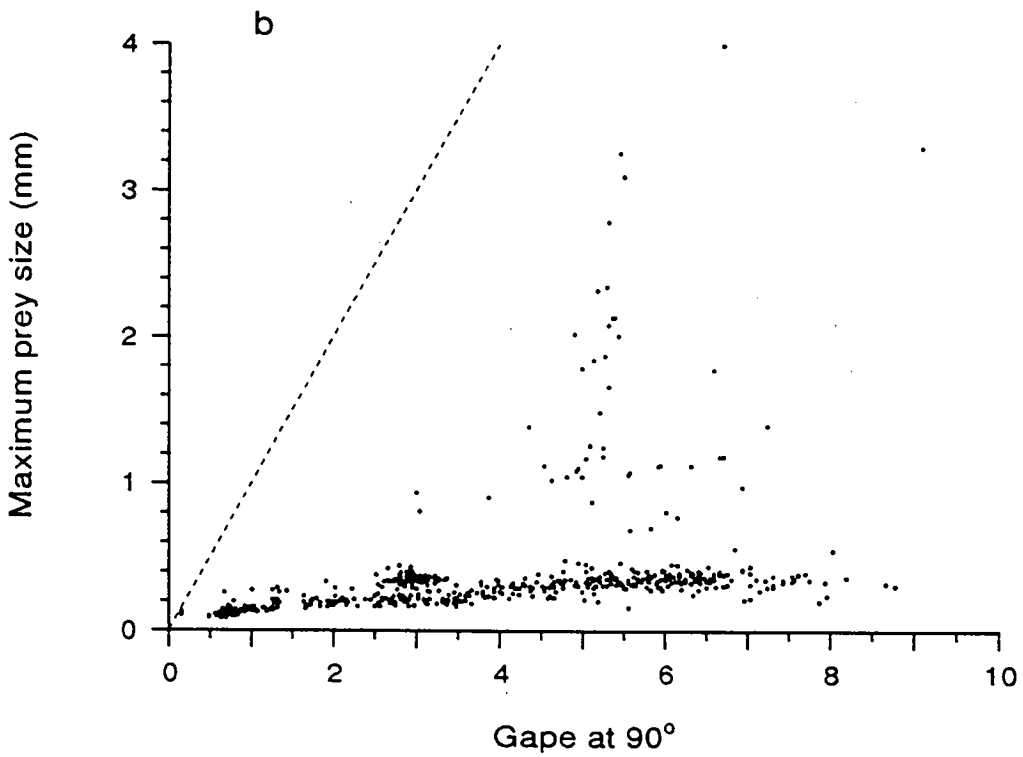

b
Mean prey size (µm)
3000
2000
1000
0
P8
P9
P12
0
10
20
30
40
Total Length (mm)

Figure 8.12 Relationship between prey size and mouth size, measured as (a) jaw gape with the jaws at a 45° angle of opening and (b) jaw gape with the jaws at a 90° angle of opening, for barramundi from pond trials P8, P9, and P12. The dotted line represents the 'line of equivalence', i.e. when prey size is equivalent to mouth size.


a
Maximum prey size (mm)
0
1
2
3
4
0
1
2
3
4
5
Gape at 45°


b
Maximum prey size (mm)
0
1
2
3
4
0
2
4
6
8
10
Gape at 90°

Cannibalism

Only 5 fish were found with teleost remains in the gut contents during this study. Of these 5 fish, only 2 had remains which could positively be identified as barramundi in their guts. The fish remains found in the other 3 fish may have been barramundi, or may have been the remains of small gobies (Family Gobiidae) which were sometimes found in the pond. All the fish found with teleost remains were relatively large, ranging from 24.1 to 39.8 mm TL.

Discussion

Diel Variation

Barramundi larvae sampled on days 7 and 13 had significantly higher numbers of prey in the gut at dusk, than during the rest of the day. This pattern appears to be related to feeding activity, rather than diel patterns of prey availability, since prey density did not vary throughout either day 7 or day 13. Barlow *et al.* (1993) found a similar pattern of feeding activity in barramundi reared in freshwater ponds, with a peak in feeding activity at dusk. Any pattern in feeding activity during day 20 was obscured by the high degree of variability in these samples, and, as noted previously, the influence of the dry weight of individual chironomid larvae. This variability was possibly due to the relatively low densities of zooplankton available at this time and consequent competition for prey.

Feeding Patterns

The gut contents of barramundi larvae sampled during four extensive rearing trials indicated that the larvae feed primarily on zooplankton, but will switch to benthic prey once zooplankton densities decline to very low levels. Generally, the progression of dominant prey types eaten by barramundi larvae in these extensive rearing trials was:

Rotifers / Nauplii → Copepods → Chironomids

Other studies on the diet of barramundi in brackishwater ponds have found a similar progression of prey from small zooplankton to larger invertebrates, albeit with some differences from the results of this study. De (1971), using an 'index of preponderance' which incorporated both relative occurrence and relative volume of different food items, found that copepods formed 75% of the diet of barramundi from 10-15 mm in length. This proportion decreased to 35% in barramundi 16-45 mm; the major component of the diet in this size class was notonectids (43%). Ghosh and Pandit (1979) found that barramundi up to 50 mm in length consume diatoms, copepod nauplii and copepods, while barramundi 51-125 mm in length fed on benthic diatoms, prawn larvae and copepods.

Although these studies show substantial differences in the relative proportions of various food items in the gut of barramundi larvae and juveniles, they may primarily reflect differing availability of these food items during the

different studies. For example, notonectids, which comprised the major component of the diet of 16-45 mm barramundi in De's (1971) study, were not found in the pond used for the extensive rearing trials described in this thesis. However, the overall pattern of a progression from smaller zooplankton to larger zooplankton, then to larger invertebrates, is relatively consistent amongst all these studies.

While the barramundi examined in the present study also followed this general feeding pattern, there was considerable variation in feeding patterns between individual pond trials. A major difference in feeding patterns was observed between trials with and without pond aeration. In the unaerated trials (P8 and P9), barramundi larvae commenced feeding on rotifers, which were the dominant prey type for about the first week after the larvae were stocked into the pond. In the aerated trials (P12 and P14), barramundi commenced feeding mainly on copepod nauplii and ate only small numbers of rotifers throughout the trial.

This change in feeding pattern required selection of copepod nauplii in preference to rotifers. In trials in which the pond was not aerated (P8 and P9), barramundi larvae initially showed a low degree of prey selectivity, which later increased as the fish preyed almost exclusively on copepods (Figs 8.8-8.9). In trials in which the pond was aerated, prey selection was initially high as the barramundi larvae selected nauplii in preference to rotifers (Fig. 8.10).

The reasons for this change in diet are unclear. Aeration apparently altered the spatial distribution of barramundi larvae within the pond, but had no

obvious effect on zooplankton distribution (Chapter 7). These results suggest that the currents induced by aeration may have made capture of nauplii easier than the capture of rotifers, although the mechanism whereby this may occur is not apparent. Intensively reared barramundi larvae successfully capture rotifers in aerated tanks where the larvae are subjected to current velocities equivalent to, or greater than, those experienced in aerated ponds. Since both predator and prey are subjected to the same currents in aerated ponds, both should be almost stationary relative to each other, and thus increased current should not affect feeding behaviour dramatically.

An alternative hypothesis is that the changed spatial distribution of the larvae allowed increased activity (e.g. by providing access to surface waters with higher levels of dissolved oxygen) which enabled greater prey selection by the larvae. However, further physicochemical data on various habitats within the pond, and on the distribution of barramundi larvae in aerated and unaerated larval rearing ponds, are necessary before this hypothesis can be examined in detail.

A second difference between trials was the change from planktonic to benthic prey, principally chironomid larvae, following the exhaustion of zooplankton prey. Barramundi larvae in pond trial P9 did not commence feeding on chironomids, as was seen in trials P8 and P12, but continued to feed on copepods up until harvest. This was despite the presence of large quantities of chironomid larvae in the pond at this time. Overall survival in pond trial P9 was only 2% (equivalent to 9,200 fish/ha), whereas in trials P8 and P12 survival was

70% (equivalent to 467,000 fish/ha) and 86% (equivalent to 637,000 fish/ha) respectively (Table 7.2). The difference in feeding patterns seen in pond trial P9 appears to be associated with the lower density of fish in this trial, and the impact this lower density had on prey populations.

In pond trial P8, chironomids first entered the diet of barramundi on day 17 from hatching (Fig. 8.7) when the fish averaged 16.0 mm TL. At this time, the density of zooplankton in the >250μm size class was 1.7 organisms/litre, and the density of this size class continued to decrease until harvest (Fig. 7.29). In pond trial P12, chironomid larvae first entered the diet on day 13 from hatching (Fig. 8.11) when the barramundi larvae averaged 13.2 mm TL. At this time the density of zooplankton in the >250μm size class was 17.5 organisms/litre, but the density of this size class of organisms dropped to 2.4 organisms/litre by day 15 from hatching and subsequently to zero for the rest of the trial (Fig. 7.48). In contrast, in pond trial P9 the density of zooplankton in the >250μm size class reached a minimum of 1.9 organisms/litre on day 15 from hatching, then began to increase, reaching 15.7 organisms/litre 2 days prior to harvest (Fig. 7.34).

This pattern suggests that barramundi larvae select zooplankton in preference to benthic prey such as chironomid larvae when zooplankton prey is available. In pond trial P8 and P12, the relatively high densities of barramundi reduced the density of zooplankton in the preferred size class (>250μm) to such a low level that the populations of organisms which comprise this size class could

not recover in the face of continued heavy predation. Once zooplankton densities declined, the barramundi concentrated on the only remaining prey type of suitable size in the pond: chironomid larvae. In pond trial P9, densities of zooplankton in the $>250\mu$m size class also dropped to low levels, but the populations of zooplankton comprising this size class recovered rapidly, possibly due to relatively light predation pressure due to the poor survival of barramundi in this trial. Although the fish from pond trial P9 were large enough to consume chironomid larvae (mean length at harvest was 21.5 mm TL), they continued to prey almost exclusively on copepods. Overall, this pattern suggests that even relatively large juvenile barramundi (>20 mm TL) remain zooplanktivorous, and commence feeding on benthic prey only in the absence of adequate quantities of zooplankton of suitable size.

This result superficially conflicts with that of Barlow *et al.* (1993) who found that juvenile barramundi change feeding habit from 'roving zooplanktivore' to 'lurking predator' at about 18 mm TL. In the present study, juvenile barramundi over 18 mm TL were still almost exclusively zooplanktivorous. This discrepancy appears to be related to the difference in prey items available in brackishwater ponds (this study) and in freshwater ponds (as studied by Barlow *et al.* 1993). Barramundi in freshwater ponds showed a decrease in the proportion of zooplankton eaten, and a corresponding increase in the proportion of insects (mainly Ephemeroptera, Odonata, and Notonectids) eaten, with increasing size. Because these insect groups were not represented in the brackishwater pond

during this study, this pattern of dietary change was not possible. Instead, the barramundi continued to prey on zooplankton.

However, the continued consumption of zooplankton by relatively large fish, compared with the consumption of chironomid larvae, appears to be energetically inefficient. Even the largest crustacean zooplankters in the pond have a dry weight of only 3.5% of the average chironomid (Table 8.1). Thus a fish would have to eat 29 copepods to ingest the equivalent dry weight of one chironomid. Griffiths (1975) suggests that predators may switch from an 'energy maximiser' mode of predation (i.e. selecting relatively large prey to maximise the energy gained to compensate for the energy spent on pursuit and capture) to a 'number maximiser' mode of predation (i.e. simply catching prey as they are encountered) when prey densities are high. Prey densities may be considered high in most aquacultural rearing situations, including extensive rearing, compared to those found in natural systems (Leis 1992), which may explain why barramundi prefer to remain as 'number maximisers' even when larger benthic prey organisms are present at high density. Additional studies of predator and prey behaviour are necessary to further examine modes of predation in barramundi larvae and juveniles.

