SALINITY SENSITIVITY IN EARLY LIFE STAGES OF AN AUSTRALIAN FRESHWATER FISH, MURRAY COD (*Maccullochella peelii peelii* Mitchell 1838)

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Abstract

The Murray cod (*Maccullochella peelii peelii* Mitchell 1838) is Australia's largest freshwater fish. Once highly abundant in the Murray-Darling river system, populations have drastically declined in recent decades. Many causes for this decline have been proposed, including over-fishing, habitat loss and altered river flow regimes. This study hypothesised that elevated salinities have led to selective mortality in some developmental stages, which have in turn depleted stock recruitment and adult populations.

The objectives of this study were to determine the optimal, threshold, upper sublethal and lethal salinities for development of eggs, yolk-sac larvae, fry and fingerlings of *M. peelii peelii*. Investigation the impact of salinity on fertilisation utilised gametes of trout cod (*M. macquariensis*, Cuvier 1829) instead of *M. peelii peelii*. Studies were carried out in a controlled laboratory environment using test media prepared from commercial sea salt.

The results showed that the eggs of the trout cod hatched only when fertilised and incubated in freshwater, and only larvae hatched in freshwater survived through the yolk absorption period of 12 days. Yolk utilisation efficiencies were not significantly different among the salinities of 0-0.30 g/L. There was no effect of pre- or post-fertilising processes on the salinity tolerances of yolk-sac larvae. No larvae survived at salinities higher than 0.30 g/L during the yolk utilisation period.

Lethal salinity concentration in Trout cod and Murray cod larvae was exposure time dependent. The 1 day LC50 of the larvae was 1.97 and 2.33 g/L respectively, compared with the 12 day LC50 values of 0.50 and 0.35 g/L respectively. The threshold (no effect level) salinities of larvae of Trout cod and Murray cod were 0.46 and 0.34 g/L respectively at 12 days exposure. The salinity sensitivities of fry of Murray cod were moderated by increasing pH between pH 6.2 and 8.8, and stimulated by increasing temperatures from 15 to 30°C. The optimal salinity was only slightly affected by temperature. The threshold and upper sublethal salinities varied slightly depending on feeding regime. The salinity sensitivities of fingerlings of Murray cod

were: LC50 = 13.7 g/L; optimal salinity from 4.6 to 5.0 g/L ; threshold salinity from 5.9 to 7.4 g/L, and upper sub-lethal salinity from 9.2 to 9.9 g/L – with the range in all cases affected by acclimation period salinity.

The blood osmolality at LC50 of the fingerlings was 444 mOsmol/kgH_2O or equivalent to 14.2 g/L, and the dehydration rate was 4.8%. The osmolality increased significantly in salinities higher than 9.0 and 6.0 g/L when fish were exposed for a period of 1 day and 41 days respectively. The oxygen consumption increased significantly in salinities higher than 8.0 g/L. Distortion of the notochord and corrosive skin syndrome were major symptoms describing sub-lethal effects found in the embryos, and fry and fingerlings of Murray cod respectively.

Noting the risks of extrapolating directly from laboratory to field conditions, it is predicted that when salinity in natural habitats increases above 0.34 g/L a significant impact on Murray cod recruitment will result.

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CHAPTER ONE

Introduction

1.1 Significance of this study

Of the significant investments in salinity investigation and amelioration works, little attention has been focused on the impacts of salinity on ecosystems or ecological processes of aquatic environments. Hart et al. (1991) stated that there is also little information available on the sublethal effects of salinity on fish. These may include shifts in abundance and distribution, or behavioural responses. There is need for more research in this area

Although some preliminary work has been done, the sensitivity of Murray cod to salinity is relatively undetermined (Ryan and Davies, 1996). Murray cod (*Maccullochella peelii peelii*) are the most abundant and least threatened compared to the other three *Maccullochella* species in the same region, namely trout cod (*M. macquariensis*), Mary River cod (*M. peelii mariensis*) and eastern freshwater cod (*M. ikei*) (Koehn, 1995; IUCN, 2002). The greater numbers of Murray cod allow detailed studies to be undertaken of their biology. Information obtained from this study will enable better understanding of Murray cod biology, which should enable better management of river systems for both this species and their close relations.

Although it has been known that many adult Australian freshwater fishes can tolerate salt levels up to or greater than 10,000 mg/L TDS (approximately 15,000 µS/cm EC) (Hart et al., 1991), for many adult species this is the part of the life cycle when they are most tolerant to increased salt levels (ANZECC, 1999). Many fish species may be able to survive at elevated salt levels during the adult stages, but reproduction, recruitment and growth of juveniles may become substantially reduced, while eggs and larvae of many species are generally more susceptible (Nielsen and Hillman, 2000). Despite the causes of declines in Murray cod population, i.e. removing of snags and altering of natural flows and temperature regimes of rivers, appearing to have been corrected with recovery programs in place, some cod populations fail to recover (Kearney & Kildea, 2001). This study hypothesised that the present salinities in the Murray-Darling river system have been the cause of mortality in some developmental stages of Murray cod.

1.2 Background

1.2.1 Significance of Murray cod

Murray cod are legendary and the mere mention of their name provokes public interest. They have been of unequalled significance to traditional, recreational and commercial fishers and, unfortunately, the target for much illegal poaching (Kearney & Kildea, 2001). Murray cod are native only to Australia (Lake, 1978) and are among the world's largest freshwater fishes (Kailola et al., 1993) growing up to 114 kg and capable of living longer than 80 years (Llewellyn & MacDonald, 1980). Murray cod are carnivorous fish, the top predator of the food chain in the freshwater ecosystem and the keystone species of the Murray-Darling basin ecosystem (Fishbase, 2000).

Kearney & Kildea (2001) state, "As the apex aquatic predator in the Murray-Darling freshwater system (Fig. 1.1), Murray cod is a unique contributor to the aquatic ecosystem and the outstanding indicator of the total well-being of that system. The difference in life history stages of Murray cod such as egg, larva, juvenile and adult life cycles requires integration of many aspects of habitat availability, water quality and quantity, and impacts of directed targeting. As a high level consumer it provides a good indicator of overall system health, integrating the impacts of water resource development and habitat modification on lower trophic levels. Its complex life history with widely varying requirements of different life history stages is such that it can be regarded as an umbrella species. Catering for Murray cod in management will bring a range of attendant benefits to other species with similar or less stringent habitat requirements. Managing for Murray cod will equate to managing for ecosystem integrity".

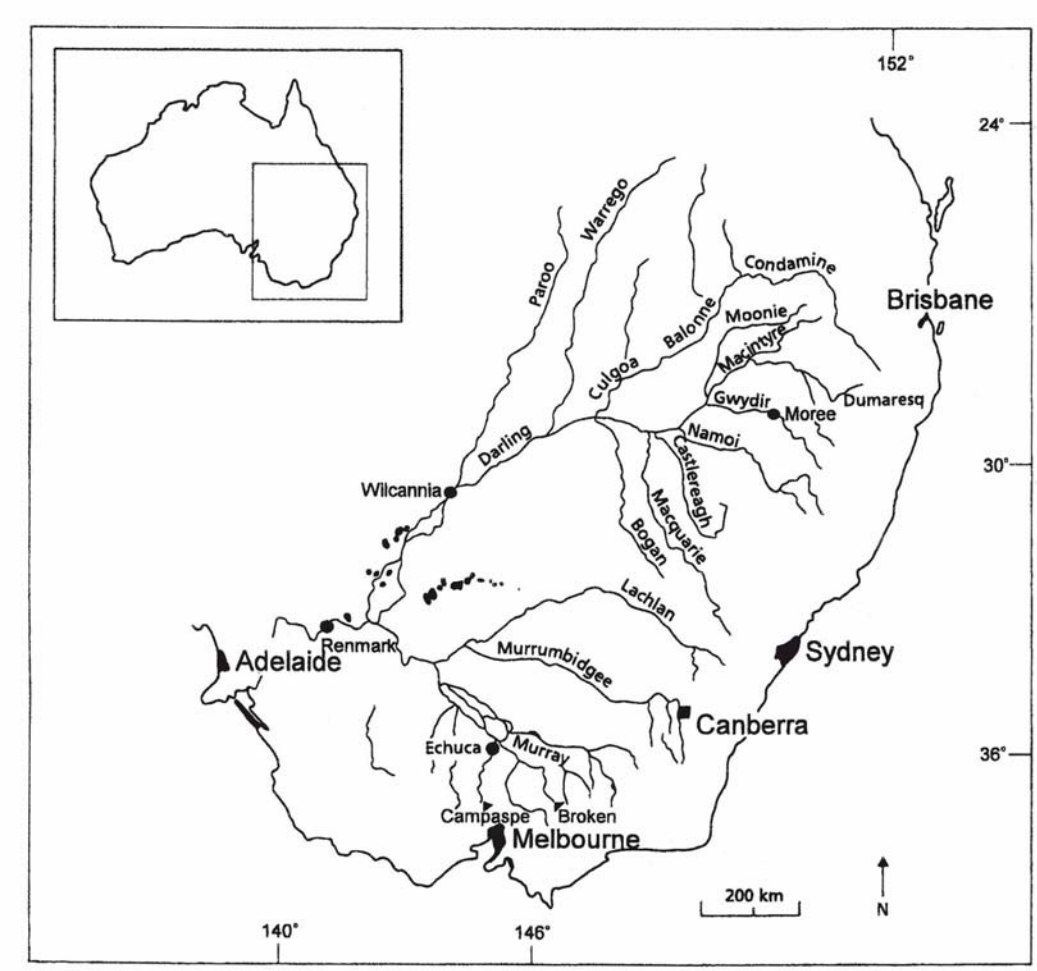

Figure 1.1 The Murray-Daring River system (Humphries et al., 1999).

1.2.2 Declining Murray cod population and threats to survival

Declining Murray cod population

At present Murray cod are considered to be a 'threatened species' (Classon & Booth, 2002). The International Union for Conservation of Nature and Natural Resources (IUCN) has classified the term 'threatened' into three consecutive classes, according to the degree of threat, as critically endangered, endangered and vulnerable (IUCN, 2002). However, Murray cod are not in the IUCN Red List Threatened Species (IUCN, 2002). Murray cod are listed as vulnerable in Victoria under the Flora and Fauna Guarantee (FFG) Act 1988 and are being considered for listing under the

Commonwealth Environment Protection and Biodiversity Conservation (EPBC) Act 1999 (MDBC, 2002).

Before European settlement, Murray cod were abundant and spread widely throughout the Murray-Darling River system from north to south from Queensland, New South Wales and Victoria to South Australia (Kailola et al., 1993). However, recently the species was found to be drastically reduced in abundance over all of its natural range (Pierce, 1989). Its population has declined dramatically since European settlement to the extent that cod are no longer common in parts of the Murray-Darling Basin (Harris & Rowland, 1996; Kearney & Kildea, 2001), and are rare in Victorian tributaries of the Murray river (MDBC, 2002). Fishing records showed a rapid fall over the period 1960 to 1967 from 80 tonnes to less than 10 tonnes per annum, and catches have remained relatively stable over the last 30 years to 1996; however, fishing efforts were also reduced over this period (Reid et al., 1997). These declines can be attributed to several categories of anthropogenic influence, including habitat degradation, pollution, reduced environmental flows, barriers to migration, and fishing (Kearney & Kildea, 2001), and recently, cold water released from dams (Classon & Booth, 2002).

Threats to survival of Murray cod

Kearney and Kildea (2001) have concluded in the report on the status of Murray cod in the Murray-Darling Basin that threats to the survival of Murray cod can be divided into three categories:

- Threats to the existence of the species (species biodiversity),
- Threats to the genetic integrity of the species (genetic biodiversity) and
- Threats to the integrity of the ecological systems to which the species contributes (ecological biodiversity).

According to a national survey, the threats to the integrity of ecological systems can be segregated in a priority order of habitat degradation, pollution - including salinity

(which is relevant to the aims of this study), reduced environmental flows, barriers to migration, introduced species, and fishing (Davis et al., 2000).

Intrusion of saltwater into the freshwater ecosystem is also a major concern to the integrity of aquatic habitats. It has been reported that within each catchment and between sub-catchments, the expected increase in the level of salinity will vary from moderate to severe, and will increase by two to three times by the year 2100 (MDBC, 1999). The Salinity Audit has claimed that the threat of the salinity of rivers has recently focused on the lower Murray. However, it predicts that more significant rises in salinity will occur in the other river valleys including those in the upland regions (MDBC, 1999).