Most barramundi sampled after being harvested from the pond had empty guts. Although this may have been related to low levels of prey availability prior to harvest, it may also have been due to the stress of the harvest procedure preventing feeding. Pond draining commenced approximately 6-8 hours prior to

harvesting the fish. Observation of barramundi during pond draining indicated that the stress associated with this procedure precluded feeding. Juvenile barramundi, 37 mm TL, have been found to have a median stomach evacuation rate of 180 minutes in non-feeding conditions at 28°C (Barlow *et al.* 1993), so fish stressed, and not eating, during harvest would have had ample time to evacuate their gut contents prior to sampling.

Cannibalism

The low level of cannibalism observed in extensively reared barramundi has also been noted in other studies on barramundi in brackish and freshwater ponds. De (1971) did not record fish remains in the gut contents of barramundi less than 50 mm, and Ghosh and Pandit (1979) considered barramundi to become piscivorous only after reaching a length of 125 mm.

This result contrasts with the situation found in intensive rearing tanks, where barramundi may cannibalise fish up to 61-67% of their own length (Parazo *et al.* 1991) and consequently must be graded at regular intervals to reduce the incidence of cannibalism (Parazo *et al.* 1990). Barramundi removed from extensive rearing ponds and placed in nursery facilities immediately adopt cannibalistic habits if not graded, suggesting that cannibalism is primarily a function of density. The highest density of barramundi obtained in these trials (in pond trial P12) would have been equivalent to 43 fish/m^3 in the pond, whereas densities of 5,000 fish/m^3 are routinely reached in nursery facilities.

Pond Management Implications

The results described in this chapter indicate that barramundi are capable of utilising a wide range of prey types, and that the pond management regime used for these trials adequately supports the production of suitable prey organisms in suitable quantities for effective production of barramundi fingerlings. It was not necessary to supplement natural zooplankton production with other prey organisms, such as brine shrimp, as has been found necessary in the extensive larval rearing of other Australian marine fishes (Battaglene *et al.* 1992).

However, one possible alteration to the existing pond management strategy that may be beneficial is the inoculation of the pond with rotifers prior to stocking the barramundi larvae, as has been trialled with red drum (Sturmer 1990), to provide a higher density of first feed prey organisms. Red drum rearing ponds stocked with rotifers in log-phase growth exhibited greater fish survival and production than control ponds (Sturmer 1990).

Based on the results of the present study, stocking rotifers into larval rearing ponds used for barramundi would only be worthwhile in unaerated ponds, because rotifers are of negligible importance as prey items for barramundi larvae in aerated ponds. For aerated rearing ponds, it may be advantageous to stock copepods to provide a population of copepod nauplii to act as prey for barramundi larvae. Unfortunately, copepod culture techniques are much less reliable than those for rotifers (Rimmer and Semmens 1993), and a great deal of further

research would be necessary to develop reliable techniques for inoculating larval rearing ponds with copepods to increase the availability of copepod nauplii.

Aeration confers several advantages to pond management: circulation is improved, which may assist with nutrient mixing in the water column, and diurnal dissolved oxygen fluctuations may be reduced. However, the findings of the present study indicate that pond aeration may be successful only when the pond contains high densities of copepods. Thus, the decision to use aeration in extensive rearing of barramundi may depend on the dominant zooplankton fauna at first feed. Additional research is required to further examine the role of artificial aeration in extensive larval rearing applications.

CHAPTER 9

Discussion: Extensive Larval Rearing of Barramundi

The results described in the previous two chapters indicate that the techniques developed for the extensive larval rearing of other marine fishes, particularly red drum, are suitable for use in rearing barramundi larvae, although some modification is necessary to adapt to local conditions. As noted in Chapter 6, the essential requirements for efficient fish production are the provision of a suitable environment and the provision of prey organisms of optimal size which are available in sufficient quantity to provide for survival and growth of the fish throughout the rearing period (Geiger 1983a,b, Sturmer 1990). The results of the extensive rearing trials undertaken with barramundi also indicate the importance of the pond environment, particularly during early larval development, and the production of suitable prey to support survival and growth of the larvae.

Environmental Conditions

The results of the analyses reported in Chapter 7 indicate that temperature and pH during the first week after hatching are important factors in determining larval survival in rearing ponds. As noted in Chapter 7, there may be other variables which also affect the survival of extensively reared barramundi larvae which could not be determined in this study because of the relatively low number of trials undertaken. In addition, water quality parameters which were not

measured during this study, such as ammonia, may also affect larval survival in extensive rearing ponds (Anderson 1993a, Bergerhouse 1993).

However, the results of the present study clearly indicate that most mortality is related to conditions in the pond during the first week after stocking. Similar studies on extensive rearing of other marine fish species are lacking, but Rutledge (1988) and McCarty and Gregg (1992) found a close correlation between the density of red drum in ponds two days after stocking and the number of fish harvested at the end of each pond run. This correlation suggests that most mortality occurs of red drum larvae within two days after stocking. It is possible that barramundi larvae also suffer most mortality during the first few days after stocking. Testing of this hypothesis was not possible during this study because larvae could not be sampled prior to about day 10 from hatching until aeration was introduced for pond trial P12. Future research will examine the correlation between larval density following stocking and survival to harvest.

Temperature appears to play a critical role in determining the survival of barramundi larvae reared extensively. Although there is little that can be done to effectively control water temperature in larval rearing ponds, control of the extremely high water temperatures that result from ectogenic meromixis may be possible. Since the occurrence of this phenomenon in pond trial P7, I have had reports of similar meromictic events in commercial barramundi larval rearing ponds, including one pond which was described as having water 'too hot to stand in' (D. Hart, pers. comm.). Dor and Paz (1989) reported a similar example of

ectogenic meromixis in experimental solar ponds in Israel, but this phenomenon in aquaculture ponds does not appear to have been previously reported (C.E. Boyd, pers. comm.).

The occurrence of ectogenic meromixis has important implications for mariculture ponds in the wet tropical regions where high levels of solar radiation may promote considerable heating in ponds, and wet season conditions may severely limit the availability of saline water. Without a supply of cooler saline water to replace the heated water of the monimolimnion, severe stress or death of cultured animals may frequently occur under conditions of ectogenic meromixis. Since most barramundi aquaculture is carried out in far northern Queensland (Trendall and Fielder 1991), conditions which promote ectogenic meromixis (i.e. heavy rainfall and high levels of solar radiation) may be a limiting factor in the use of extensive larval rearing techniques for barramundi.

Aeration can be used to prevent stratification of the pond, but heavy rainfall will result in a rapid decrease in salinity as the incoming freshwater mixes with the saline water used to fill the pond. Although reduced salinities may be lethal to newly hatched barramundi, larvae as young as day 8 have been successfully transferred to freshwater (S. Fielder, pers. comm.), indicating that if salinities can be kept high for the first week after stocking, barramundi larvae should tolerate any decrease in salinity thereafter. Overall, reduced salinities through mixing by aeration is a better management strategy than allowing the development of ectogenic meromixis in ponds.

The role of elevated pH as a major factor in determining larval survival has only recently been recognised in freshwater larval rearing ponds (Anderson 1993a,b, Bergerhouse 1993). Generally, the pH values measured in this study are lower than those commonly attained in the extensive larval rearing of Australian freshwater fish (up to 9.5) (S. Rowland, pers. comm.), presumably due to the better buffering capacity of sea water. The major cause of pH elevation in ponds is the utilisation of CO_2 during photosynthesis by the phytoplankton (Boyd 1990). Because the production of phytoplankton is largely controlled by the application of inorganic fertilisers, a reduction in the application rate of inorganic fertilisers should reduce pH levels. Phytoplankton forms a major source of pond productivity, and is an important food source for zooplankton, so it would seem that any reduction in the amount of inorganic fertilisers added to the pond would reduce overall pond productivity. However, Culver *et al.* (1993) demonstrated increased pond productivity of percid fingerlings using reduced applications of fertilisers, equivalent to weekly rates of 600 μg/l N and 30 μg/l P, compared with traditional weekly fertiliser application rates equivalent to 1,566-4,530 μg/l N and 326-1,225 μg/l P (Anderson 1993c).

Ammonia concentrations were not measured in the present study, and it has been suggested that high ammonia concentrations may have contributed to mortality of barramundi in the larval rearing trials (S. Rowland, pers. comm.). While ammonia may be a contibuting factor in the mortality of barramundi in larval rearing ponds, recent research at NFC has shown that pH has a much greater influence on survival of newly hatched barramundi larvae than does

ammonia at the concentrations typically found in larval rearing ponds (M. Rimmer and M. Pearce, unpublished data).

This result indicates that pond productivity is not simply linked to nutrient inputs, but is the end result of a complex pond management strategy (Culver *et al.* 1993). Assuming the relationship between elevated pH and barramundi survival in extensive rearing ponds to be real, further research into reducing nutrient inputs without decreasing overall pond productivity would be valuable.

Pond Zooplankton

As discussed in Chapter 6, extensive rearing requires the provision of prey organisms of optimal size in sufficient quantity to provide for survival and growth of the fish throughout the rearing period, and particularly during the period immediately after stocking (Geiger 1983a,b, Sturmer 1990). To ensure that this requirement is met, a predictable response by zooplankton to a particular pond management regime is necessary. The results of the pond trials described in this chapter suggest that the predictability of zooplankton populations and the structure of zooplankton communities in fertilised salt or brackishwater ponds is poor. A contributing factor to this poor predictability of zooplankton response may have been the rapid reuse of the pond for successive larval rearing trials.

(Note: data from trials P3 and P4 have been omitted from the following discussion because of the incompatibility between the 25μm plankton net samples used to estimate zooplankton abundance in those trials and the vertical tube

samples used in subsequent trials). The timing and extent of peak zooplankton abundance varied substantially between trials (Table 7.5). In particular, peak density of the size class utilised by first feeding barramundi larvae, i.e. $>37\mu m$ (Chapter 8), occurred between day 4 and day 21 (median 12.5) and varied between 611 and 9,813 organisms per litre (Table 7.5).

The predictability of zooplankton populations may be inherently poor. Relatively simple models of populations of organisms which have high reproductive rates and short life-spans (such as many zooplanktonic organisms) indicate that the future population levels are effectively unpredictable (May and Oster 1976). When additional complicating factors are added, such as the numerous biotic and abiotic factors which affect zooplankton populations in ponds (see Chapter 6), the reliable prediction of zooplankton responses to a particular management regime becomes effectively impossible. Anderson (1993c) suggests that, given the uncertainties associated with predicting zooplankton responses from sampling data, 'defining good densities of zooplankton to support good fish production may be futile'.

Further research is necessary to examine the role of zooplankton densities in determining survival of barramundi larvae reared extensively. Some trials that had good survival also had relatively low densities of $>37\mu m$ zooplankton at stocking, e.g. at the time of stocking for pond trial P8 there were only 148 organisms per litre in the $>37\mu m$ size class, yet this trial had 70% survival. Conversely, other trials which had high densities of zooplankton in the $>37\mu m$

size class at stocking had poor survival (Table 7.5). In contrast with the prey densities provided for first feeding barramundi larvae in intensive culture (10,000 - 20,000 rotifers/litre), these results suggest that extremely high densities of zooplankton may not be essential for successful rearing of barramundi larvae.

An experimental evaluation of the effects of prey density on first feeding success would provide valuable information towards developing a better management regime for extensive rearing ponds. As noted above, it may be necessary to reduce nutrient inputs into extensive rearing ponds to reduce pH elevation resulting from photosynthetic use of CO_2, but this strategy may reduce pond productivity and hence reduce zooplankton populations. Using this scenario, the development of a pond management strategy which increases the production of fingerlings becomes an optimisation exercise aimed at reducing nutrient inputs while maintaining adequate densities of zooplankton. Unfortunately, with only limited information available on pond environmental conditions and larval prey requirements, the development of better pond management strategies awaits considerable further research on these topics.