1.2.3 Threat of increasing salinity to aquatic organisms in cod habitat

Nielsen and Hillman (2000) have stated, "Vast areas of Australian farm and grazing lands are gradually surrendering to dryland salinity. It has been estimated that by the year 2050 an area the size of Victoria will be lost to traditional agriculture. The importance of rising salinity and associated deterioration in water within the Murray-Darling Basin has been identified as a priority in the Murray-Darling Basin Natural Resources Management Strategy. It is difficult to predict the effects of dryland salinisation on the ecology of the waterbodies that originate flow through and ultimately drain these areas. Main considerations include: How will the chemistry of these waterbodies change, are there critical thresholds for salt concentration and how will the biota respond? Will the Australian biota be adaptable to increased salt, or will there be large-scale changes in species composition? Will biodiversity and ecosystem function be preserved in an increasingly salty world?"

Changes in salinity can affect biota in freshwater directly by a range of effects through physiological changes (particularly osmoregulation), resulting in change in diversity by the loss (or gain) of species. Indirect changes in species' diversity can occur as a consequence of direct effects modifying the community structure and function by the removal (or addition) of taxa that provide refuge and/or food. Nielsen and Hillman (2000) have stated that an increase in salinity from less than 500 mg/L (approximately 735 µS/cm EC) to above 1,000 mg/L (approximately 1,500 µS/cm

EC) will most likely be encountered in Australian rivers and wetlands. There is some evidence that ecological effects of salinity are likely to be observed within this range.

Nielsen and Hillman (2000) have also stated that the rate of change of salinity in freshwater ecosystems, as predicted by the salinity audit, will be much higher than the rate at which freshwater biota can evolve or adapt. In lowland rivers much of the biota may be salt tolerant or have mechanisms that allow survival during periods of extreme salt concentrations. Upland rivers potentially have experienced less variation in salinity. Biota in these systems may be less salt tolerant. Changes in salinity in these upland systems may be too rapid for taxa to adapt, resulting in these taxa becoming lost from these systems. This will result in communities dominated by salt tolerant taxa.

Direct adverse biological effects are likely to occur in Australian river, stream and wetland ecosystems, if salinity is increased to around 1,000 mg/L TDS (approximately 1,500 µS/cm EC). The expected increase in the salinity levels of many of the northern rivers would be expected to have a significant impact on the biota. This would be either directly through the individual species salinity tolerance or indirectly through impacts on breeding/nesting areas or food sources (Hart et al., 1991).

Increasing salinity may indirectly modify the structure of freshwater ecosystems. Such changes may be first indicated by changes in the dominance of plants or animals (Williams, 1987). Increasing salinity reduces the diversity of macrophytes creating less complex ecosystems. Loss of habitat complexity will influence the diversity and abundance of associated taxa. Increasing salinity also modifies many of the processes that occur within freshwater ecosystems. Salinity has been shown to reduce the amount of suspended particulate and colloidal matter in the Darling River allowing increased phytoplankton production (Nielsen & Hillman, 2000).

While increasing salinity will potentially have some impacts on the biota associated with lowland permanently flowing rivers, there is likely to be greater impact on the biota associated with non-regulated low flow or intermittent rivers. In these systems

periods of higher salinity may occur that exceed the tolerance of associated biota (Nielsen & Hillman, 2000).

A threshold level of 1,000 mg/L is considered to be the level of salinity at which the direct adverse biological effects are likely to occur in a freshwater system (MDBC, 1999). Above this threshold many macrophytes and invertebrate taxa are known to have reduced abundance and diversity. For the majority of taxa, however, very little research has been done on the effects of salinity at a concentration below 1,000 mg/L on recruitment, reproduction or survival of early life stages. Changes in diversity and loss of taxa may not become apparent until after an extended period of time (Nielsen & Hillman, 2000).

1.3 Study aims

The main objectives of this study were to:

- determine the salinity sensitivity of Murray cod eggs, larvae, fry and fingerlings in terms of LC50, optimal, threshold, and upper sublethal salinities
- establish the 'salinity-growth curve' as the growth characteristics of Murray cod fry and fingerlings when reared in different salinities
- investigate the interactions between temperature and food availability on the sensitivity of Murray cod fry
- measure the blood osmolality and dehydration of fingerlings when exposed to different salinities
- measure the oxygen consumption of fingerlings when exposed to different salinities
- measure the utilisation efficiency of yolk-larvae when exposed to different salinities
- investigate the stress responses in eggs, larvae, fry and fingerlings to sublethal salinity.

1.4 Reviews of literature

1.4.1 Terminology

The following terms are standard in aquatic methods for fish biology, ecology, toxicology, evolution and systematics. The terms are used as defined throughout this thesis.

Acute : Coming speedily to a crisis or end point, or happening quickly. For fish the term acute customarily is used for effects that occur within 4-7 days. It can also refer to the duration of exposure (e.g., an acute test). An acute effect may be lethal or sublethal.

Chronic : Long-lasting or continued. The term can refer to the effect or the duration of exposure. In aquatic toxicology, it has sometimes been used to mean a full life cycle test (Sprague, 1990); practically that is often not possible, so it is taken to mean a 'significant' portion of a life cycle.

EC50 : Median effective concentration: the concentration of a substance estimated to have an effect – lethal or nonlethal – on half of a group of organisms. The effect as well as the exposure time must be specified. (Note that this statistic must be calculated on the basis that 50% of the individuals showed the specified effect, and hence it should be used for such things as a 50% reduction in growth of an algal population, or 50% reduction in photosynthesis activity.) The term IC50 – inhibition concentration – is currently being used for such a reduction in activity or other biological attributes (Sprague, 1990). It could also be 50% growth reduction in all individuals.

LC50 : Median lethal concentration (=TLm of older literature): the concentration of a substance that is estimated to kill half of a group of organisms. The duration of exposure must be specified (Sprague, 1990).

Lethal : Causing death, or sufficient to cause death (Sprague, 1990).

Optimal : Most favourable; used of the levels of environmental factors best suited for growth and reproduction of organisms (Lincoln et al., 1998).

Sensitivity : The capacity of organisms to respond to a stimulus (Lincoln et al., 1998).

Sublethal : Toxigenic response that is pathological but not lethal, or an effect that is not directly lethal (Sprague, 1990).

Threshold :The minimum level or value of a stimulus necessary to elicit a response (Lincoln et al., 1998).

Tolerance :Highest concentration of a substance in which the median tested organism could live for a definite time (the highest concentration that does not cause an acute LC50) (Sprague, 1990).

1.4.2 Classification of early life stages in fish

The process of formation of an embryo to create new phenotypes is under the instructions given by the genome that is selected by the past environment. But the phenotype – the sum of observable structural and functional properties of an organism (Lincoln et al., 1998) – itself is formed by an interaction between the genotype and the present environment (Balon, 1985). Therefore, attributions of the present environment will have impacts on the development of an individual organism.

The course of growth and development of an individual organism is called ontogeny (Lincoln et al., 1998). But when does ontogeny begin? Balon (1985) has stated that the most common answer would undoubtedly be ‘with fertilisation’. However, fertilisation is not an instantaneous event, but a process that lasts for a period of time. It starts with insemination during which gametes are brought together. The process continues with activation that releases the egg’s developmental block and triggers irreversible changes. The changes comprise the growth of the fertilisation cone, cortical reaction, perivitelline space formation and bipolar differentiation. The fertilisation process then ends with the fusion of male and female pronuclei. Only the latter process can be considered fertilisation in a strict sense. If fish gametes are stripped and mixed with the coelomic fluid of the female out of water, the sperm are activated to penetrate, but become arrested half way down the micropyle and the egg remains unchanged. Under these circumstances this insemination cannot be considered the beginning of ontogeny.

Table 1.1 Terminology of early life history of Murray cod used in this study.

Developmental stages	**Egg**	**Larva**	**Fry**	**Fingerling**
Observable descriptions	Extruded egg prior to beginning of ontogeny and embryo (Blaxter, 1988)	Hatch, endogenous feeding, include preflexion and flexion stages; also called free embryo, yolk-sac larva or sac fry (Blaxter, 1988)	Completion of yolk absorption, beginning of exogenous feeding; feeding exclusively on zooplankton; includes early preflexion and post flexion stages (Cadwallader & Gooley, 1985; Blaxter, 1988)	Completion of appendage assembly to adult stage; sexual gametes absent, confined to the juveniles of smaller size than young-of-the-year
Approximate age (day)	0	1-10 (Neira et al., 1998)	10-46 (Neira et al., 1998)	46-200
Approximate total length (mm)	3-4 (Lake, 1978)	(5-9)-10 (Cadwallader & Gooley, 1985)	10-19 (Cadwallader & Gooley, 1985)	19-127*
Approximate weight (g)	NA	0.004 (Cadwallader & Gooley, 1985)	0.004-0.09 (Cadwallader & Gooley, 1985) 0.01-0.50*	0.09-50*

* The values are estimated from a relation equation obtained in this study, $W=0.1118\ e^{0.0482L}$, where W is weight (g) and L is length (mm).

In the course of natural mating, the gametes are released into water and insemination coincides with the activation of ovum by water (Brummett & Dumont, 1979). The cortical granules of the egg release their content thus forming the perivitelline fluid and space. This cortical reaction is followed by rapid changes of external tension and density and simultaneous continuity of meiosis. Along with changes in ion concentration there is the subsequent formation of pronuclei. However, these developments can also be initiated by contact with water without insemination. Therefore, activation in the above sense should be considered the beginning of ontogeny (Balon, 1985).

The terminology used to name and describe the different developmental stages in teleost fishes varies greatly depending on the author. To make the specific terms recognizable it is essential to review the terminology used in various works.

Blaxter (1988) uses the term “embryo” to denote fish after hatching. The term “larva” covers development from hatching to metamorphosis and the term “juvenile” describes the state from metamorphosis to the first spawning. Some workers may use the term “fingerling” to name the juvenile that has a long body, or the term “young-of-the-year” to cover the juvenile stage of a species that has a long life span (Blaxter, 1988), such as Murray cod. The term “yolk-sac larva” may be used as an additional state for the larva that does not hatch in an advanced state of development. To provide more comprehensive descriptions of larval development, Blaxter (1988) divides the larval stage into “preflexion”, “flexion”, and “postflexion” substages, referring to the elevation of the notochord tip during the first stage of development of the caudal fin (Fig. 1.2).

Some species, especially those that migrate between freshwater and seawater, such as trout and salmon, undergo a more complicated metamorphosis. Balon (1985) states that development in the embryo period comprises seven consecutive steps: 1) formation of the perivitelline space and bipolar differentiation; 2) first multiplication of cells, cleavage, and the formation of morula; 3) gastrula, the beginning of epiboly, the formation of the germ ring and embryonic shield; 4) closure of the germ ring, formations of brain, optic and auditory vesicles, separation of tail-end; 5) the beginning of trunk movements, heart beats, the circulation anlage and the appearance of the first melanophores; 6) rapid expansion of the respiratory yolk plexus of subintestinal, and mix hepatic descent; and 7) function of hepatic-vitelline and caudal respiratory plexuses, rapid expansion of pigment and hatching.

The free embryo phase comprises three developmental steps: 1) differentiation of embryonic finfold, formation of first dorsal rays in unpaired fins, development of first chondrified skeleton structures and growth of pelvic fins beyond the finfold edge; 2) onset and duration of strong photophobia, transfer to mainly branchial respiration and calcification of jaw tooth points; and 3) the active free embryo; complete absorption of yolk, calcification of skeleton structures and rapid expansion of iridocyte distribution on the body flanks.

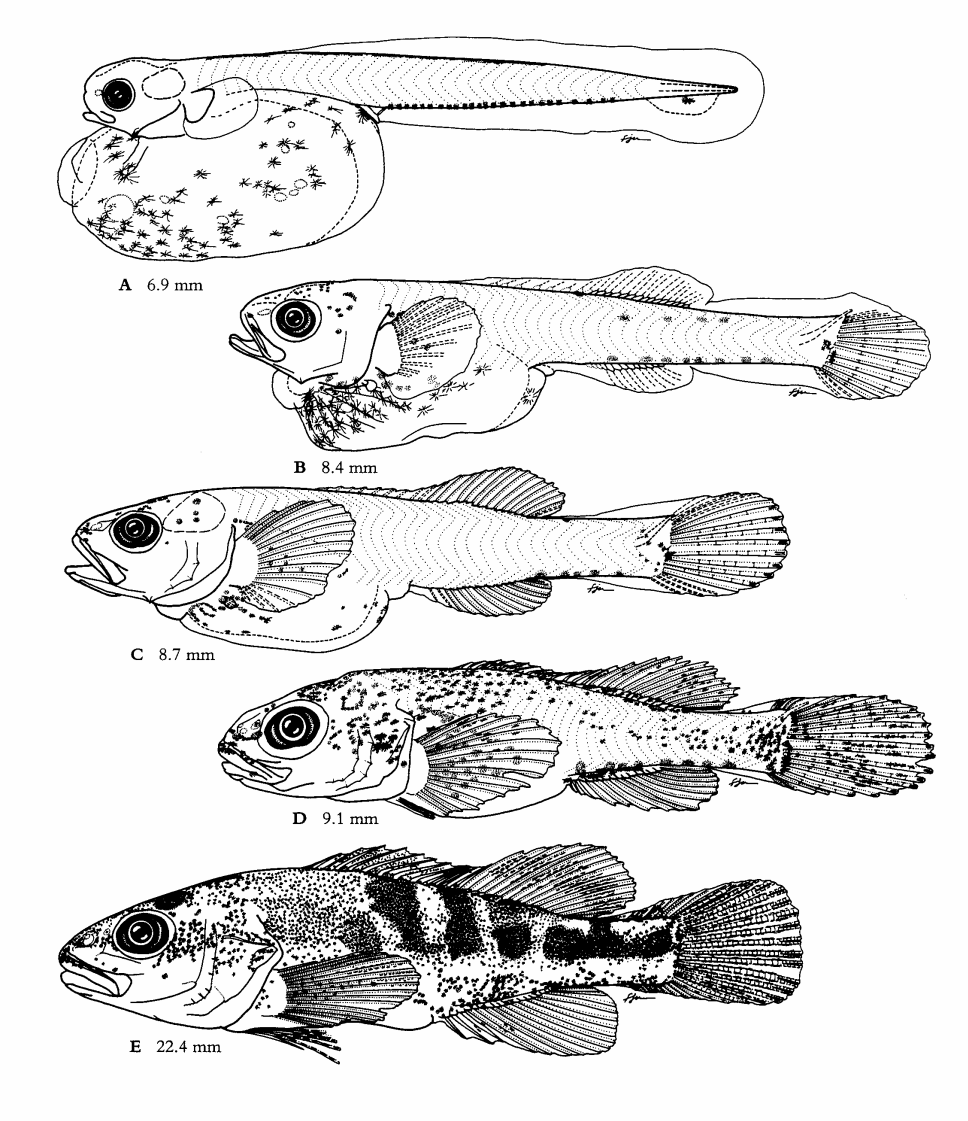

Figure 1.2 Larvae and juvenile stages of Murray cod (*Maccullochella peelii peelii*). **A** Preflexion, 1 day old; note large yolk sac. **B** Flexion, 8 days old; note pelvic-fin bud. **C** Early postflexion, 11 days old. **D** Postflexion, 20 days old; note remnants of yolk sac. **E** Juvenile, 46 days old (Neira et al., 1998).

This study has adopted the attributions that appropriately define the early life stages of Murray cod as concluded in Table 1.1. These include egg (prior to ontogeny), larva (free swimming yolk-sac), fry (postflexion, exogenous), and fingerling (sexual gametes absent, smaller than young-of-the-year). However, the transition stage of late-fry to early-fingerling is too intricate to determine. This study designated the fry stage of Murray cod to plankton feeder in which stage fish might grow up to 0.50 g in laboratory rearing, fed extensively with *Artemia* nauplii.

1.4.3 Aspects of salinity in fish

Osmoregulation and salinity stresses

Osmoregulation is the process by which the total electrolyte content and water volume in an organism are held relatively constant. The overall salt concentration of fish blood is approximately one third that of seawater (35,000 mg/L). Marine fish have slightly more salt in their blood than freshwater species (Heath, 1987). Since gills are permeable to water as well as oxygen, carbon dioxide, etc., there is an osmotic flow of water out of the marine fish because the salt concentration of its blood is less than of the ocean, and into the freshwater fish because of the opposite osmotic gradient. The diffusion gradient for sodium chloride (NaCl – the primary salt) is in the direction opposite to the osmotic diffusion of water across the gill epithelium (Heath, 1987). In the marine environment the blood has a relatively low osmotic pressure so that, by passive diffusion, water is lost through the gills and ions are gained (Ellis et al., 1989). Urine is produced in small quantities with low or negligible glomerular filtration rates. The urine is approximately *i*so-osmotic or slightly hypo-osmotic and contains mainly the divalent ions Mg^{2+} and SO_4^{2-} (Ellis et al., 1989). The rate of urine production is low (about 0.05% body weight per hour) and the urine is *i*so-osmotic with the body fluid (Eddy, 1981).

The water loss by diffusion from a marine fish is replace by the animal drinking the equal amount of seawater which amounts to a rate of about 0.5% of the body weight per hour. In order for the seawater to actually enter the blood from the gut and lumen, there must be an active transport of NaCl from the lumen into mucosa, which sets up a local osmosis gradient to pull water into the blood (Heath, 1987). By this mechanism salt, along with water, is also transported into blood. Due to this uptake of salt into the blood and the passive diffusion of salt into the gills, marine fish must constantly excrete by active transport a considerable amount of salt (Heath, 1987).

Key structures in ion exchange across the gills in teleost fish are the chloride cells, which are implicated in pumping salt inwards in freshwater and outwards in seawater species. They are located on both primary and secondary lamellae, principally at the junction between the two, and are found in greater densities in marine adapted fish. It

has been suggested that salt pumping from the gills of marine fish results from diffusion from a high concentrate of salt in a specialized space between adjacent chloride cells, which are open to the sea. The salt gradient is produced by a very high density of sodium pumps on the membranes adjacent to these intercellular spaces. Chloride cells are well provided with mitochondria to generate the active transport – an energy-requiring process that requires the utilisation of ATP (Conte, 1969) and the enzyme Na-K activated ATPase (Heath, 1987). The movement of Na^+ and Cl^- out of marine fish is a complex process involving a combination of electrical potential and a Na/K exchange (Heath, 1987). The mechanism of inward pumping of salt in freshwater is thought to be essentially the same as in amphibian skin (Ellis et al., 1989).

In freshwater the environment is hypo-osmotic and water tends to pass into body fluids through the gills and permeable surfaces of the pharynx. This is compensated by the kidney producing large volumes of dilute urine, so that glomerular filtration is of great importance (Ellis et al., 1989). Body fluids in freshwater fishes are much more concentrated than the surrounding medium and thus water enters fish osmotically and salts diffuse outwards. Excess water is removed as copious hypotonic urine (0.15-0.04% body weight per hour) whilst salt losses are replaced by active uptake mechanisms in the gills. Branchial ionic uptake increases with the external salt concentration up to about 2 mM/L when the mechanism becomes saturated. The neutrality is maintained by the exchange of Na^+ for NH_4^+ and H^+, and Cl^- for HCO_3^-. For reasons not yet understood, freshwater fish drink the medium at a rate of approximately 0.05% body weight/h. The gills of freshwater fish contain the enzyme Na-K activated ATPase as well as an ion-stimulated enzyme Cl-K ATPase, which is possibly involved in the proposed Cl/HCO_3 exchange (Eddy, 1981).

Euryhaline species can alternate between the mechanism of freshwater and marine species according to their requirements, although the glomerular apparatus of some undergoes degeneration in a marine environment. There are also basophilic primordials, adjacent to the glomeruli, in teleost nephrons, which may be the site of the origin of new glomeruli (Ellis et al., 1989).

Osmoregulation is mainly under endocrine control via the posterior pituitary and internal corticoid hormones and possibly the caudal neuro-secretory system and the juxtaglomerular apparatus of the kidney (Ellis et al., 1989). A number of hormones have been shown to be involved in osmotic and ionic regulation, but particular attention has been paid to cortisol, prolactin and the catecholamines. Stimuli that have the capacity for altering any aspect of the metabolism of these hormones must, therefore, be potentially capable of influencing osmotic and ionic regulation in fish (Eddy, 1981).

The animals of freshwater ecosystems can be divided into two groups based on their ability to regulate their internal osmotic concentration against the external environment. Good regulators are termed euryhaline and can adapt to a wide range of salinities. Poor regulators are termed stenohaline and are restricted to only a narrow range of salinities (Hart et al., 1991).

In normal habituating conditions, freshwater fish regulate the plasma ions equivalent to approximately 10,000 mg/L (Brett, 1979). When freshwater fish are placed in seawater the high osmotic pressure of the medium results in extensive withdrawal of water from the gill tissues, with the result that the afferent and efferent arteries are obliterated by the pressure of shrinking surrounding tissues, and death results from the arrest of branchial circulation. Freshwater fish will readily tolerate diluted seawater, or similar balanced solutions, of an osmotic pressure equal to that of their blood, but higher concentrations of salt are rapidly fatal (Jones, 1964). Maximum growth rates can also be obtained when fish are acclimated to some certain salinity concentrations in a range of the optimum temperature. However, in the salinities that are not necessarily lethal, abnormalities may occur (Brett, 1979). Salinity impacts on a species depend on the specific tolerance of the species and the regional variations and tolerance within the species. Salinity requirements can also vary for particular species depending on their lifecycle stage (Jones, 1964). The variations in salinity tolerances between fish species are also attributed to aquatic types of the ancestors, the length of time their lineage has existed away from the sea, and the individual level that a particular fish has been subjected to over its recent history (Ryan & Davies, 1996). Sensitivity may also vary between populations of the same species depending upon the degree to which a population has adapted to a given salinity range through direct selection (Ryan & Davies, 1996).

A number of studies reported physical changes in freshwater fish caused by salinity stress as shown in Table 1.2. The common symptoms appeared to be skin lesions, cataracts and skin melanism.

Table 1.2 Some physical changes occurring in freshwater fish exposed to saline conditions.

Pathological responses	**Fish species**	**Causative**	**Source**
Cataracts	*Micropterus salmoides*	salinity	Ubels and Edelhauser (1987)
Scale deformation	*Cyprinus carpio*	salinity or other factors	Yoshitomi *et al*. (1998)
Skin lesions	*Lates japonicus*	salinity	Tashiro and Iwatsuki (1995)
	Oncorhynchus mykiss	salinity associated with bacteria	Garcia *et al.* (2000)
	Oreochromis niloticus	salinity and temperature	Likongwe *et al*. (1996)
	Oreochromis mossambicus	salinity associated with *Streptococcus iniae*	Mukhi *et al.* (2001)
	hybrid of *Oreochromis mossambicus* x *O. hornorum*	salinity	Garcia and Sedjro (1987)
Skin melanism	*Oncorhynchus mykiss*	salinity	Francis *et al*. (1997)

Development and growth

Development and growth in fish are controlled by internal factors including central nerve system, endocrinological and neuroendocrinological systems, which are highly dependent on environmental conditions such as water salinity (Boeuf & Payan, 2001). Conte *et al*. (1966) reported that salinity tolerances vary with age.

In most species, egg fertilisation and incubation, yolk sac resorption, early embryogenesis, swimbladder inflation, and larval growth are dependent on salinity. Salinity is also a key factor in controlling growth in larger fish (Boeuf & Payan, 2001). The larvae of some species of fish that spawn in freshwater migrate relatively early in their life histories to marine or estuarine conditions (Holliday, 1969). This demonstrates the essential nature of salinity to the development of some freshwater species. A lightly enhanced salinity can also be favourable to the development of eggs of some freshwater fish because of the effects of saltwater on certain freshwater diseases (Holliday, 1969).

The impacts of salinity on eggs and larvae have been largely worked on with migratory and marine fishes because the changes in salinity environment are part of their life cycle. However, even in marine species, increased salinity outside their optima also affects their development. The gametes, i.e. egg and sperm, are generally *i*so-osmotic with, or slightly hypoosmotic to the body fluids of the parent fish. At spawning the gametes are often subjected to an abrupt change, which might be expected to result in death, or at least considerable impairment of the ability to produce fertile eggs (Holliday, 1969). However, the gametes of some species may be highly tolerant of salinity change (Holliday, 1969).