Comparison with other species

To put the results of these extensive rearing trials in a wider perspective, it is useful to compare the results of the barramundi trials with published results for extensive larval rearing of other marine species. Survival of barramundi in these pond trials ranged from 0 to 86% (Table 7.2) and averaged 22.3% overall for

pond trials P1-P12. Although there are relatively few published estimates of survival of other extensively reared marine fishes (Table 9.1), the survival figures obtained for barramundi are better than those published for spotted seatrout and comparable with those of red drum. Note that the average survival of extensively reared red drum improved from about 20% in 1976 to about 50% by 1985. This increase in survival is attributable to increased experience in managing extensive rearing ponds used for red drum (McCarty *et al.* 1985). It is expected that further research on extensive rearing techniques for barramundi will also result in increased average survival and greater predictability of success.

Production of barramundi fingerlings (ignoring those trials that had survival $<1\%$) ranged from 9,215 to 637,037 fish/ha, and from 0.05 to 7.10 kg/ha/day. While these production figures, like the survival rates discussed above, are highly variable, they also compare favourably with published information on other fish species reared in saltwater ponds (Table 9.1).

Table 9.1 Summary of published data on survival and production of fish fingerlings reared in saltwater ponds, and comparative data on barramundi fingerlings from this study. Production data for barramundi are from those pond trials that had survival >1%. Superscripted numbers indicate sources of data for other species (see key to sources below).

Species	Survival		Production	
	Range	Mean	fish/ha	kg/ha/day
Barramundi *Lates calcarifer*	0 - 86%	22%	9,215 - 637,037	0.05 - 7.1
Red Drum *Sciaenops ocellatus*	2-65%[1] 38-66%[2]	20%[1] 40-53%[2]	61,483[1]	0.13 - 2.17[1] 3.2-6.1[2]
Striped Bass *Morone saxatilis*	17-72%[1]	56%[1]	52,450 - 126,000[1]	0.75 - 1.68[1]
Spotted Seatrout *Cynoscion nebulosus*	0-19%[1] 6-11%[3] 26-100%[4]	3%[1] 8%[3] 51%[4]	5,065[1]	0 - 0.89[1] 1.58 - 2.53[3] 0.63 - 1.68[4]
Orangemouth Corvina *Cynoscion xanthulus*	0-100%[4]	44%[4]		0 - 8.5[4]
Cynoscion hybrids	27-100%[4]	47%[4]		0.25 - 1.41[4]

Sources: 1: Colura *et al.* 1976; 2: McCarty *et al.* 1985; 3: Porter and Maciorowski 1984; 4: Bumguardner *et al.* 1992.

CHAPTER 10

Synthesis: Larval Rearing of Barramundi

This thesis examines aspects of both intensive and extensive larval rearing techniques for barramundi. At this point it is instructive to compare intensive and extensive larval rearing techniques in order to examine the advantages and disadvantages inherent in these dissimilar approaches to larval rearing, to suggest avenues of future research, and to examine how the development of larval rearing procedures for barramundi may impact on the development of culture technologies for other finfish species.

Intensive vs. Extensive Larval Rearing

The comparative advantages and disadvantages of intensive and extensive larval rearing systems are summarised in Table 10.1

Table 10.1 Comparison of intensive and extensive larval rearing techniques for barramundi.

	Intensive	Extensive
Survival:		
Research	50%	22%
Commercial	20-50%	c. 20%
Growth	0.4-0.5 mm/day	1.1-1.7 mm/day
Capital costs[1]	High	Low
Labour costs	High	Low
Fingerling costs	25c each[2]	16c each[2]

(1) Excluding land purchase.
(2) Data from Lobegeiger (1993)

Survival and Growth

As noted in Chapter 9, survival of extensively reared barramundi (currently c. 20%) compares favourably with survival of other marine fishes reared extensively, particularly in the early stages of the development of larval rearing techniques. Adoption of extensive rearing techniques by commercial producers has also seen an average survival rate of about 20% (C. Phillips and M. Fantin, pers. comm.). In comparison, survival of intensively reared barramundi larvae at the small-scale hatchery established at NFC, following adoption of the nutritional enhancement techniques described in Part One of this thesis, consistently averaged about 50%. Survival of intensively reared barramundi in large-scale production facilities is reported to average 20-30%,

although survival as high as 50% for some production runs have been recorded (S. Fielder, pers. comm.). Further refinement of extensive rearing techniques should see average survival approaching 50%, as has been experienced with extensively reared red drum (W. Rutledge, pers. comm.) and golden perch (S. Rowland, pers. comm.).

A common criticism of extensive larval rearing techniques for barramundi is that survival is highly variable and that the technique is therefore unreliable. This view is supported by the results of this study which indicate survival ranging from 0 to 86% in individual trials (Table 7.2; p. 122). However, further research into extensive rearing techniques, as outlined in Chapter 9, is expected to increase the reliability of extensive larval rearing and also to increase survival.

An important difference between barramundi larvae reared intensively and those reared extensively is growth rate. Although the use of nutritionally supplemented brine shrimp significantly increased the growth rate, as well as survival, of intensively reared barramundi (Chapter 3), growth rates in intensive culture were dramatically lower than those recorded in extensive rearing trials. During the present study, overall growth in pond trials ranged from 1.1 to 1.7 mm/day and barramundi harvested after about 3 weeks in the pond averaged 21.5 to 40.8 mm TL (Table 7.2; p. 122). In comparison, barramundi larvae reared intensively in the NFC hatchery reached only about 10 mm TL after 3 weeks. Growth rates for intensively reared *L. calcarifer* in Asia are comparable

with those recorded for Australia: about 30 mm after 45 days (0.7 mm/day) (Maneewong 1987).

Intensive rearing of barramundi larvae to a size greater than about 10 mm TL requires considerable food resources. Because barramundi can be easily weaned to artificial diets only when they are about 16 mm TL or larger (Barlow *et al.* 1993), weaning smaller barramundi often results in considerable mortality because of the large proportion of fish which do not accept an artificial diet and subsequently die of starvation. Barramundi can be reared to 16 mm TL using brine shrimp as the food source, although this is costly (in terms of both the cost of brine shrimp cysts and the labour required for feeding) and, because of the larger size of the fish, may require the brine shrimp to be grown out using microalgae or yeast. Use of the latter may result in nutritional deficiencies in the diet of the barramundi larvae, as noted in Part One of this thesis.

To rear barramundi to a size suitable for conversion to artificial diets or for stocking, barramundi reared intensively at NFC were converted to freshwater when they had reached about 10 mm TL (usually about day 20 from hatching) and were transported to the QDPI freshwater hatchery at Walkamin Research Station (Walkamin, Queensland) where they were reared on freshwater zooplankton cultured in earthen ponds (Barlow *et al.* 1993). This approach requires further rearing in the hatchery because barramundi juveniles less than about 18 mm TL were found to be subject to heavy predation by dragonfly (Odonata) nymphs. Consequently, barramundi could not be released into

freshwater rearing ponds until they had reached a size where behavioural changes reduced this mortality (Barlow *et al.* 1991).

Extensive rearing overcomes these problems, since the fish harvested from the pond are large enough (i.e. >16 mm TL) to wean immediately to an artificial diet. Barramundi harvested from the pond used for this study were retained at NFC on numerous occasions and began accepting an artificial pellet diet within a few hours of relocation to NFC. Most of the barramundi were feeding on the artificial diet within a few days of harvesting.

Production Costs

Production costs associated with extensive larval rearing of barramundi are substantially lower than those associated with intensive larval rearing because of the fewer, and cheaper, facilities required and the fewer staff required to carry out the operation. Production costs for intensively reared fingerlings could be reduced if live food organisms were replaced, at least in part, with artificial diets. However, the results discussed in Chapter 6 of this thesis suggest that this methodology is unlikely to be developed for practical use in the short term.

Economic modelling of barramundi aquaculture has shown that fingerling cost is one of the most sensitive factors affecting profitability (Treadwell *et al.* 1991). Consequently, lower fingerling cost substantially increases the profitability of barramundi aquaculture. An estimate of the costs associated with intensive and extensive larval rearing of barramundi derived production costs of 25c and 16c

each for intensively and extensively reared fingerlings respectively (Lobegeiger 1993). In support of these estimates, production costs of barramundi fingerlings produced intensively at 'Sea Harvest' (Mourilyan, Queensland) were estimated at about 25c each in 1993 (D. Hallam, pers. comm.). Commercial aquaculturists using extensive rearing techniques have sold fingerlings for 20-25c each, suggesting that Lobegeiger's (1993) figure of 16c is reasonably accurate, or even slightly high.

Prior to the commercial use of extensive rearing techniques commencing in the 1990-91 barramundi breeding season, barramundi fingerlings were sold by hatcheries at 50-80c each. Subsequently, barramundi farmers have been able to produce their own fingerlings for around 16c each or less. The economic model developed by Treadwell *et al.* (1991) indicated that a substantial increase in the profitability of barramundi aquaculture would occur if fingerling costs were reduced from 50c each to 25c each, as a result of the lower production costs associated with extensive larval rearing. The substantially lower estimate of 16c, compared with the estimate of 25c used by Treadwell *et al.* (1991), suggests that the actual reduction in fingerling production costs to 16c each or less has provided a significant economic benefit to the barramundi aquaculture industry.

Further support for the cost-effectiveness of extensive larval rearing, in comparison with intensive techniques, is the enthusiasm with which extensive rearing techniques have been adopted by the barramundi aquaculture industry and the impact that this change has had on fingerling production. Prior to the

development of extensive rearing techniques during the 1989-90 barramundi breeding season, all commercial barramundi fingerling production in Australia was undertaken intensively at two hatcheries, one at Mourilyan and the other at Weipa. Adoption of extensive larval rearing by a large proportion of barramundi producers has resulted in increasing numbers of fingerlings being produced using extensive rearing techniques.

At the time of writing (March 1995), 12 of the 14 major Australian barramundi fingerling production facilities were using extensive rearing techniques (Table 10.2). In comparison, only 3 facilities were using intensive rearing techniques (including the 'green water' modification developed by Palmer *et al.* [1992]). One Queensland fingerling producer uses both methods.

Table 10.2 Number of Australian barramundi larval rearing facilities using intensive and extensive rearing techniques for production of barramundi fingerlings for grow-out and stocking. Facilities included are those used by commercial barramundi farmers, as well as those used by government and community groups for producing barramundi fingerlings for stocking. Facilities listed as using intensive rearing methods includes those using the 'green water' modification developed by Palmer *et al.* (1992). One commercial producer in Queensland uses both intensive and extensive techniques.

State	No. facilities	Larval rearing methods	
		Intensive	Extensive
Qld	9	2	8
NT	4	0	4
SA / NSW	1	1	0
Total	14	3	12

Future Research

Barramundi aquaculture is a growing industry in Queensland and there is general agreement amongst researchers and industry that it will continue to expand as production costs, particularly fingerling costs, decrease (Quinn *et al.* 1991, Trendall and Fielder 1991). Consequently, it is relevant to examine research directions which will assist in making barramundi aquaculture a more competitive industry.

The details of the requirements of barramundi larvae for intensive rearing are now well established, due to the nutritional work described in Part One of this thesis and other research carried out at NFC. Established intensive rearing methods allow the reliable production of large numbers of barramundi fingerlings, albeit at relatively high cost. Future research on intensive rearing techniques would have to examine ways of reducing production costs, while not adversely affecting growth and survival. However, the reduced utilisation of intensive rearing methods for barramundi in Australia makes research into such methods a relatively low priority.

Because of the economic advantages provided by extensive rearing, this method of larval rearing will continue to be the technique most commonly used in Australia for production of barramundi fingerlings. In comparison with intensive larval rearing methods, extensive rearing results are far more variable and average survival of fingerlings is lower. Future research on larval rearing of barramundi should concentrate on refinement of extensive larval rearing techniques, since this is now the most commonly used procedure for larval rearing.

The research described in Part Two of this thesis examines factors affecting survival and growth, and the diet of extensively reared barramundi larvae. A limitation to the correlative approach used here is the difficulty in establishing precise cause and effect relationships between variables. In particular, the results of the pond trials carried out during this study (Chapter 7) suggest that water quality is a major factor in determining larval survival.