Increased salinity has been documented as having impacts on larval survival in marine fish. Holliday and Jones (1967, cited in Holliday, 1969) reported dehydration in the embryos of *Pleuronectes platessa* in increased salinity. Weisbart (1968) showed the changes in serum osmotic concentration in the embryos of five species of fish kept in seawater. The effect of salinity on the percentage hatching and abnormalities of larvae was also recorded in saltwater species. Rosenthal and Alderdice (1976) stated that severe morphological aberrations (e.g. distorted notochords) have been found in embryos whose normal environments have been altered by low oxygen level and/or extreme salinity levels or temperature variations. Santerre and May (1977) also reported high percentages of morphologically abnormal larvae of moi (*Polydactylus sexfilis*) at several salinity levels at extreme temperature ranges.

A number of studies have demonstrated the enhancing effects of water salinity on the growth of freshwater fish as shown in Table 1.3. It was found that most of the tested species grew better in mild saline water than in fresh water. The best growth of carp should be obtained in salinities between 5 and 7 g/L, while tilapias preferred a slightly higher salinity. Silver perch is the only native freshwater species of Australia to date that has undergone growth trials tested against different salinities (Kibria et al., 1999), though Murray cod have been successfully grown in saline farm dams (Cadwallader & Gooley, 1985).

Ryan and Davies (1996) have reviewed publications on the salinity tolerances of eggs and larvae of some Australian native fish. It can be concluded that the LC50 of eggs of the five species (i.e. silver perch, river blackfish, common galaxias, crimson-

spotted rainbow fish and Australian graying) is in a range of 5 to 17 g/L. The LC50 of the larvae of the six species (i.e. river blackfish, common galaxias, Murray cod, Macquarie perch, crimson-spotted rainbow fish and Australian graying) is in a range of 4 to 30 g/L, and the LC50 of Murray cod larvae is less than 7 g/L.

Table 1.3 Growth of some freshwater fish reared in saline conditions.

Fish species	**Growth characteristics**	**Source**
Catfish (*Mystus vittatus*)	growth reduced at 10 g/L compared to 0 g/L	Arunachalam and Reddy (1979)
Channel catfish (*Ictalurus punctatus*)	grow in 3 g/L better than freshwater	O'Neal and Weirich (2001)
Common carp *(Cyprinus carpio)*	grow well up to 7 g/L acceptable up to 8 g/L Juvenile, best in 5 g/L Juvenile: fluctuation of salinity 0-2% accelerates growth	Garg (1996) Qiu and Qin (1995) Konstantinov and Martynova (1993)
Grass carp (*Ctenopharyngodon idella*)	grow best in 6 g/L Juvenile: fluctuation of salinity 0-2% accelerates growth	Routray and Routray (1997) Konstantinov and Martynova (1993)
Largemouth bass, freshwater (*Micropterus salmoides*)	growth decreases in 4 to 8 /L, stops feeding at 12 g/L	Meador and Kelso (1990)
Russian sturgeon (*Acipenser gueldenstaedti*)	Juvenile: fluctuation of salinity 0-2% accelerates growth	Konstantinov and Martynova (1993)
Silver carp (*Hypophthalmichthys molitrix*)	grows well in 5.1 g/L	Falk (1986)
Silver perch (*Bidyanus bidyanus*)	grows best in 4 g/L	Kibria et al. (1999)
Striped bass (*Morone saxatilis*)	Juvenile: non-anadromous, grows best at 7 g/L	Secor et al. (2000)
Tilapia, (*Oreochromis spilurus spilurus*), and *Oreochromis mossambicus*	fairly constant growth in 6-14 g/L	Payne et al. (1988)
Tilapia (*Tilapia nilotica*)	grows best in 50% seawater	Kang (1985)
Tilapia (*Oreochromis niloticus*)	grows better in 15 g/L than in freshwater (15 g/L is iso-osmotic medium)	Woo et al. (1997)
Tilapia, hybrid (*Oreochromis niloticus* x *O. mossambicus*)	grows best in 0-15 g/L	Alfredo and Hector (2002)

Table 1.3 continued

Tilapia, hybrid (*Oreochromis niloticus* x *O. aureus*)	grows best in 0-6 g/L	Payne et al. (1983)
Tilapia, Ivory strain (*Tilapia nilotica*)	juvenile: weight gain depresses at 10 g/L; fry gradual transferred improves weight gain and survival; hardened eggs: hatching viability decreased from 0 to 10 g/L, extremely low at 15 g/L	Wardoyo (1991)
Tilapia, Nile (*Oreochromis niloticus*)	Juvenile- grow well up to 8g/L	Likongwe et al. (1996)
Tilapia, red hybrid	fairly constant growth in 6-14 g/L	Payne et al. (1988)
Tilapia , red (*Oreochromis* spp)	grow better in 10 g/L than in 20 g/L	Yi et al. (2001)
Tilapia, red hybrid (*Oreochromis urolepis hornorum*, x *O. mossambicus*)	Juvenile - sex-reversed male, grow better in 10g/L than in freshwater	Watanabe et al. (1988)

1.4.4 Characteristics of saline waters

Naturally occurring freshwaters may vary enormously in mineral content depending on the source and location. Seawater and freshwater are clearly different in their mineral contents. Aquatic ecologists have generally accepted a value of 3,000 mg/L TDS (approximately 5,000 µS/cm EC) as conventionally dividing fresh from salt water (GHD, 1999). Waters with salinity above 3,000 mg/L TDS are typically inhabited by a distinct biota unlike that found in waters of low salinity (GHD, 1999). Above 3,000 mg/L TDS, communities comprise only halo-tolerant (or saline tolerant) freshwater forms or forms restricted to saline waters, and both species richness and diversity decrease (GHD, 1999).

Salt from different sources also impacts differently on aquatic animals. O'Brien and Ryan (1999) reported a relative different salinity sensitivity of *Daphnia carinata* to various salt solutions among which Pacific Sea Salt, which was utilised to make up the test solutions in this study, was more lethal than two other test salt sources (Table 1.4).

Table 1.4 Relative sensitivity of *Daphnia carinata* to various salt solutions (O'Brien & Ryan, 1999).

Salt Source	48 h EC_{50}
Ocean Nature	9,800 EC
Lake Charm	4,980 EC
Pacific Sea Salt	4,380 EC
Cheethams	4,180 EC

Inland saline water results primarily from either groundwater or terrestrial material via the weathering of rocks. Some salt may also be transported with wind and rainfall. The contributions of these sources depend on factors such as distance inland, climate and geology (Williams, 1987). Because of this, the ionic composition of water varies between localities. Bicarbonate is typically the dominant anion in the headwaters of rivers, whereas chloride is the dominant anion downstream. The proportion of sodium to total cations increases with distance downstream. The dominant salt found in the groundwater is sodium chloride (Close, 1990). Groundwaters may contain significant quantities of dissolved minerals, different from those of seawater, that cause the high salinity of freshwater sources (Munro & Roberts, 1989). The saline groundwater from Wakool, New South Wales, Australia is low in K^+ compared to the level in seawater. It is not suitable for aquaculture unless fortified with potassium salts (Fielder et al., 2001). However, Ingram et al. (2002) documented the growth of some fish species reared in saline basins in the Goulburn-Murray Irrigation District, south-eastern Australia where K^+ concentrations were significantly lower than in seawater (Table 1.5). Table 1.5 clearly shows that the major ion composition (i.e. Ca^{2+}, Mg^{2+}, K^+, Cl^- & HCO_3^-) of the groundwater differs from that of seawater at same salinity. The significance of cation and anion concentrations in fish were explained in section 1.4.3 under 'Osmoregulation and salinity stress'. Ca^{2+} and Mg^{2+} are important for ionic regulation of freshwater fish because they influence the permeability of biological membranes, preventing high ionic loss to water. Hatching of some freshwater species such as silver catfish (*Rhamdia quelen*) was improved when water hardness was increased from 20 to 70 mg/L $CaCO_3$ by using Ca^{2+} or Mg^{2+} (Silva et al., 2003). In seawater, fish such as marine red drum (*Sciaeops ocellatus*) did not survive when calcium concentration reduced from 340-465 mg/L to 176 mg/L (Willam & Stickney, 1989). Salinity can therefore be regarded as a determinant, either directly or indirectly, of biological

community structure in inland waters, with the degree of importance being dependent upon the complexity of community structures and salinity level (GHD, 1999).

Table 1.5 Differences in concentrations of major anions and cations between the saline groundwater in the Goulburn-Murray Irrigation District, south-eastern Australia and seawater at a salinity of 10 g/L (Ingram et al., 2002).

Anion/cation	Saline water (mg/L)	Seawater (mg/L)
Chloride	4,130	5,527
Fluoride	0.2	0.4
Sulphate	1,280	775
Bromide	<0.4	19
Bicarbonate	190	41
Boron	1.0	1.0
Calcium	350	118
Potassium	25	114
Magnesium	470	370
Sodium	2,700	3,078
Strontium	5.2	2.0

1.4.5 Murray cod biology

Diagnostic features

Murray cod is registered as an Australian species code no. 311076 (Kailola et al., 1993), and FAO species no. 611 (Fishbase, 2000).

Murray cod (*Maccullochella peelii peelii* Mitchell 1838) (Teleostei: Actinopterygii: Perciformes: Percichthyidae) (Fishbase, 2000) have a broad, depressed head with small eyes, a rounded snout and a concave forehead profile. Their jaws are usually equal in length but sometimes the lower jaw protrudes. The mouth is large, extending beyond the eye. There are 65-81 scales in the lateral line. The body is covered by small scales, mostly ctenoid, some cycloid (Cadwallader & Backhouse, 1983). The caudal fin is rounded. Body colour is variable with the back and upper sides usually olive-green to yellow-green and the upper flanks mottled brown to pale green. The belly is generally white to light grey (Allen, 1989; Kailola et al., 1993; Llewellyn & MacDonald, 1980). The maximum size is about 1800 mm; but the length is commonly about 500-700 mm (Allen, 1989). Small Murray cod up to 30 mm may be

confused with young golden perch, pigmy perch and possibly some gudgeons; the adult is similar to trout cod (Llewellyn & MacDonald, 1980).

Murray cod has a various common names, i.e. cod, codfish, goodo or goodoo, ponde (Kailola et al., 1993), and Murray perch (UK) (Fishbase, 2000). It has also been found published under a number of synonyms (Fishbase, 2000): *Maccullochella peelii peelii* a validated new combination (Fishbase, 2000), *M. peelii* (Mitchell 1838) a non validated new combination (Fishbase, 2000), *M. peeli* (Mitchell 1838) a non validated misspelling (Fishbase, 2000), *Acerina peelii* (Mitchell 1838) a non validated original combination (Fishbase, 2000), and *M. macquariensis* (Cuvier & Valencinnes) a formerly used name (Dakin & Kesteven, 1939; Roughley, 1966).

Age and growth

Murray cod grow up to a weight of 114 kg and may reach 1800 mm in length (Llewellyn & MacDonald, 1980) at an estimated age of 80 years (NSWF, 2000). The oldest Murray cod ever caught was 48.6 years, weighing 31.8 kg and 1280 mm in length (Anderson et al., 1992). By using the maximum recorded size of 1280 mm reported by Anderson *et al.* (1992) the mean generation time is estimated at 9.6 years with a life span of 29.0 years and a natural mortality of 0.17 per year (Fishbase, 2000). These estimates are based on the von Bertalanffy growth parameters.

Growth rates can be extremely variable depending on location and conditions. For example, Murray cod in the Darling River have less weight at a particular length than Murray cod in the Murray River (Roughley, 1966), and Murray cod caught in the Murray River in the 1960s showed significantly slower growth rates and were heavier for a given total length than cod caught in the 1990s (Anderson et al., 1992). Only the fastest growing fish in the 1960s have similar length-at-age as fish in the 1990s (Anderson et al., 1992).

Murray cod grow rapidly in their first three years (Cadwallader & Gooley, 1985). They generally attain a length of 203-254 mm at one year and 305-356, 406-483, 533-584 and 610-660 mm at the end of their second to fifth years respectively (Roughley, 1966). The yearly average rates of weight gain may be roughly given at

0.43 - 2.00 kg per year, and the length-weight relationships may be described as $W = (0.36 \times 10^{-4})L^{2.91}$ where W is weight in g and L is total length in mm (Anderson & Gooley, 1992).

There is considerable variation in length-at-age of Murray cod. For example, a fish at 800 mm may be aged 7 years or 22 years; likewise a fish at 1150 mm may be aged 11 years or 40 years (Anderson & Gooley, 1992). Weights are even more variable, and the variance in weights of fish at the same total length or estimated age increases substantially with length and age. According to the otoliths' growth annuli, the growth of cod is promoted in spring and continues throughout their lives (Anderson & Gooley, 1992).