However, the tolerances of barramundi larvae to various water quality parameters are, at best, poorly known. Research into the physicochemical tolerances of barramundi larvae are essential in order to develop a set of operational parameters which can be used to ensure that the pond environment is suitable for larval survival.

Another major area which requires research is the processes which affect zooplankton production in larval rearing ponds. This covers a wide range of topics, including nutrient availability and cycling, dynamics of phytoplankton, bacteria and protozoans, and diet, growth and reproduction of zooplanktonic organisms. As noted in Chapter 7, the zooplankton of Australian marine and estuarine waters are poorly known, to the point where even identification is difficult. Consequently, any research into zooplankton dynamics in larval rearing ponds has little basic information from which to begin. However, the continuing development of barramundi aquaculture depends, at least in part, to the continued refinement of existing techniques, including extensive larval rearing.

Application to Other Species

The results of the research described in this thesis have application not only in barramundi aquaculture, but also in the culture of other finfish species. There is considerable interest throughout Australia in developing aquaculture techniques for a range of marine finfish species. Nutritional deficiencies during larval development affect survival and growth in a range of marine fish species

(see Chapter 2); thus the nutritional enhancement techniques developed for barramundi may also be of use for other species. In addition, the biochemical information obtained from this work can be used for comparison with other species, and with other treatments for enhancing the nutritional composition of live food organisms.

The development of larval rearing techniques for barramundi provides a useful model for aquaculture researchers and managers to develop larval rearing technologies for other marine finfish species. The experience of the barramundi farming industry has shown that the economic viability of the industry relies partly on the development of cost-effective larval rearing techniques. Intensive larval rearing techniques require relatively large amounts of labour, and because of the high labour costs in Australia, this method of fingerling production will always be relatively expensive.

The use of intensive rearing techniques can be justified on economic grounds for high value species, such as barramundi cod (*Cromileptes altivelis*) and maori wrasse (*Cheilinus undulatus*), which bring A$100-150/kg live in Asian markets. However, these highly valued species are the exception rather than the rule: most finfish species in Australia bring considerably lower prices. As in the case of barramundi, economically viable larval rearing of most Australian cultured finfish species will have to be based on lower cost techniques, such as extensive rearing, if Australian finfish aquaculture is to develop as a viable industry.

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APPENDIX 1

Fatty Acid Nomenclature

Fatty acids are described by several systems of nomenclature, although the most common is the shorthand system previously known as the *omega* nomenclature. For example, the fatty acid with the chemical name Eicosatetraenoic acid is also known by its trivial name, Arachidonic acid, and more commonly by its shorthand nomenclature, 20:4*n*-6 (previously 20:4ω6). Less common variations of shorthand nomenclature are 20:4(*n*-6) and 20:4(6).

The shorthand nomenclature indicates the structure of the molecule by describing:

1. the number of carbon atoms in the chain;
2. the number of double bonds;
3. the inclusive number of carbon atoms from the terminal methyl group to the carbon atom of the first double bond.

Thus, Arachidonic acid (20:4*n*-6) has 20 carbon atoms and four double bonds with the first double bond located on the 6th carbon atom from the terminal methyl group:

$CH_3 - CH_2 - CH_2 - CH_2 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - COOH$

A fatty acid can be elongated by adding further carbon atoms two at a time to the carboxyl end of the molecule, and desaturated by forming double bonds between the carboxyl terminal and the nearest double bond to it along the carbon chain. Consequently, the value (*n*-*x*), where *x* is the number of carbon atoms between the terminal methyl group and the nearest double bond, is fixed. Thus, fatty acids are frequently described as belonging to a particular series, such as the '*n*-3 series' or the '*n*-6 series'.

A fatty acid such 18:3*n*-3 can be elongated and desaturated to form 20:5*n*-3 which in turn can be metabolised to form 22:6*n*-3, but cannot form a fatty acid from another series such as 20:4*n*-6:

18:3*n*-3 (Linolenic acid)

$CH_3 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - COOH$

20:5*n*-3 (Eicosapentaenoic Acid)

$CH_3 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - COOH$

22:6*n*-3 (Docosahexaenoic Acid)

$CH_3 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - COOH$

Fatty acids without a double bond are termed saturated fatty acids, those with one double bond are termed monounsaturated, and those with two or more double bonds are termed polyunsaturated (PUFA) or highly unsaturated fatty acids (HUFA).

In addition, the chemical names of fatty acids regarded as essential fatty acids for marine fishes are frequently abbreviated: EPA for eicosapentaenoic acid (20:5*n*-3) and DHA for docosahexaenoic acid (22:6*n*-3). More general references to fatty acids with the same number of carbon atoms, regardless of their degree of saturation, are also abbreviated (e.g. C18 for fatty acids with 18 carbon atoms).

References

Christie, W.W. (1982). 'Lipid Analysis - Isolation, Separation, Identification and Structural Analysis of Lipids'. (2nd. ed.). Pergamon Press, Oxford.

Cowey, C.B. and Sargent, J.R. (1972). Fish nutrition. Advances in Marine Biology 10, 383-492.

Sargent, J.R. (1976). The structure, metabolism and function of lipids in marine organisms. In: Maling, D.C. and Sargent, J.R. (eds). Biochemistry and Biophysical Perspectives 3, 149-212.

APPENDIX 2

25μm Plankton Net Sample Data

The figures in this appendix show the densities of the major groups of zooplankton sampled using a 25μm plankton net during pond trials P5 to P12. As previously mentioned (Chapter 7), this particular zooplankton sampling technique was replaced by one which used a PVC tube to obtain water samples which were later sieved to separate various size ranges of zooplankton. The 25μm net sample results are included for comparison with those from the vertical tube sampler. The advantages and disadvantages of the two techniques are discussed in Chapter 7.

Figure A2.1 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P5 (14 November - 11 December 1990). The day of stocking is marked with an arrow.

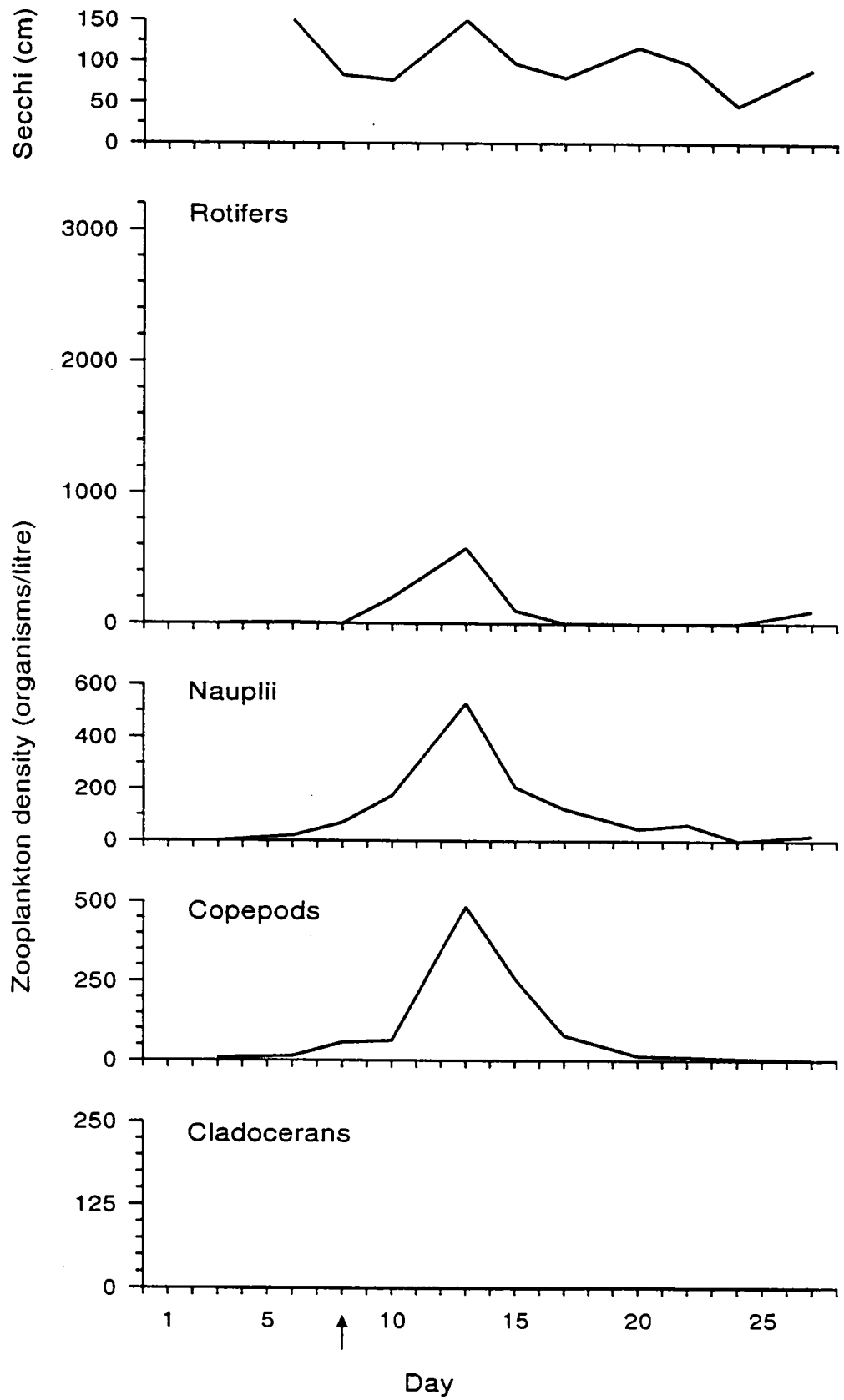

Secchi (cm)
150
100
50
0
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
500
250
0
Cladocerans
250
125
0
Zooplankton density (organisms/litre)
1
5
10
15
20
25
Day

Figure A2.2 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P6 (3 January - 24 January 1991). The day of stocking is marked with an arrow.

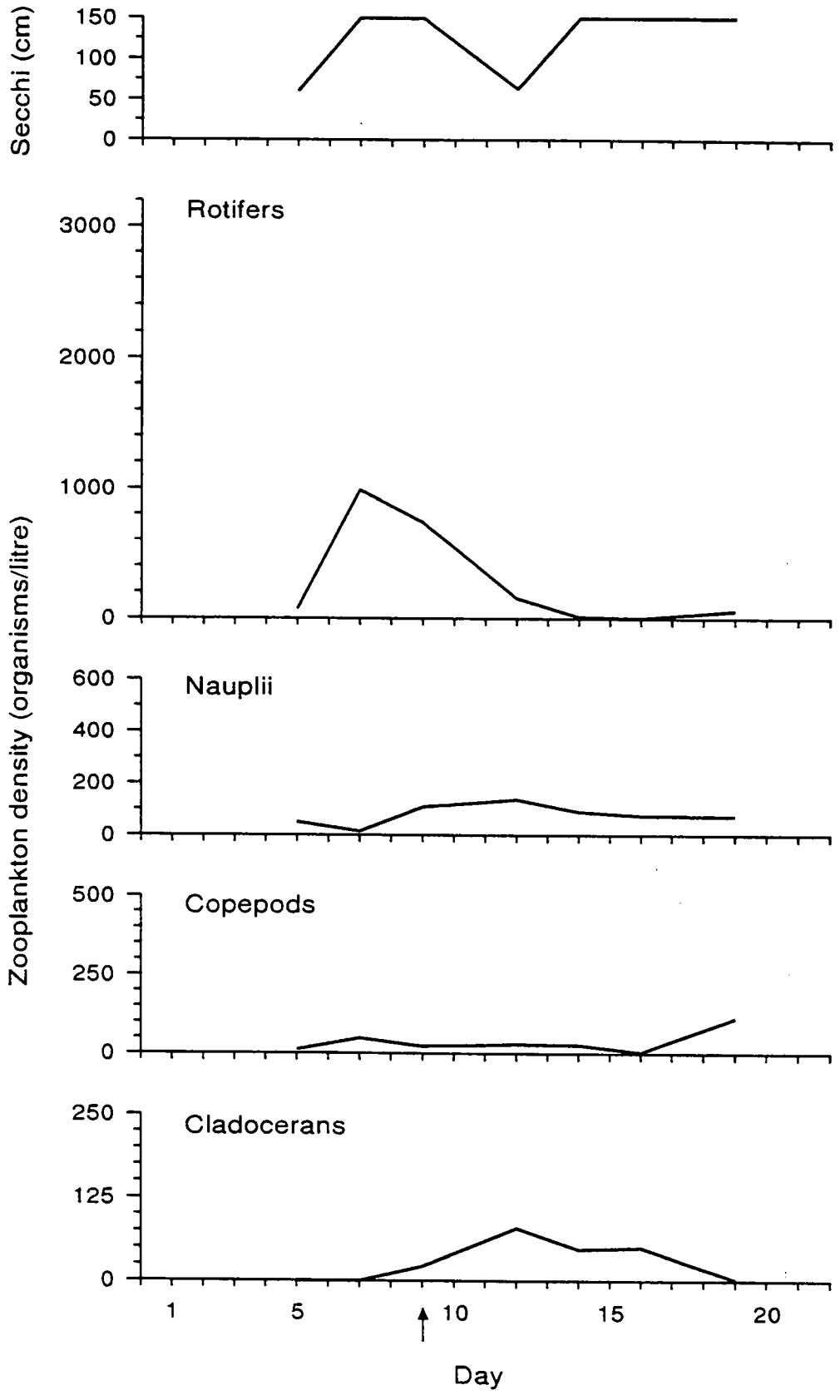

Secchi (cm)
150
100
50
0
Zooplankton density (organisms/litre)
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
500
250
0
Cladocerans
250
125
0
1
5
10
15
20
Day

Figure A2.3 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P7 (29 January - 27 February 1991). The day of stocking is marked with an arrow.