Reproduction

Murray cod mature at various sizes and ages, from 381 mm to 560 mm or 1.14 kg to 2.2 kg at age 3-4 years. Most cod mature at 4-6 years at a size greater than 2.5 kg and 500 mm in length (Cadwallader & Gooley, 1985; Lake, 1978; Llewellyn & MacDonald, 1980; Roughley, 1966; Scott et al., 1974).

Gooley *et al*. (1995) has reported a relative delayed age at first maturity of Murray cod in Lake Charlgrark of 6 years for female fish (2,000 g), and 3-4 years (700 g) for males. However, Anderson & Gooley (1992) have stated that the estimated growth rates of Murray cod in Lake Charlgrark are significantly low compared to fish from other waters.

It has been known that Murray cod spawn annually during spring (Rowland, 1995a) and early summer, depending on prevailing climatic conditions (Gooley et al., 1995). Spawning usually occurs in September to October, or is delayed to January (Roughley, 1966). Spawning is triggered when water temperature approaches 20°C (Roughley, 1966). It has been found recently that the rate of rise of water temperature may be more important than a particular temperature; generally a range of 18-25°C is required (Codwatch, 1993a). The raising of water levels is not required to stimulate the spawning of Murray cod (Rowland, 1995a), but it has been found to stimulate its

upstream migration for spawning (Codwatch, 1993e). Cadwallader and Gooley (1985) have suggested that depth and water clarity are not significant factors in stimulating spawning, and that increasing daylight in the spring does not by itself stimulate spawning, but does contribute to a rise in water temperature.

Murray cod commonly lay adhesive eggs inside hollow objects (Roughley, 1966). In the Murray-Darling River, the submerged hollow logs of river red gum (*Eucalyptus camaldulensis)*, which grow along the banks, provide an ideal substrate for Murray cod (Lake, 1978). They also occasionally lay eggs attached to open substrates in shallow water in pools and farm dams (Cadwallader & Gooley, 1985). Murray cod regularly breed in impoundments and dams (Rowland, 1995a) provided with a plentiful food supply and suitable substrates (Lake, 1978). Individual Murray cod at approximately 2.3 kg spawn 20,000 eggs; at a weight of 4.1 kg they can produce 30,000 eggs (Roughley, 1966) and, depending on size, can produce up to 200,000 eggs (Codwatch, 1993a). Relative fecundity is around 5,000 eggs per kg (Rowland, 1995a). A female fish might lay her eggs in several plots of substrate at a time, and these possibly are fertilized by more than one male (Cadwallader & Gooley, 1985). It has been shown that the male cod protects (Rowland, 1995a) and probably fans the eggs during incubation (Fishbase, 2000).

Eggs after fertilizing swell to 3-4 mm in diameter (Roughley, 1966). The eggs hatch within 5-14 days in natural conditions depending on the water temperature (Lake, 1978): for example, 13 days at 16°C, 6-9 days at 20°C (Codwatch, 1993a) and 5 days at 20-22°C (Rowland, 1995a). In artificial incubators at a temperature range of 18.4-24.4°C the eggs hatch in 6-11 days, taking 4 days to complete (Rowland, 1995a) with 50-60% of eggs hatching in the third day (Cadwallader & Gooley, 1985).

Newly hatched larvae are about 5-9 mm long, with a relatively large yolk sac (Lake, 1978) as shown in Fig. 1.2. Their colour can be amber to pale orange and they are mainly translucent. The larvae apparently gather in clumps, and then scatter over the substrate. With yolk sacs absorbed within 8-16 days they start feeding on zooplankton (Cadwallader & Gooley, 1985). On reaching 15-20 mm, they are able to feed on aquatic insects (Kailola et al., 1993). However, little is known about this aspect of

their life cycle under natural conditions (Codwatch, 1995a). The larvae drift downstream from their hatching sites into new habitats (Codwatch, 1998). Larvae survive well when natural food is abundant during floods (Lake, 1978). There is limited documented information on Murray cod growth rates in the early developmental stages in natural conditions. Cadwallader and Gooley (1985) found that fry-cod reared in aquaria fed on brine shrimp grew 27-33 mm, 0.2-0.4 g in 115 days.

Diet

Murray cod are carnivorous at the trophic level of 3.8, having a diet mainly of food organisms at a trophic level of 2.8 and up (Fishbase, 2000). They consume food in large amounts at about 3.7 times body weight each year (Fishbase, 2000). Murray cod feed on invertebrates, fishes, amphibians and occasionally reptiles, birds and aquatic mammals (Lake, 1978). They may also occasionally eat their own kind up to half their length (Dakin & Kesteven, 1939). The fry fish feed on zooplankton (Llewellyn & MacDonald, 1980). Fry reared in earthen ponds feed on various zooplankton suitable to their sizes (O'Sullivan & Walker, 1991; Rowland, 1992). Fry feed on copepods and cladocerans, and shift to chironomid larvae and aquatic insects at 15-20 mm in length (Harris & Rowland, 1996).

Habitat and environmental conditions

Murray cod prefer slow, sluggish, turbid water (Llewellyn & MacDonald, 1980), but they live in a wide range of habitats from clear rocky streams to slow flowing, turbid rivers and billabongs (Fishbase, 2000). Murray cod are demersal fish, active at night especially in the summer months, seeking shelter under snags and logs during daytime (Roughley, 1966). They are territorial fish (Rohan, 1989) living individually in sheltered areas with cover from rocks, timber or overhanging banks, slothful in cold water (Dakin & Kesteven, 1939; Llewellyn & MacDonald, 1980; Roughley, 1966), in various depths up to 5 m. Snags are the primary habitat determinant of Murray cod as they support shelters and spawning sites, and provide attaching substrates for food organisms (Codwatch, 1995b). Eighty three percent of Murray cod are found within 1 m of snags and 97% have some form of wood debris within 6 m (Codwatch, 1995b).

Submerged bank vegetation such as river bottlebrushes have also been widely utilized as shelter during high flows. Murray cod do not prefer the deepest areas of a river (Codwatch, 1993b). During high flow they generally move closer to the bank and will inhabit anabranches and smaller channels as soon as they contain flows, but they do not move onto the flood plain (Codwatch, 1993b). Murray cod sometimes make localized movements over a distance up to 200 m between two favorite sites (Codwatch, 1998).

No published work has recorded the presence of larvae in temporary billabongs or floodplains (Humphries et al., 1999). Adult Murray cod have been reportedly caught in billabongs (Cadwallader & Lawrence, 1990, cited in Humphries et al., 1999), but no juveniles have been recorded in such a habitat (Humphries et al., 1999). It is likely that Murray cod using temporary floodplain habitats move between those habitats and the main channels, but move out before the connections are severed (Humphries et al., 1999).

Murray cod are potamodromous fish and migrate wholly within freshwater (Mallen-Cooper, 1989). Some earlier studies have suggested that Murray cod do not migrate but make only random forays (Reynolds, 1983), usually moving less than 10 km along the river (Berra, 1998). Radio-tracking studies have suggested that the movements of Murray cod consist of an upstream migration during high flows followed by a return journey after spawning has been completed (Koehn, 1995). The major migration commences in early spring, appearing to be stimulated by elevated water levels. Many fish utilize smaller creeks and flooded anabranches (Koehn, 1995). In summer, following spawning, fish move rapidly downstream and return to their previous locations, with many resuming residences in exactly the same sites (Codwatch, 1993e). The longest recorded return trip is 130 km (Codwatch, 1993e). Juvenile cod may also undertake migration (Kailola et al., 1993).

Murray cod are commonly found in turbid water (turbidity 5.8-300 NTU), with temperature ranging from lower than 6°C in winter to above 29°C in summer, pH 7.6-9.6 and dissolved oxygen at 7.2-12.0 mg/L (Cadwallader & Gooley, 1985).

There are varying opinions on salinity tolerances of Murray cod. Though Murray cod are an entirely freshwater species that do not tolerate high salinity levels (NSWF, 2000), it has been claimed that Murray cod tolerate a relatively high salinity, which may relate to marine ancestors having been exposed to episodic high environmental salinity in their habitats (Jackson & Pierce, 1992). Murray cod have been reported to grow in a saline farm pond in the Wimmera, Victoria where the salinities varied from 1,080 EC in spring to 9,900 EC in autumn. There, fingerlings at 26 mm in length were stocked, and they grew to 90 mm within 485 days (Cadwallader & Gooley, 1985). The growth rate of the fish is apparently low compared with the common growth of Murray cod in the first year at 203-254 mm (Roughley, 1966).

Murray cod may tolerate parameters outside the preferable range for a short period of time (NSWF, 2000). However, prolonged exposure to unsuitable or undesirable conditions will stress the fish and leave them open to attack from a number of parasites, fungal and bacterial infections, and viruses (NSWF, 2000). This may cause mass mortalities both in the wild and in culture conditions (Rowland & Ingram, 1991).

It has been claimed that the natural habitats of Murray cod are degrading. According to the NSW Rivers Survey, Murray cod have been found in rivers ranging from fair to poor quality, namely Horton, Naomi, Gwydir, Mehi, Little, Darling, and Macquarie rivers respectively (Harris & Silveira, 1997; Schiller et al., 1997). The survey by Harris and Gehrke (1997) has found that 27.7% (13 out of 47 individual fish) of Murray cod caught in the Darling River and its tributaries present visible abnormalities. The percentages of abnormality are high in Murray cod compared to other native fish caught in the same areas.

Predators and pests

Murray cod are predatory fish. However, other animals, such as aquatic birds (cormorants, *Phalacrocorax* sp.) and large fish, namely golden perch (*Macquaria ambigua*) and English perch (or redfin perch *Perca fluviatilis*) can prey on juvenile Murray cod (Cadwallader & Gooley, 1985; Kailola et al., 1993). Man is probably the only predator of large cod (Kailola et al., 1993).

An epizootic ectoparasitic protozoan, *Chilodenella* sp., is the most vital parasite of Murray cod. It has been reported causing mass mortality of wild Murray cod as well as aquaculture brood fish (Langdon, 1989; Rowland & Ingram, 1991). Other ectoparasites such as *Icthyophthirius* sp. and *Lernacea cyprinacea* are also commonly found infecting fish when they are in stressed conditions due to crowding, low temperature and poor oxygen supply (Langdon, 1989; Rowland & Ingram, 1991). *Trichodina* spp. is also a common parasite found in Murray cod (Rowland & Ingram, 1991).

Stock structure

Murray cod presumably colonized Australian freshwater from a marine origin (MacDonald, 1976). It has been suspected that the Murray cod inhabiting the Murray-Darling basin are a different species from those occurring in the two coastal rivers, the Clarence and Mary Rivers of eastern Australia. Rowland (1993 cited by Musyl & Keenan, 1997) has revealed that cod found in the Clarence River are actually the eastern freshwater cod (*M. ikei*), and the cod of the Mary River are the Mary River cod (*M. mariensis*, or *M. peelii mariensis,* Rowland 1993 cited by Fishbase, 2000). The relationships between Murray cod, eastern freshwater cod and Mary River cod remain unclear (Kailola et al., 1993). Trout cod (*M. macquariensis*) is probably a relative of Murray cod. Despite differences in the genetic electrophoresis (MacDonald, 1976), a natural hybridisation between the two species has been found in the Murray River (Douglas et al., 1995). Fertile hybrids are also commonly found in the Cataract Dam near Sydney (Kailola et al., 1993).