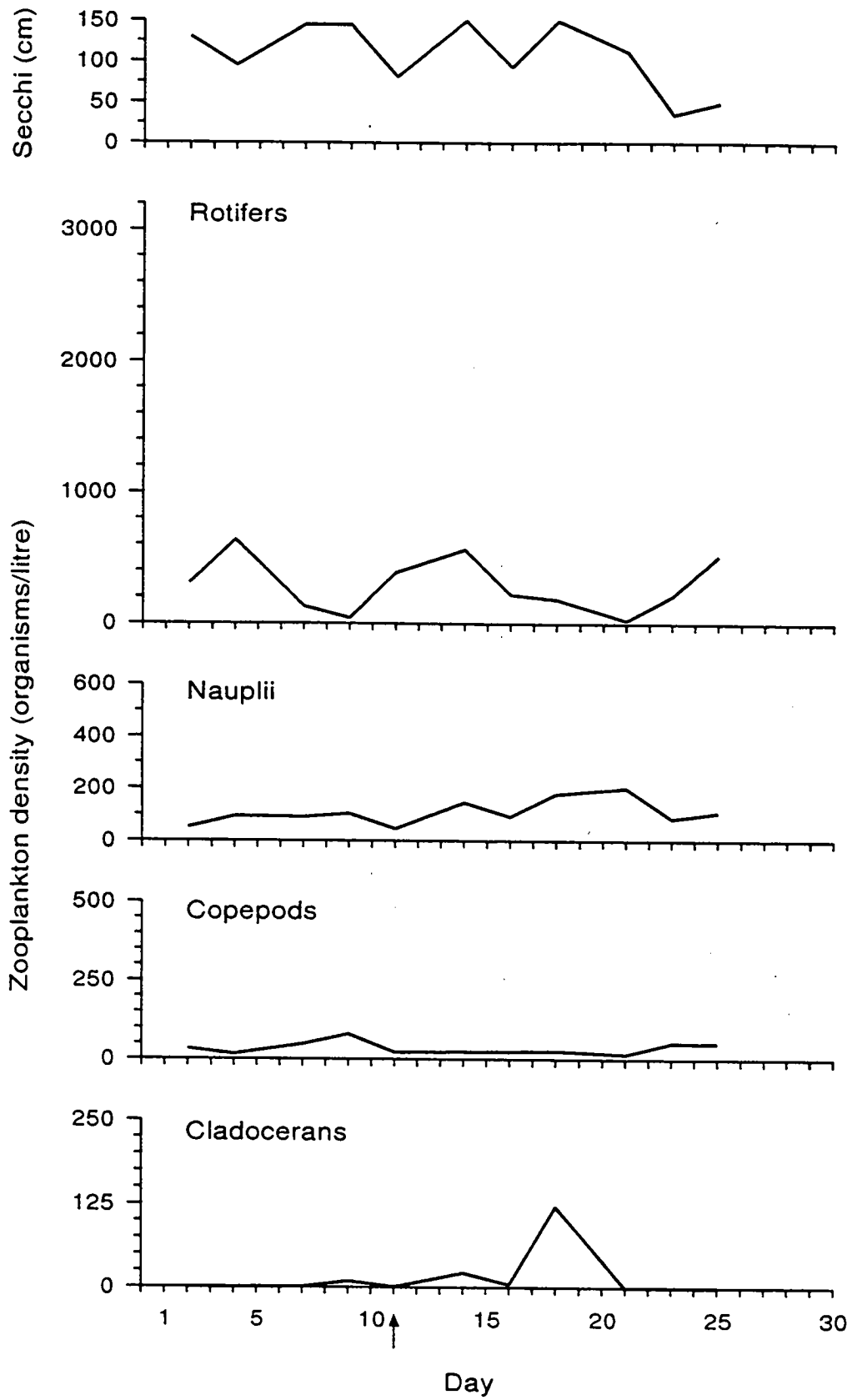

Secchi (cm)
150
100
50
0
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
500
250
0
Cladocerans
250
125
0
Zooplankton density (organisms/litre)
1
5
10
15
20
25
30
Day

Figure A2.4 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and ostracods sampled using a 25μm plankton net during pond trial P8 (8 October - 11 November 1991). The day of stocking is marked with an arrow.

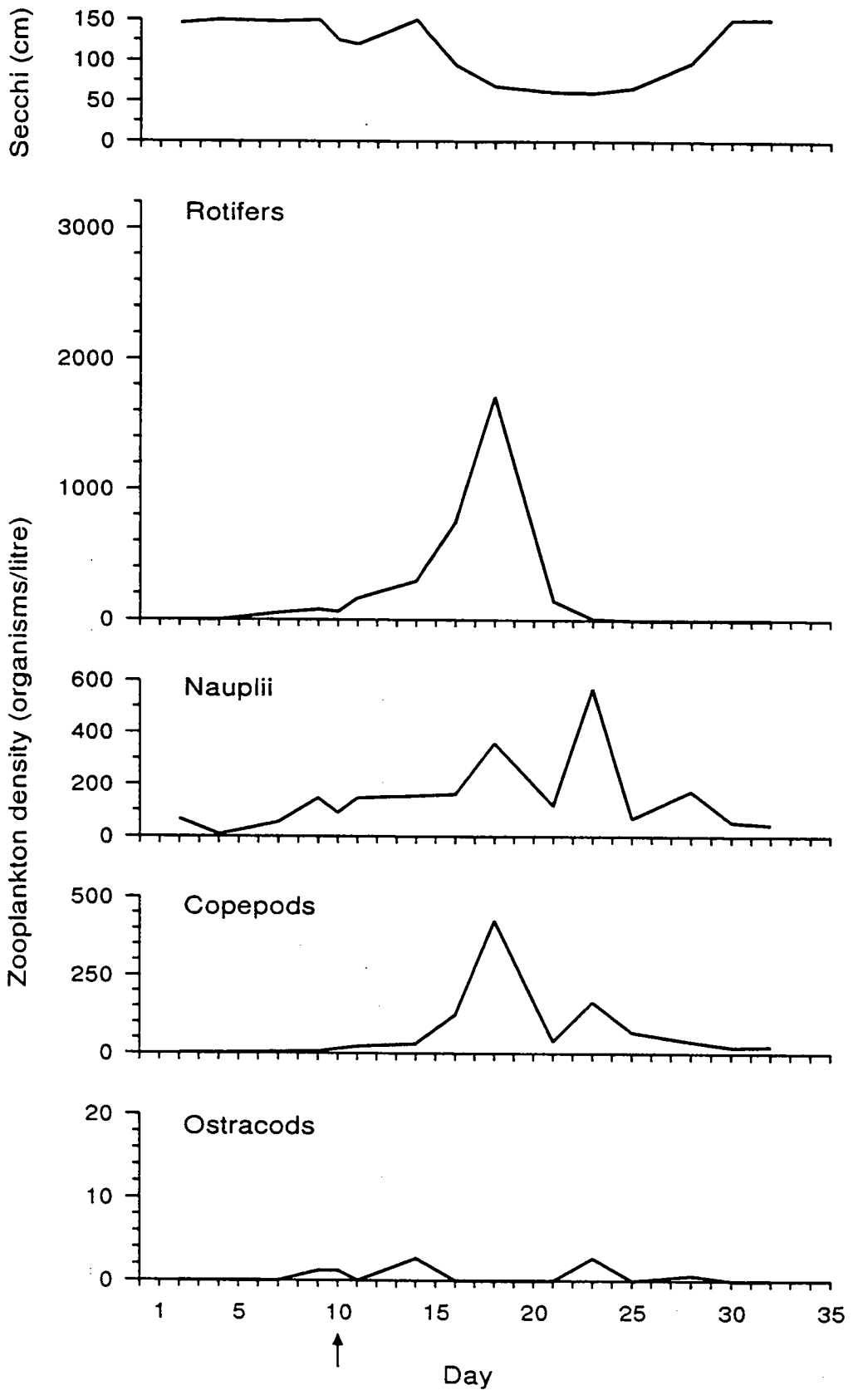

Secchi (cm)
150
100
50
0
Zooplankton density (organisms/litre)
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
500
250
0
Ostracods
20
10
0
1
5
10
15
20
25
30
35
Day

Figure A2.5 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and ostracods sampled using a 25μm plankton net during pond trial P9 (14 November - 11 December 1991). The day of stocking is marked with an arrow.