Distribution

The Murray cod is native to Australia (Lake, 1978). It formerly spread widely and was abundant throughout the Murray-Darling River system of South Australia, Victoria, New South Wales, and Queensland except for the upper reaches of the Southern tributaries (Kailola et al., 1993). However, currently they are found drastically reduced in abundance over all of their natural range (Pierce, 1989). The geographical distribution of Murray cod is shown in Fig. 1.3

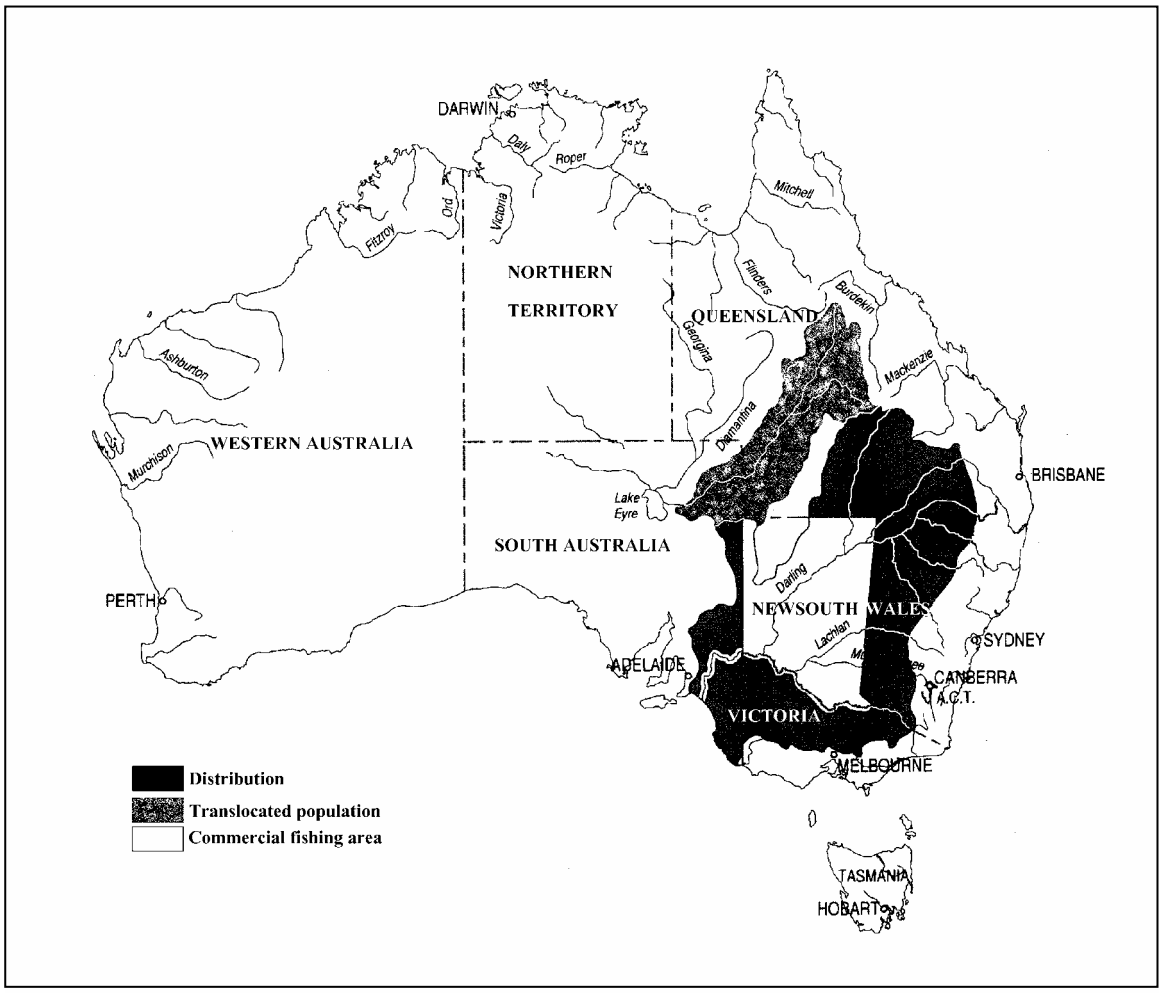

Figure 1.3 Geographic distribution and commercial fishing area for Murray cod (modified from Kailola et al., 1993).

In the last century Murray cod were widely translocated to waters within their natural range and introduced to waters outside their range (Kailola et al., 1993). In 1990, Murray cod were introduced to the Cooper Creek system in central Australia (Kailola et al., 1993). Few of the earlier stocked populations have survived, but more recently stocked populations were found to be still present in many farm dams and water supply reservoirs (Berra, 1998).

In New South Wales, they have been recorded throughout the Murray-Darling river system and its tributaries (Roughley, 1966) except at the highest altitudes (Lake, 1978), and they are also found in the headwaters of the Richmond and Clarence rivers (Roughley, 1966). However, the NSW Rivers Survey, of fish, caught relatively few

Murray cod (Harris & Gehrke, 1997). Murray cod found in the Hawkesbury River system are introduced stock (Lake, 1978).

In Victoria, Murray cod occur naturally north of the Great Dividing Range in tributaries of the River Murray (Roughley, 1966). They have been introduced into the Yarra River and into several waters in the Wimmera catchment (Cadwallader & Backhouse, 1983). Presently there are very few localities in Victoria where Murray cod can be considered common (Cadwallader & Backhouse, 1983).

In Queensland, Murray cod inhabit the Condamine River in great numbers, and are also found in the Dawson and Mary rivers (Roughley, 1966). The fish found in Western Australia are a successfully acclimated stock translocated from Adelaide in 1893 (Roughley, 1966). There is no evidence of cod found in or introduced to the Northern Territory.

Resource status

Murray cod are more highly esteemed for their edible qualities than any other Australian freshwater fish (Roughley, 1966). Murray cod are also popular among anglers as a recreational fish (Gooley et al., 1995). Thus, they are keenly sought as a food fish and sport fish and have considerable value for exploitation by commercial and recreational fishers (Cadwallader & Gooley, 1985).

It has been claimed that the natural abundance of Murray cod no longer supports the commercial fishery that it once did (Berra, 1998). Since 1937, the depletion in their stocks has been noted (Cadwallader & Gooley, 1985). It has been claimed that the abundance and distribution of Murray cod in the Murray-Darling river have been permanently reduced (Cadwallader & Gooley, 1985), and it is now considered rare in many Victorian tributaries (Fishbase, 2000).

In Victoria, Murray cod are considered to be potentially threatened and it has been listed for protection under the Flora and Fauna Guarantee Act (NSWF, 2000). In South Australia, populations of Murray cod have suffered from recruitment failure

since the 1980s (Rohan, 1989). Victoria, New South Wales and Queensland all have minimum legal size of 500 mm in length (Codwatch, 1993c).

Pierce (1989) has suggested various causes of the decline in Murray cod, including modification of the flood regime, introduction of foreign species and pathogens, fish passage restrictions, over-fishing, pollution and change in water quality and habitat alteration. While much of the decline in Murray cod numbers up until the 1930s can be attributed to over-fishing, environmental changes such as those caused by removal of snags or by construction of dams, levee banks, and high level weirs throughout the Murray-Darling River system have undoubtedly had a major effect since the 1950s (Kailola et al., 1993). From 1976 to 1987, in excess of 24,500 snags were removed from some sections of the Murray River (Hinson, 1999). Removed of snags ravages Murray cod shelters and spawning sites (Lake, 1978). Constructions have effects in altering natural flows and temperature regimes of rivers and reducing the incidence of seasonal flooding (Codwatch,1993d; Pierce, 1989), hence preventing spawning and recruitment of young fish. Natural flows and temperature regimes are essential to stimulate spawning (Codwatch, 1993d,e), while flooding promotes the production of food organisms for larvae (Kailola et al., 1993).

Alteration of the environmental conditions has reduced larval recruitment and, in consequence, reduction in Murray cod numbers. It has been stated that the estimated yield recruitment of Murray cod is only 0.0382 per year (Fishbase, 2000), with a generation time of 3.9 years (Fishbase, 2000). If this decline in biomass or numbers exceeds the threshold of 0.95 over 10 years or 3 generations, Murray cod will be considered to be vulnerable to extinction (Fishbase, 2000).
While poor juvenile recruitment is probably the main reason for the continued decline in Murray cod populations within the river system (Kailola et al., 1993), another possible factor contributing to the decline of Murray cod in the Murray-Darling River system is introduced fish, including English perch (*Perca fluviatilis*) (Kailola et al., 1993) and European carp (*Cyprinus carpio*). English perch spawn earlier in water temperatures of around 12°C, hence there is competition for resources, as the diets of Murray cod and English perch overlap extensively, and it is possible that juvenile English perch prey on cod larvae (Kailola et al., 1993). While carp are spreading

throughout Australian waters, still there is no scientific evidence that the increase of carp has affected Murray cod numbers. Although carp may compete with cod for space and food, young carp may provide a source of food for native species (Codwatch, 1993d).

Recently, development of reliable culture techniques has resulted in relatively large-scale production of hatchery-bred juveniles for release into public and private waters in Australia for conservation and recreational purpose (Rowland, 1995b).

CHAPTER TWO

Principal methodology

2.1 Conceptual framework

Studies concerned with effects of stressors on fish in the wild must consider and deal with a variety of interacting environmental factors. Fish in nature may be exposed to sublethal levels of contaminants and to unfavourable or fluctuating temperatures, water velocities, sediments loads, dissolved oxygen concentrations, food availability, and other variables. These factors, individually or together, can impose considerable stress on physiological systems in fish.

Adams (1990) states that stress exceeding the tolerance of organisms is obvious because it is normally lethal, while sublethal stress is not readily observed. Lethal or acute stress usually occurs quickly in response to short-term perturbations. Acute stress responses may result from either single or several rapid exposures to a stressor. These responses usually occur immediately, but some may be delayed for short periods after exposure to a stressor. Sublethal or chronic stress is more common, but subtle because adverse effects are generally manifested first at the suborganism level. Chronic stress results from either continuous or periodic exposure to low levels of stressors over time. Chronic stressors usually produce long-term effects and typically involve the entire reproductive life cycle of an organism, whereas acute stressors affect only a short part of the life cycle. Depending on its severity, sublethal stress may load or limit physiological systems, reduce growth, impair reproduction, predispose organisms to disease, and reduce the capacity of fish to tolerate subsequent stress.

In the natural environment, responses to the increase of salinity should contribute to both direct and indirect effects as shown in Fig. 2.1. In such conditions Lloyd (1992) suggests that the measurement of the effects of salinity on fish can be made at different levels of organisation from cellular functions within the body up to the community level composed of populations of different fish species.

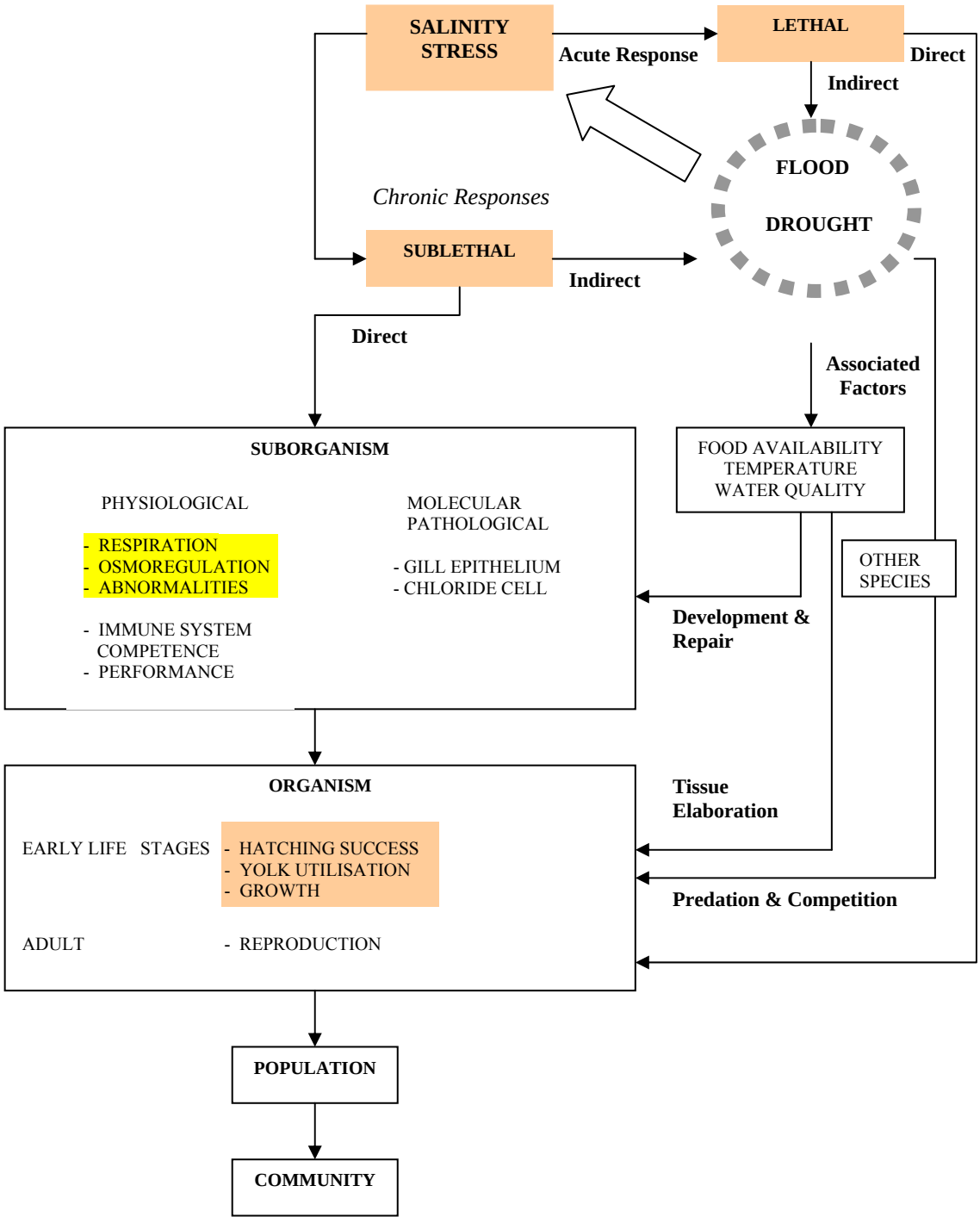

Figure 2.1 Direct and indirect pathways affecting responses to increased salinity in Murray cod habitat, adopting Adams's (1990) model. Effects of salinity on fish can be measured at different levels – suborganism, organism, population, and community. The selected areas – highlighted with dark colour – are included in this study.

This study has applied those concepts to the laboratory experiment. The environmental salinity is artificially made up in the laboratory using crystals of a commercial sea salt. The investigation of tolerances is measured both at lethal and sublethal levels, from the stage of the newly extruded egg to the juvenile stage. The measurements of respiration (of oxygen consumption rate), osmoregulation (of blood osmolality and dehydration), and physical abnormalities define investigation of body functions, whereas measurements of hatch, food uptake (of yolk utility and ingestion), and growth describe investigation of whole body responses.

2.2 Materials

2.2.1 Test media

The test media were prepared using a commercial sea salt (Pacific Sea Salt, PSS) and the freshwater used at the experiment sites as diluent. Salt was added to the diluent to make up desired salinities. In order to obtain the precise content of salt, the concentration of the solution was measured as conductivity equivalent to salinity level using the relationship between salinity and conductivity as

$$1\ TDS\ (\text{mg/L}) = 0.58 \times EC\ (\mu\text{S/cm}) \quad (n = 16,\ R^2 = 0.9951)\ . \tag{2.1}$$

The equation was drawn from the actual measurements of conductivity and salt contained in the standard solution made from PSS and distilled water. Conductivity was measured using an Activon conductivity meter (Model 301). The conductivity sensor was calibrated with KCl standard solutions.

Initially, salt compositions of PSS were analysed from the media prepared using PSS solid salt and deionised as a diluent. The salt trace detected in the solvent used was then subtracted from the salt figures of the media to give true result of the PSS composition. It has been found that sodium contained in PSS was compatible to the constituent of ocean water reported by Munro and Roberts (1989), while potassium, calcium and strontium levels were much lower (Table 2.1). Magnesium was not present in PSS. However, magnesium is not involved in osmoregulation processes in fish. The study of William and Stickney (1989) also reported the insignificance of

magnesium in red drum (*Sciaenops ocellatus*) when reared in freshwater and saltwater.

Table 2.1 The constituents in solution in ocean water compared to the Pacific Sea Salt (PSS) used in this study.

Constituent (g)	**Ocean water** (salinity 35‰) (Munro & Roberts, 1989)	**PSS** (35 g/1,000 mL solution
Sodium (Na)	11.1	11.56
Magnesium (Mg)	1.33	Not detected
Calcium (Ca)	0.42	0.23
Potassium (K)	0.39	0.13
Strontium (Sr)	0.01	< 0.01

2.2.2 Experimental organisms

Eggs and larvae

Murray cod (*Maccullochella peelii peelii*) and trout cod (*M. macquariensis*) eggs were used. The experiments were carried out at the Snobs Creek fish hatchery, Marine and Freshwater Resources Institute, Victoria. Eggs and larvae of Murray cod were obtained from a female fish spawned naturally in the earthen pond of the institute. Eggs and larvae of trout cod were obtained from an artificial spawning from a male and a female using Chorionic Gonadatrophin hormone. Only the eggs of a single female and a single male were used in this study. These made for homogeneity of the genetic characteristics among the experimental organisms.

Fry fish

Only Murray cod were utilized in this part of the study. The fry – 0.021 g, and about 2 weeks old – were obtained from the Snobs Creek fish hatchery. Fry were obtained from a male and female fish spawned naturally in the brood pond of the institute. The fish were transferred in plastic bags to the experimental site at Freshwater Ecology Laboratory, Arthur Rylah Research Institute, Department of Natural Resources and Environment in Victoria. The fish were acclimated to the laboratory conditions, and experimental trials were commenced with fish of 0.086 g.

Fingerling fish

Only Murray cod were utilised in this part of the study also. Fingerlings – 1.04 g – were purchased from a warm water fish farm in New South Wales (Uarah Native Fish Farm). The fish had been reared in the earthen pond at the farm a few weeks before purchasing, however the history of the brood fish was not known. The fish were transferred in plastic bags to the experimental site at the Arthur Rylah Research Institute. The fish were acclimated to the laboratory conditions, and experimental trials were commenced with fish of 2.0 g.

2.2.3 Fish stocking facilities

There were two types of fish stocking facilities used in this study. The experimental fish were reared in either 20-L self contained-biofilter glass aquaria or 5-L glass aquaria without a biological filter. When fish were reared in the aquaria without biofilter, water was changed regularly. In contrast, water in the aquaria containing a biofilter was recycled with limited change if necessary.

The biofilter aquaria consisted of a 16-L animal chamber and a 4-L biofilter chamber separated by an acrylic sheet. The biofilter consisted of 2 L of foam beads (the refill used for bean-bags) covered on the top with a square metre of fine-mesh polyethylene net. The net was folded to fit the chamber surface. A piece of clay-brick was used to sink the biofilter materials. A mat of aquarium fibre, lying underneath the brick and used as a mechanical filter, was cleaned regularly to prevent clogging of the biofilter. Water in the aquaria was circulated between the biofilter chamber and the animal chamber through a 10 mm diameter airlift tube and a slit beneath the partition. The aquaria were filled with test water at the desired salinities. The nitrifying bacteria in the biological filters were established using inoculations of bacterial culture and solutions of NH_4Cl and $NaNO_2$ following the method used by Forteath (1990). Fish were transferred into the aquaria when the biological filters were properly established. However, to maintain the desired water quality the water in the aquaria was partially changed daily. Water chemistry parameters were recorded once a week.

2.3 Methods

2.3.1 Measurement of salinity sensitivity

Sprague (1990) suggests that most of the work in aquatic toxicology requires conventional statistical analysis such as the Probit method and the usual regression technique. Within this study the measurement of salinity sensitivity is divided into two response levels – lethal and sublethal. The lethal level is calculated using the Probit procedure, and the sublethal level is calculated using regression analysis.

The measurements include,

- hatching viability of egg exposed to salinity on the onset of fertilisation
- hatching viability of egg exposed to salinity after the completion of fertilisation
- yolk utilisation of larvae while developing to fry
- dehydration of larvae
- interaction of salinity and pH on mortality of fry
- interaction of salinity and temperature on the growth and mortality of fry
- interaction of feeding regimes on growth and mortality of fry
- growth and mortality of fingerlings in different modes of transfer
- increased blood osmotic concentration in fingerlings
- dehydration of fingerlings
- oxygen consumption of fingerlings
- pathological responses.

2.3.1.1 Lethal salinity

Lethal level is estimated in terms of median lethal concentration (LC50) using the Probit method. This method was used to calculate the likelihood response data obtained from biological assays. The probability (p) of a positive response is modelled, using SAS Software Release 8.2 (SAS Institute Inc., USA 1999-2001), as

$$P = F\,[b_0 + b_1 \times \log_{10}(Dose)]\,, \qquad (2.2)$$

where F is the normal cumulative distribution function, $b_0 = -\,\mu/\sigma$ is the intercept parameter, $b_1 = 1/\sigma$ is the regression parameter, and *Dose* is the concentration level of salinity. The estimated values calculated by this method included threshold salinity of egg, threshold salinity of larva, LC50 of fry, and LC50 of fingerling.

2.3.1.2 Optimal, threshold and upper sublethal salinities

Threshold salinity in this study determined the minimum concentration of salt that caused a significant growth response. There were two measurements of the threshold salinity: one measured the test organism during development in a short period of time, i.e. the embryo and yolk larva, the other measured growth of the test organism, i.e. the fry and fingerling.

a) Threshold salinity of larvae

Investigation of salinity responses in the larvae was carried out in a short period of time (12 days). The salinity sensitivity of yolk larvae was measured from the number surviving until completion of yolk adsorption. The threshold salinity was then estimated from the survival rate using the Probit method. The threshold salinity was determined by the lowest effect level (NEL), which is the estimate of 1% mortality (99% survival), while LC50 is designated at 50% mortality as shown in Fig. 2.2.

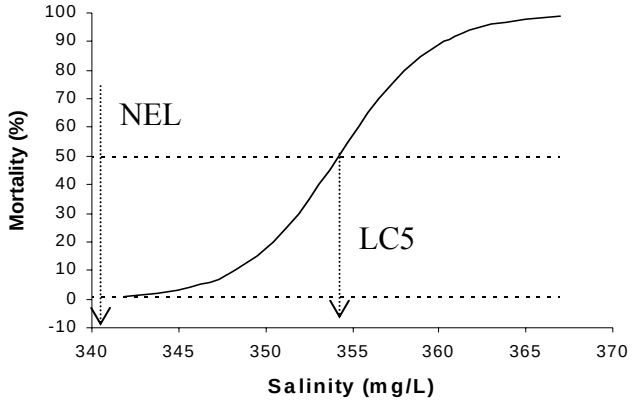

Figure 2.2 Estimates of LC50 and NEL (threshold salinity) of Murray cod larvae from the data in Table 3.8 using the Probit procedure in SAS software.

b) Threshold salinity of fry and fingerlings

Like most freshwater fish, Murray cod was found to tolerate some degree of salinity. Generally the hypertonic regulator species that require energy to extract salt from the immersing solution grow better in a certain concentration of saline medium than in freshwater medium (see also growth of freshwater species in Chapter 1). Therefore, when fish are placed in saline media, growth would initially increase following the increasing salinity to a certain level indicating optimal salinity. The growth rate would then gradually decrease from the optimal level and drop below the rate found in freshwater. In this study, as shown in Fig.2.3, the concentration at which the growth rate starts falling below the growth rate found in freshwater is referred to as the "threshold salinity (THS)". The threshold salinity is estimated from the freshwater growth rate using the regression relationship between salinity and growth rate from the function proposed in this study.

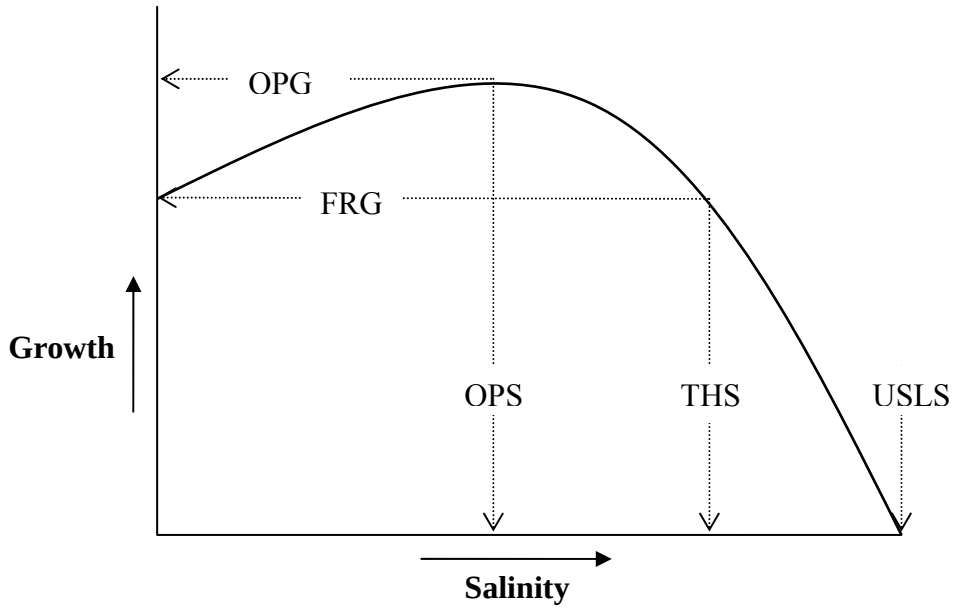

Figure 2.3 The proposed salinity-growth curve of freshwater fish shows the threshold salinity (THS) and optimum salinity (OPS). THS is calculated from freshwater growth rate (FRG) while the OPS is calculated from optimum growth rate (OPG) using regression equation of the relationship between salinity and growth rate. The upper sublethal salinity (USLS) determines the concentration in which fish stop growing and eventually die after long exposure.