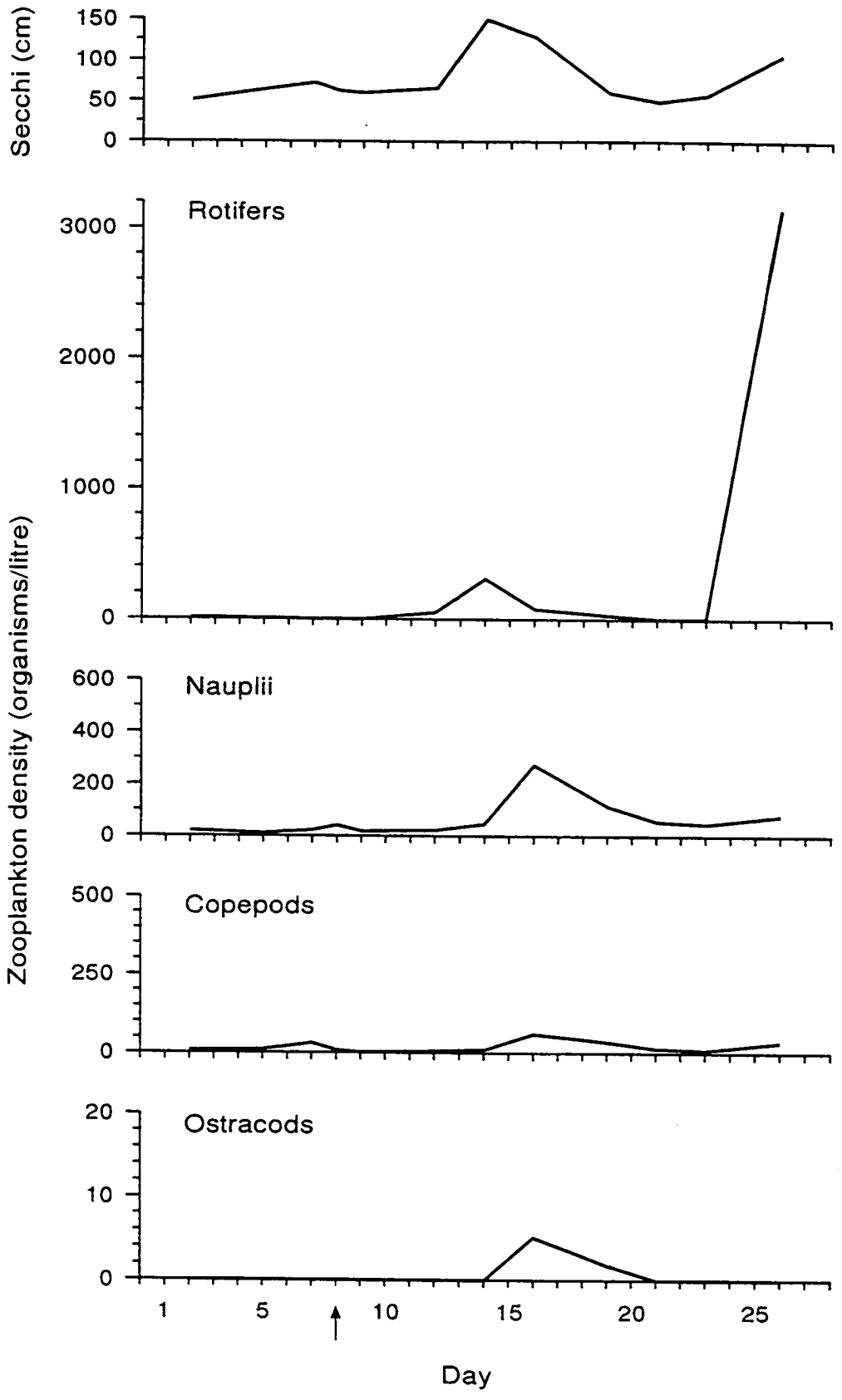

Secchi (cm)
150
100
50
0
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
500
250
0
Ostracods
20
10
0
Zooplankton density (organisms/litre)
1
5
10
15
20
25
Day

Figure A2.6 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P10 (12 December 1991 - 7 January 1992). The day of stocking is marked with an arrow.

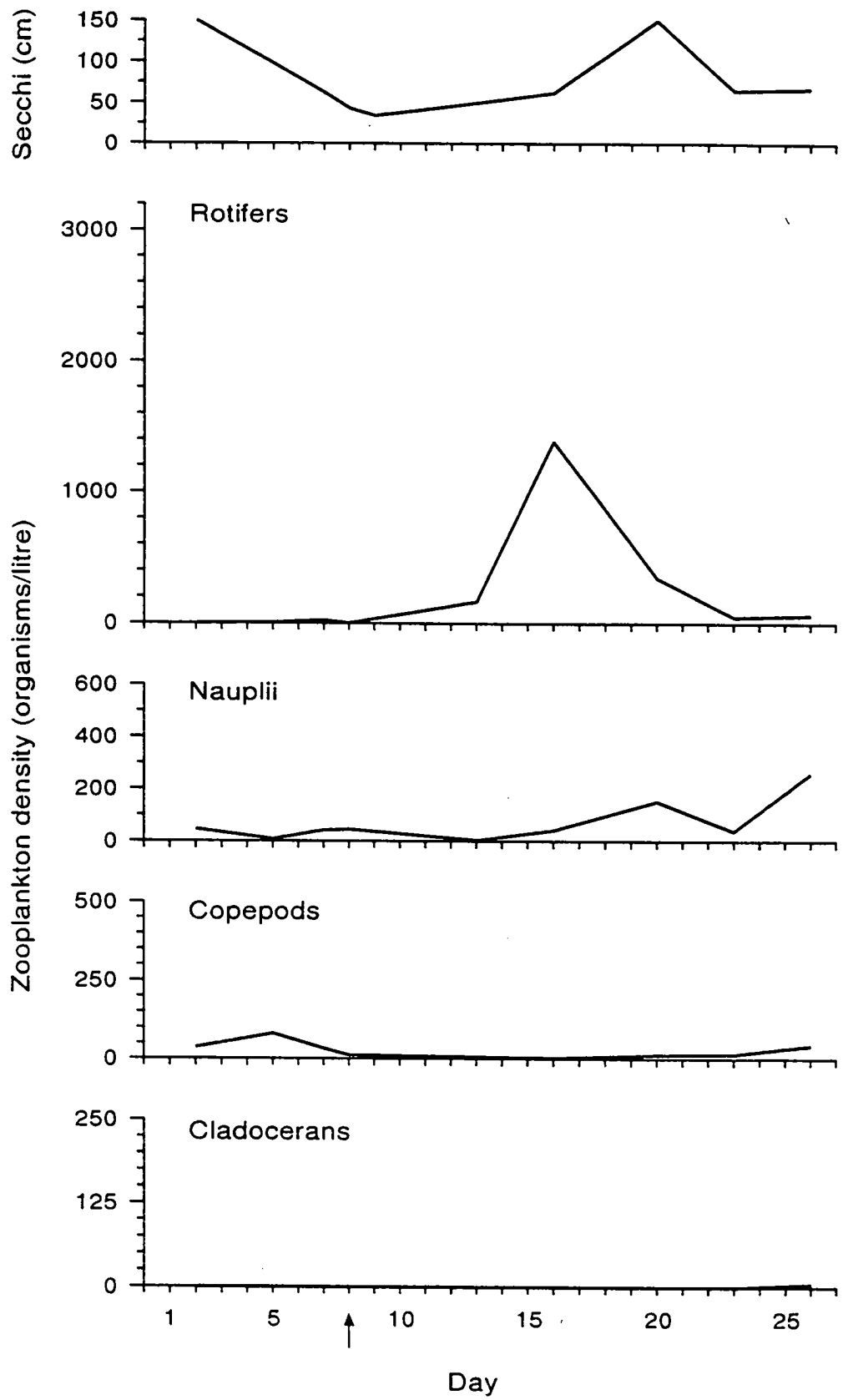

Secchi (cm)
150
100
50
0
Zooplankton density (organisms/litre)
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
500
250
0
Cladocerans
250
125
0
1
5
10
15
20
25
Day

Figure A2.7 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P11 (14 January - 14 February 1992). Note the changed y-axis ranges for rotifer and nauplii density in this figure due to the high densities of zooplankton during this trial. The day of stocking is marked with an arrow.

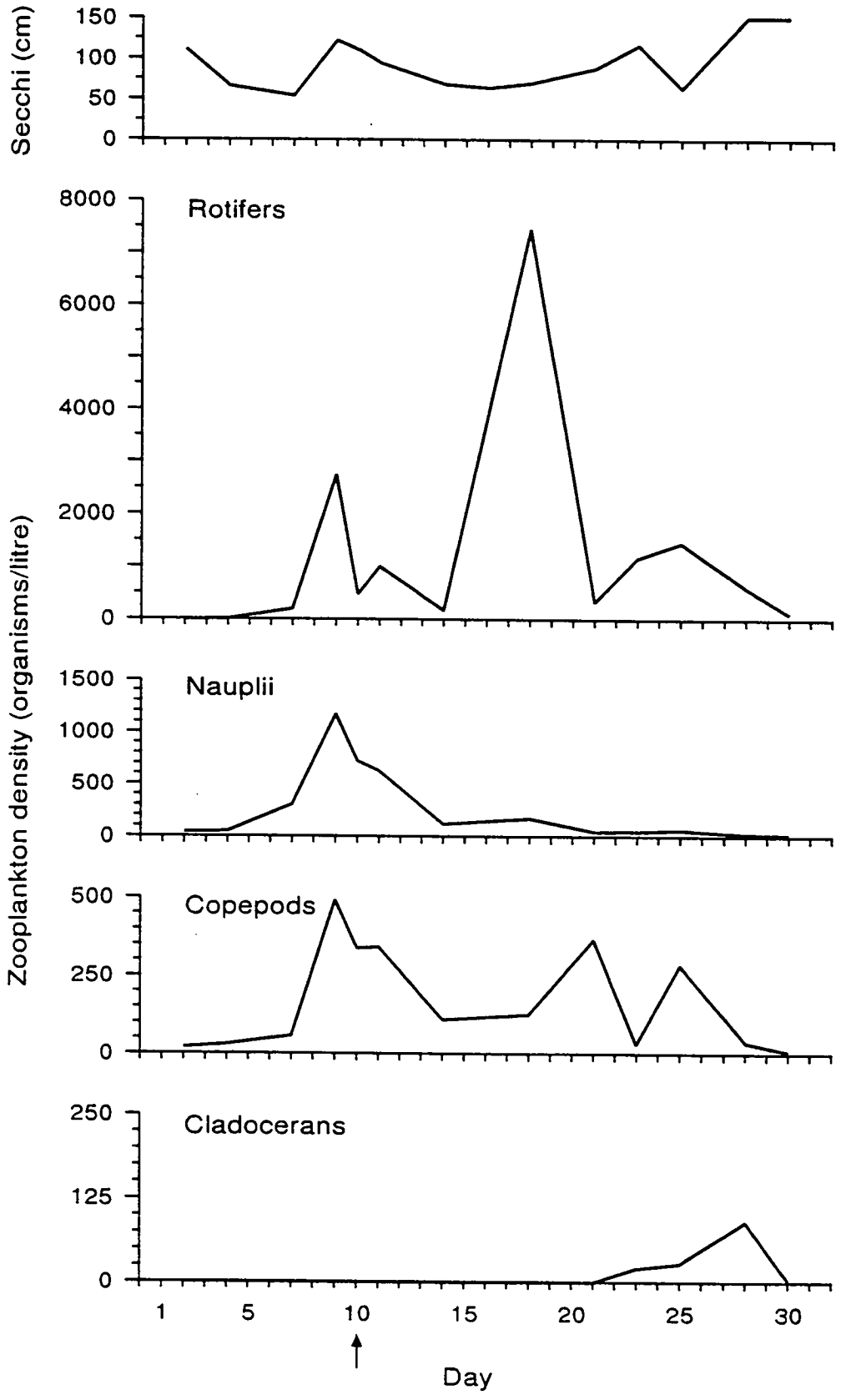
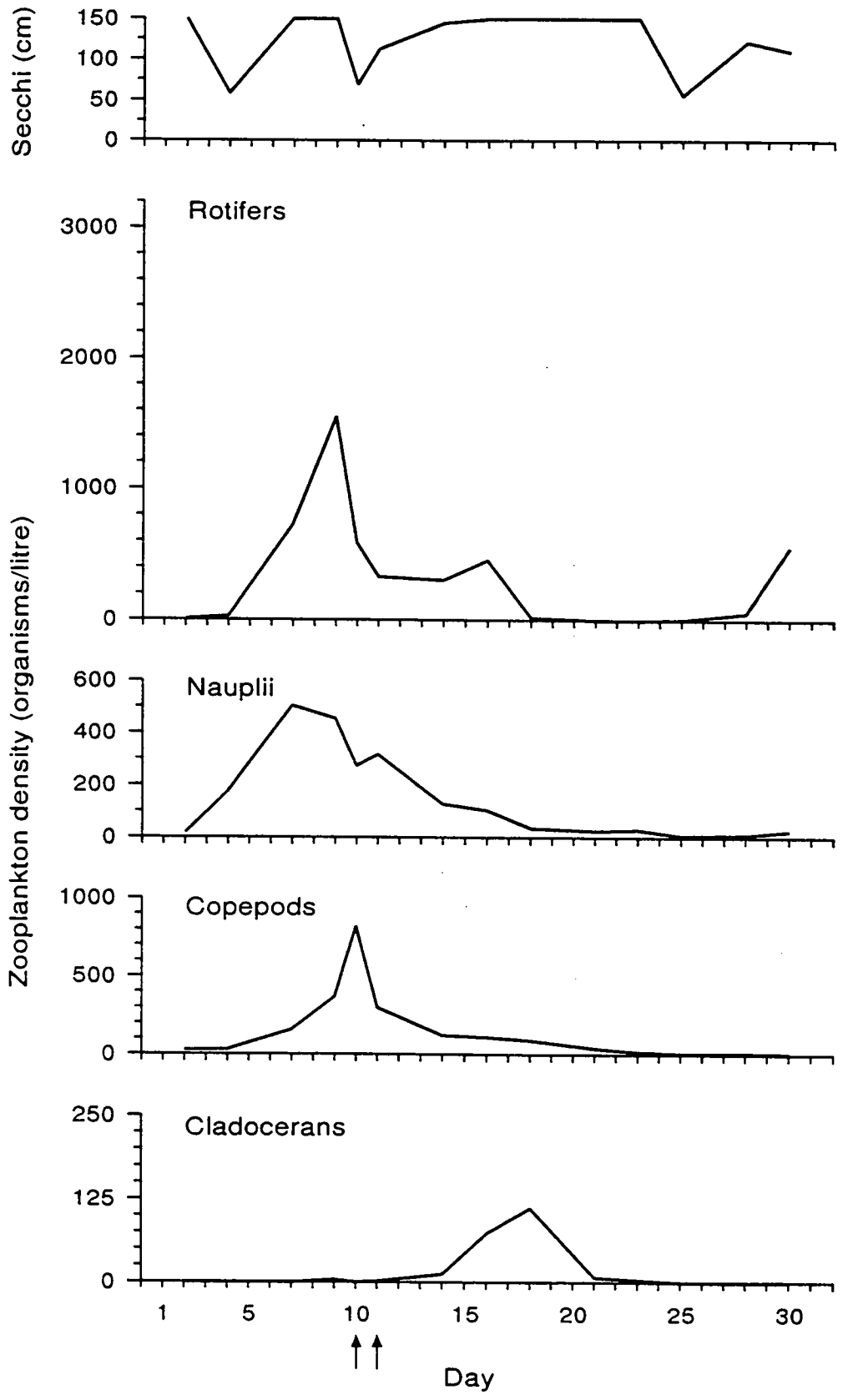

Secchi (cm)
150
100
50
0
Zooplankton density (organisms/litre)
Rotifers
8000
6000
4000
2000
0
Nauplii
1500
1000
500
0
Copepods
500
250
0
Cladocerans
250
125
0
1
5
10
15
20
25
30
Day

Figure A2.8 Secchi visibility and densities of rotifers, copepod nauplii, copepods (copepodites and adults), and cladocerans sampled using a 25μm plankton net during pond trial P12 (18 February -20 March 1992). Note the changed y-axis range for copepod density in this figure due to the high densities of zooplankton during this trial. Larvae were stocked on 2 separate days (indicated by arrows) for this trial.


Secchi (cm)
150
100
50
0
Rotifers
3000
2000
1000
0
Nauplii
600
400
200
0
Copepods
1000
500
0
Cladocerans
250
125
0
Zooplankton density (organisms/litre)
1
5
10
15
20
25
30
Day

APPENDIX 3

Box and Whisker Plots

Although box and whisker plots (or box plots) provide more information about a sample distribution than the traditional plot of means and confidence limits, they are still relatively uncommon in the biological literature. Box and whisker plots have been used in several figures in this thesis in order to provide additional detail of sample distributions where such detail is of relevance, but in view of the comparative rarity of this form of data presentation, this appendix provides a brief explanation of this plotting technique.

Box and whisker plots provide a simple summary of a sample distribution by indicating both the variability and the symmetry of the data. The upper and lower quartiles of the data are portrayed by the top and bottom of a rectangle (i.e. the 'box'), and the median is portrayed as a horizontal line within the rectangle. Vertical lines (the 'whiskers') extend from the rectangle to the adjacent values. The upper adjacent value is the observed value which is less than or equal to the upper quartile plus 1.5 times the interquartile range; the lower adjacent value is the observed value which is greater than or equal to the lower quartile minus 1.5 times the interquartile range. Any observed values which lie outside the range of the 'whiskers' are termed 'outside values' and are plotted as individual points, usually as asterisks.

The median line indicates the central point of the distribution, and the box itself indicates the extent of 50% of the data. The 'whiskers' indicate the tails of

the distribution and their relative length provides an estimate of the skewness of the distribution. Outside values are not necessarily outliers, but give an indication of unusually small or large data which may affect the distribution.

Reference

Chambers, J.M., Cleveland, W.S., Kleiner, B. and Tukey, P.A. (1983). 'Graphical Methods for Data Analysis'. Wadsworth and Brooks/Cole Publishing Company, Pacific Grove, California.

APPENDIX 4

Barramundi Jaw Length, Width and Gape Regressions

The mouth size parameters (width and gape) used to compare prey size and mouth size in Chapter 8 were derived from the equations listed in this appendix.

Morphometric data comprising measurements of TL (measured using vernier calipers) and jaw length and jaw width (measured using a binocular microscope) for 700 barramundi up to 9 mm TL were supplied by M. Pearce (NFC) from his study on intensively reared barramundi. Another 95 barramundi juveniles sampled from the pond trials described in Chapter 7 were similarly measured to extend the range of measurements to 50 mm TL. Consequently, most of the data are in the lower part of the TL range, but this is valuable in determining more precisely the inflection point in each regression line (Fig.'s A4.1, A4.2).

As explained in Chapter 8, mouth gape was estimated from measurements of jaw length because direct measurement of gape was found to be highly inaccurate. Gape was estimated using the jaw length measurement as two sides of an isocoles triangle (assuming both jaws to be effectively the same length), then deriving the length of the base of the triangle using the formula:

$$G = 2L (\sin \Theta),$$

where G is jaw gape (mm), L is jaw length (mm) and Θ is ½ the opening angle of the jaw. The derived value for jaw gape thus represents a straight line between the upper and lower jaws when viewed laterally and represents the maximum width of a prey organism which can be ingested at a given angle of jaw opening.

Jaw gape was derived for two jaw opening angles, 45° and 90°, based on visual observation of barramundi larvae. 90° represents the maximum probable distension of the jaw, while 45° represents a more likely 'typical' gape during feeding.

'Bent stick' regression models (Griffiths and Miller 1973) were fitted to ln transformed data using the nonlinear regression procedures in the statistical package GenStat. The resulting regression equations (Fig.'s A4.1, A4.2) were used to predict fish jaw length, jaw width and jaw gape at 45° and 90°, from measurements of TL.

Reference

Griffiths, D.A. and Miller, A.J. (1973). Hyperbolic regression - a model based on two-phase piecewise linear regression with a smooth transition between regimes. Communications in Statistics 2, 561-569.

Figure A4.1

Relationship between TL and mouth size parameters in barramundi larvae:

(a) Jaw length:

$$JL = e^{-0.92+4.81(\ln TL-0.94)-3.79\sqrt{(\ln TL-0.94)^2+(3.5*10^{-5})^2}}$$

where JL = jaw length (mm) and TL = total length (mm);

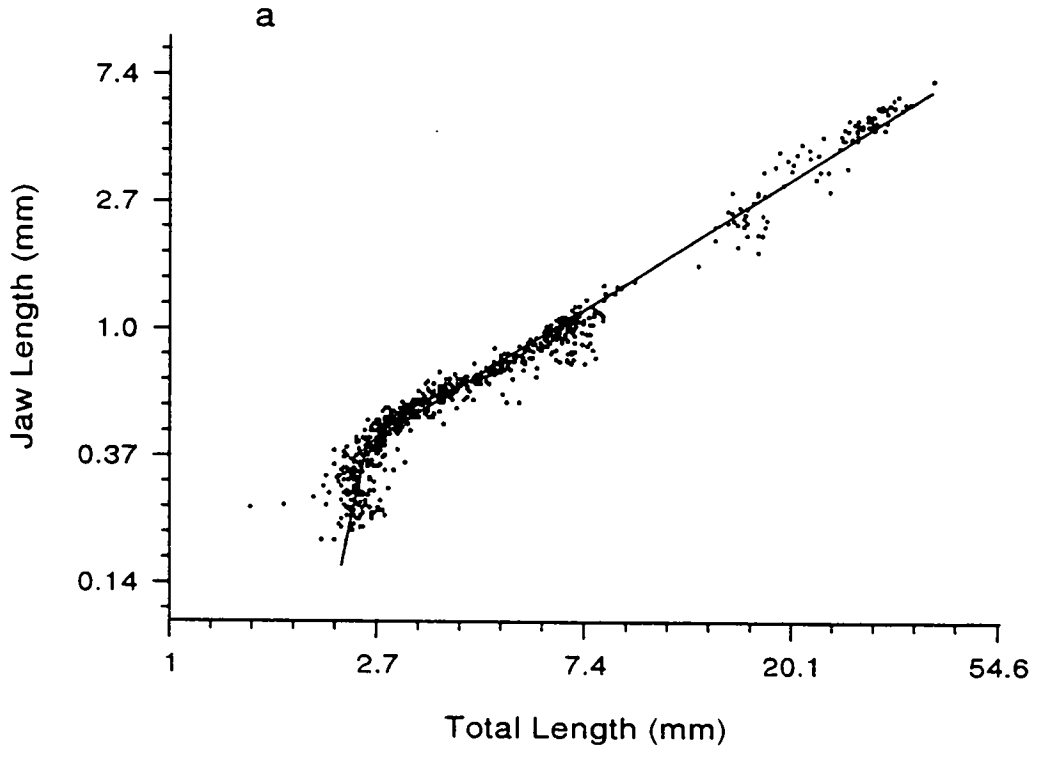
adjusted r^2 = 0.98; F = 9352.4, $P<0.01$.

(b) Jaw width:

$$JW = e^{-1.58+12.70(\ln TL-0.85)-11.52\sqrt{(\ln TL-0.85)^2+(1.2*10^{-3})^2}}$$

where JW = jaw width (mm) and TL = total length (mm);

adjusted r^2 = 0.97; F = 6513.1, $P<0.01$.


a
7.4
2.7
1.0
0.37
0.14
Jaw Length (mm)
1
2.7
7.4
20.1
54.6
Total Length (mm)

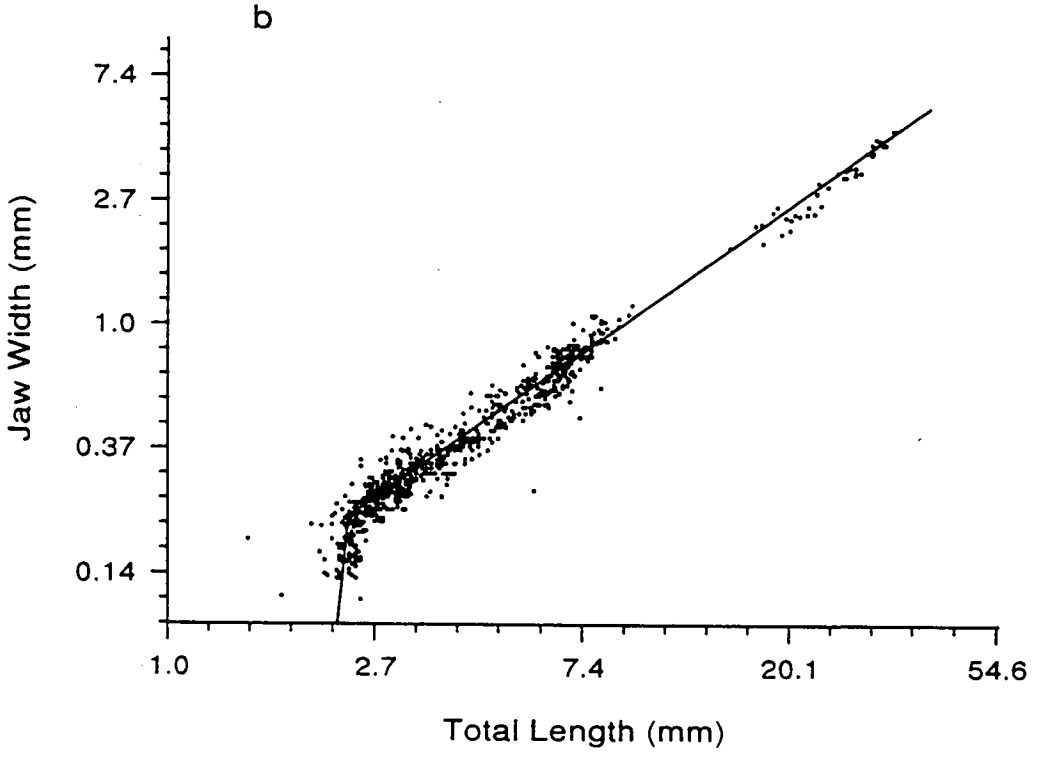

b
7.4
2.7
1.0
0.37
0.14
Jaw Width (mm)
1.0
2.7
7.4
20.1
54.6
Total Length (mm)

Figure A4.2

Relationship between TL and mouth size parameters in barramundi larvae:

(a) Gape at 45°:

$$G45 = e^{-1.19+4.75(\ln TL-0.94)-3.73\sqrt{(\ln TL-0.94)^2+(9.0*10^{-6})^2}}$$

where G45 = gape at 45° (mm) and TL = total length (mm);

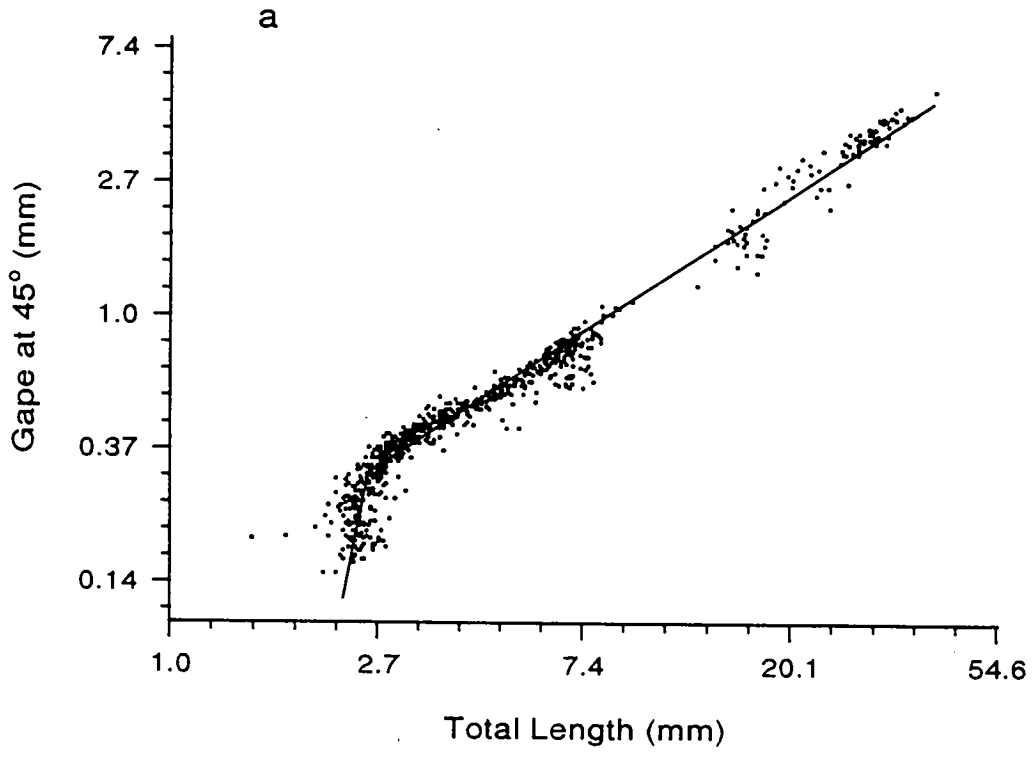
adjusted r^2 = 0.98; F = 9361.1, $P<0.01$.

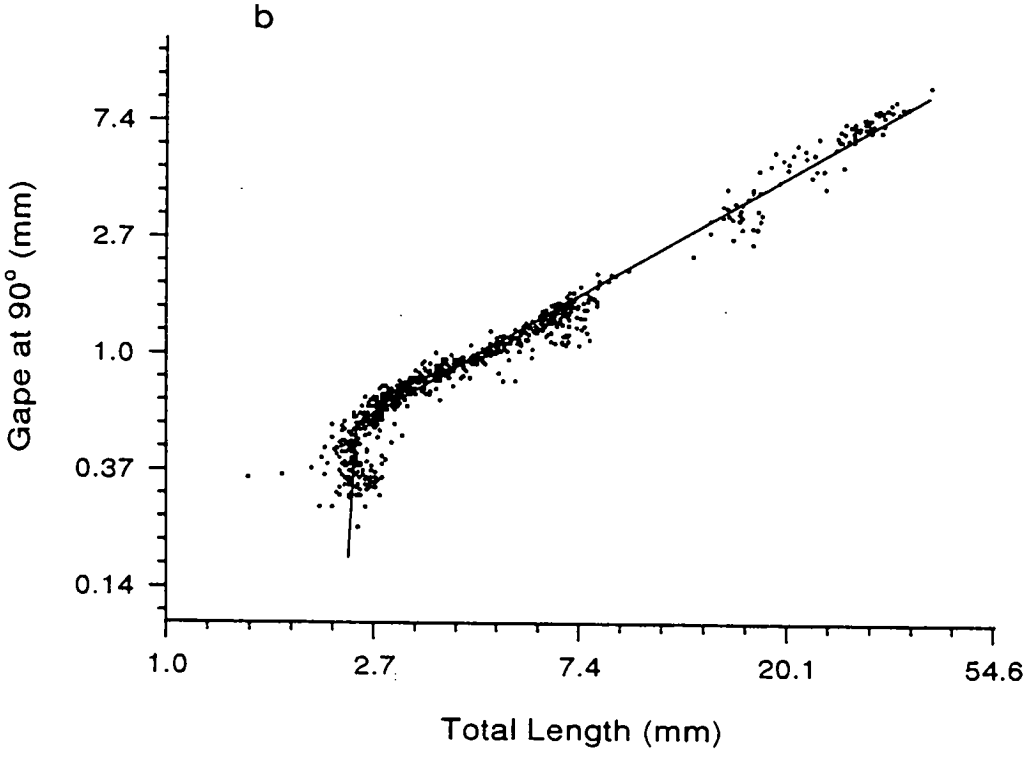
(b) Gape at 90°:

$$G90 = e^{-0.61+14.89(\ln TL-0.92)-13.87\sqrt{(\ln TL-0.92)^2+(1.7*10^{-5})^2}}$$

where G90 = jaw gape at 90° (mm) and TL = total length (mm);

adjusted r^2 = 0.98; F = 9287.8, $P<0.01$.


a
7.4
2.7
1.0
0.37
0.14
Gape at 45° (mm)
1.0
2.7
7.4
20.1
54.6
Total Length (mm)


b
7.4
2.7
1.0
0.37
0.14
Gape at 90° (mm)
1.0
2.7
7.4
20.1
54.6
Total Length (mm)

APPENDIX 5

Recommendations for Barramundi Larval Rearing

The research carried out for this degree was primarily of a practical nature, and the results are of value to aquaculture practitioners. Consequently, this appendix contains a concise list of recommendations for barramundi larval rearing procedures. Further details of these recommendations are discussed in the relevant chapters of the thesis.

Intensive Larval Rearing

1. Provided that a microalgal isolate high in 20:5*n*-3 is used for rotifer culture, additional supplementation of rotifers with HUFA's appears unnecessary. However, this is contingent on the rotifers having a relatively high level of 20:5*n*-3, i.e. c. 11% or greater.

2 Brine shrimp should be supplemented with a diet high in HUFA's, particularly 20:5*n*-3. Levels of 20:5*n*-3 in brine shrimp fed to barramundi larvae should be 8% or greater.

3. The HUFA content of the live food organisms used to rear barramundi larvae should be determined by analysis once a production and supplementation regime has been developed. The supplementation regime should be similar to that described in this thesis. The HUFA content of the live food organisms should be determined at least annually thereafter to

ensure that long-term changes in the nutritional value of the live food organisms are evaluated.

Extensive Larval Rearing

1. Extensive larval rearing provides a relatively low cost method for commercial scale production of barramundi fingerlings, although at the cost of decreased average survival and decreased reliability compared with intensive rearing techniques.

2. Extensive larval rearing of barramundi can be carried out using the procedures outlined in Chapter 7, i.e. fertilisation of a marine or brackish water earthen pond with a combination of inorganic and organic fertilisers.

3. Aeration of extensive larval rearing ponds is recommended to increase the stability of the algal bloom presumably due to better nutrient circulation in the pond. However, due to changes in the diet of barramundi larvae associated with pond aeration, aeration should be undertaken only when adequate quantities of copepod nauplii are available as prey.

4. Aeration will also assist with pond management, in particular by preventing ectogenic meromixis (the formation of a separate layer of heated water below the surface layer) which was associated with severe mortality of larvae in the present study.

5. Larvae should preferably be stocked when both water temperature and pH are optimal, i.e. when temperature is within the range 28-30°C, and pH is less than 8.4. As control of temperature and pH is difficult in earthen ponds, these conditions may only occasionally occur. However, the results of the research detailed in this thesis indicate that survival is likely to be highest when they do.

6. Phytoplankton density can be easily, and reasonably accurately, measured using a Secchi disk. However, it is important to recognise that decreasing turbidity represents a logarithmic increase in phytoplankton density, so that, at low turbidity values, even small changes in turbidity can represent dramatic fluctuations in the abundance of phytoplankton. This in turn may affect pond water quality, particularly since high densities of phytoplankton may cause a drastic decrease in dissolved oxygen levels due to overnight respiration.

7. Zooplankton density can be measured using a fine mesh plankton net (50μm, or smaller, mesh). Although these fine mesh nets clog readily, they provide an adequate estimate of zooplankton density for routine commercial use.

8. The use of lift nets to sample barramundi in larval rearing ponds is recommended in order to accurately follow growth of the larval and juvenile fish. These nets do not provide an accurate estimate of fish

density, but, with experience, will provide a rough guide to the abundance of fish in the pond.

9. The stomach contents of larval and juvenile barramundi should be monitored throughout each larval rearing 'run'. Barramundi larvae commence feeding on rotifers and copepod nauplii, progressing to larger zooplankton (copepod adults, and, when present, cladocerans) and finally benthic prey such as chironomid larvae ('bloodworms'). Because barramundi only commence feeding on chironomid larvae once zooplankton prey has been exhausted, the presence of chironomid larvae in the gut contents indicates that the zooplankton has been eaten out and the fingerlings will soon need to be harvested, since they rapidly exhaust supplies of chironomid larvae in the ponds.

10. Larval rearing ponds should be thoroughly dried and limed between uses. Although the evidence was not conclusive, the failure to dry and lime the pond used in this study was associated with decreased zooplankton density in subsequent larval rearing trials.

APPENDIX 6

Published Papers

This appendix contains copies of the two papers that were published based on results reported in this thesis. The papers are:

Rimmer, M.A. (1993). Ectogenic meromixis in a brackish water pond in tropical northern Australia. Journal of Applied Aquaculture 2, 125-130.

Rimmer, M.A., Reed, A.W., Levitt, M.S. and Lisle, A.T. (1994). Effects of nutritional enhancement of live food organisms on growth and survival of barramundi, *Lates calcarifer* (Bloch), larvae. Aquaculture and Fisheries Management 25, 143-156.

Ectogenic Meromixis in a Brackish Water Pond in Tropical Northern Australia

Michael A. Rimmer

Journal of Applied Aquaculture, Vol. 2(1) 1993

Aquaculture and Fisheries Management 1994, **25**, 143–156

Effects of nutritional enhancement of live food organisms on growth and survival of barramundi, *Lates calcarifer* (Bloch), larvae

M. A. RIMMER *Fisheries Branch, Department of Primary Industries, Northern Fisheries Centre, Cairns, Queensland, Australia*
A. W. REED *International Food Institute of Queensland, Department of Primary Industries, Brisbane, Queensland, Australia*
M. S. LEVITT *Biochemistry Branch, Department of Primary Industries, Animal Research Institute, Brisbane, Queensland, Australia*
A. T. LISLE *Biometry Branch, Department of Primary Industries, Mareeba, Queensland, Australia*