Calculation of growth rates

Growth rates of fish were determined in terms of the relative weight gain (RWG:%/day) following the method used by Guo et al. (1995),

$$RWG = (W_t - W_0) \times 100/W_0/t \,, \qquad (2.3)$$

and the specific growth rate (SGR:%/day) was calculated using equations following the method used by Weatherley (1972),

$$SGR = (\ln W_t - \ln W_0) \times 100/t \,, \qquad (2.4)$$

where W_0 is the average initial weight (g/fish) and W_t is the final average weight (g/fish) in a period of time t.

The total body length (from the tip of snout to the caudal fin tip) of individual fish was measured using a measuring scale or a vernier caliper. The length-weight relations were calculated using the exponential fitting equation

$$W = a\, e^{bL} \,, \qquad (2.5)$$

where W is the wet weight, L is the total length, a and b are constants.

Calculation of optimal and threshold salinities

The relation between growth and salinity is drawn from the salinity-growth curve in Fig. 2.3 by an equation,

$$G = aS^3 + bS^2 + cS + d \,, \qquad (2.6)$$

where G is growth rate, S is salinity (g/L) and a, b, c and d are constants.

The freshwater growth rate (FRG) is estimated by setting $S = 0$ in equation (2.6). Thus, $G = d$ when G is the freshwater growth rate.

Threshold salinity (THS) is estimated at a salinity level where the growth rate is equivalent to freshwater growth. Calculation of THS is done repeatedly by writing equation (2.6) into the Excel sheet. The salinity values that give the specific growth rate equivalent to *d* are used as threshold salinity.

Estimates of the optimal salinity (OPS) is also calculated repeatedly using the equation in the Excel sheet. The salinity value that gives the highest specific growth rate is then picked up and used as optimal salinity.

2.3.2 Sublethal effects

2.3.2.1 Yolk utilisation efficiency

The yolk utilisation efficiency (YE) determines the effectiveness of converting food to body tissue during the endogenous stage of larvae. The yolk utilisation efficiency (YE) was calculated applying the equation from Lasker (1962),

$$YE = \textit{Dry weight of yolk-adsorbed larva} \times 100 / \textit{Dry weight of egg cytoplasm} . \quad (2.8)$$

2.3.2.2 Blood osmotic concentration

The osmolality of solutions is the measurement of the freezing point (Δ) of water which contains dissolved substances and is lower than the freezing point of pure water, i.e. the freezing point is below 0ºC. The lowering of the freezing point is proportional to the amount of dissolved substances. A one-gram molecule of non-electrolytes (glucose or sucrose) dissolved in water reduces the freezing point of the solution by 1.86ºC (Veselov, 1964). Thus the osmolality of the solution is translated from the freezing point by being multiplied by 537, i.e. 1000/1.86 (Ginetsinskii et al., 1964). The osmolality per kilogram of water of the solution is usually expressed in terms of the milliosmole, which is the unit concentration of osmotically active substances equivalent to 0.001 M solution of non-electrolyte (Ginetsinskii et al., 1964).

The relationship between osmolality and sodium chloride concentration of a standard solution measured by the instrument (Osmomate 030) used in this study is expressed as

$$S = 31.954\ Os + 18.761\ , \qquad (2.9)$$

where S is salinity in mg/L and Os is osmolality in mOsmol/kgH_2O.

As it was possible to take only a small volume of blood from test fish in this study, samples of whole blood were used instead of blood plasma as suggested in most of the literature. However, the osmolality of blood plasma and the sample of whole blood, 281±10 and 285±16 mOsmol/kgH_2O (mean ± SD, n=10) respectively, were also tested and it was found that there were insignificant differences between the osmolalities of plasma and whole blood.

2.3.2.3 Dehydration

Dehydration determines loss of the body water content due to osmoregulation and diffusion. To measure the dry weight of samples, Busacker *et al*. (1990) suggests that a temperature of 60ºC for 24 h is usually adequate for samples of 0.5 g or less, but larger quantities may require higher temperatures or longer time periods. Bound water can be eliminated at 100-110ºC, but volatile oils and lipids may also be lost. The dry weight is acceptable if changes in weight between successive weighings are less than 0.1% (Busacker et al., 1990). In this study, the samples of eggs, larvae and fry fish were dried at 60ºC, and the samples of fingerling fish were dried at 105ºC following the method used by Guo et al. (1995).

The dehydration rate was calculated using the equation,

$$Dehydration = (F_w - S_w)/F_w \times 100\ , \qquad (2.10)$$

where F_w is the percentage body water content in freshwater and S_w is the percentage body water content at the measured salinity. The body water content is the result of subtracting the dry weight from the wet weight.

2.3.2.4 Oxygen consumption

Respirometer system

The flow-through respirometer system (Fig. 2.4) consisted of a 2-mm inner diameter silicone tube that fed water from a water source held in a 50-L glass aquarium. A peristaltic pump (Minipuls 2, Gilson) was set at its highest speed to deliver a constant flow to a respiratory chamber. The chamber was made of transparent acrylic sheets (two sizes of chambers were used, 664 mL and 2 570 mL) and the lid placed on the top was held to the chamber with a rubber seal and stainless steel screws.

The water was fed to the chamber through an opening on the lower edge of one side, and passed through a socket fitted with a membrane oxygen probe (YSI). The external outflow went from the chamber to an opening on the upper edge of the opposite side and passed through to another socket that was fitted with a membrane oxygen probe.

The respirometer system also comprised a flow-through acclimation line that consisted of a 3-mm inner diameter aquarium tube. The test water flowed into a respiratory chamber via a submerged aquarium pump and then passed over through an opening in the middle of the chamber lid.

Collection of oxygen consumption data

A digital oxygen meter (Oximeter, WTW Oxi 330) was used to measure the concentration of oxygen in water when necessary. The test water with different salinities was aerated to 90% saturation before it was used. 90% saturation water was also calibrated to its salinity using the procedure indicated on the meter used. The measurement of oxygen consumption rates by the fish were via a meter and signal converter (Tain Electronics, ED 500) that was connected to the oxygen probes and later recorded in a specific program by Tain Electronics on the computer. The

dissolved oxygen concentrations were recorded as percentage saturation and later converted manually to mg/L concentration by using the equation

$$C_{O2} = (14.3289 - 0.3253\ T + 0.0032\ T^2)\ S_{O2}/100\ , \qquad (2.11)$$

where C_{O2} is the dissolved oxygen concentration in mg/L and S_{O2} is the dissolved oxygen concentration expressed as percentage saturation, and T is the water temperature (ºC) in this study (Bayly & Williams, 1973). There was no correction for salinity when calculating the oxygen concentration at saturation.

Calibration of respiratory chamber

The oxygen consumption rates of the fish within the respirometer system were also measured at the onset of the experiments when the respiratory chamber was empty to ensure that the depletion of oxygen was due to organism respiration and the chemical oxidation of organic and inorganic matters. This baseline oxygen consumption was excluded when the oxygen consumption rates of fish were manually calculated.

Calculation of oxygen consumption rate

The oxygen consumption was calculated by using the equation

$$V_{O2} = (v \times dC_{O2} / dt) + (F \times C_{O2}\,in)\ , \qquad (2.12)$$

where V_O (mg/h) is the dissolved oxygen consumption, v (L) is the volume of the water in the chamber, dC_{O2} (mg/L) is the increment of dissolved oxygen concentration measured at the outlet in the period of time t, $C_{O2}\,in$ (mg/L) is the dissolved oxygen concentration of water at the inlet, dt (h) is the period of measurement, and F is the flow rate (L/h).

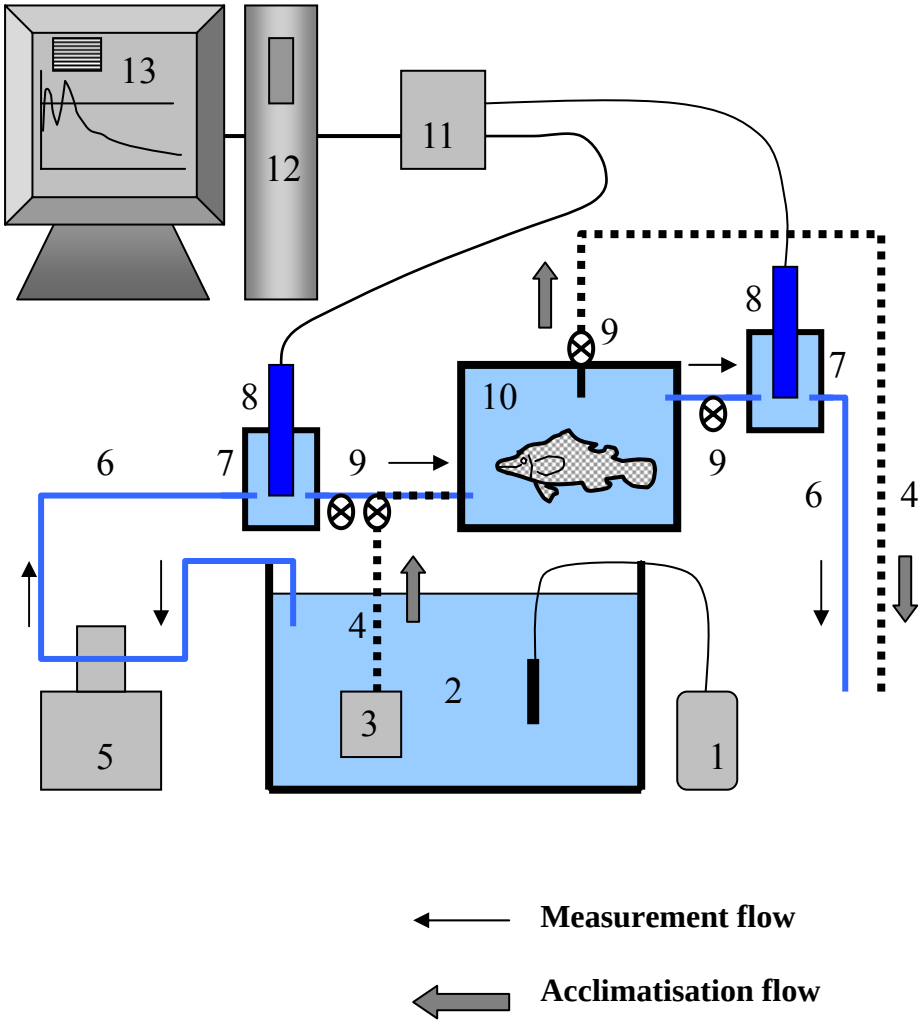

Figure 2.4 Respirometer system consisted of (1) digital oxygen meter (2) water source, (3) submerged aquarium pump, (4) plastic tubes (5) peristaltic pump, (6) silicone tubes, (7) oxygen probe sockets, (8) oxygen probes (9) valve, (10) animal chamber, (11) oxygen meter and signal converter (12) data recorder in the computer CPU, and (13) computer monitor.

2.3.2.5 Pathological responses

The pathological responses determine the apparent impacts of sublethal salinity that can be observed by the naked eye. The characteristics and numbers of the responses were observed, recorded and described.

2.3.3 Statistical method

Two-factor factorial experiments were designed to measure the interactions between two factors such as salinity and growth rate, etc. The general linear model (GLM) was used in all cases. The means of the treatments in one factor experiments were compared by a one-way analysis of variance (ANOVA) and the paired comparisons were made using Least Significant Difference (LSD). The small or large percentage values (0-30% or 70-100% that form binomial distribution) were arcsine-transformed before comparison (Zar, 1984). All tests were conducted with SAS Software Release 8.2 (SAS Institute Inc., USA 1999-2001).

It should be noted that to compare a control mean with the mean of each other group requires the use of Dunnett's test as recommended by Zar (1984) or the use of standard multiple-range tests as suggested by Sprague (1990). However, LSD is applied in this study in order to detect any minor differences between group means with particular interest, regardless of the significance of F values – even the null hypothesis is accepted.

2.3.4 Monitoring of water physico-chemistry

Stickney and Kohler (1990) suggest that it is very important when maintaining fish for research to minimise the stress placed on animals. Stressed fish may behave abnormally and are much more susceptible to diseases and parasites than fish that are held under optimal conditions. Any behavioural, disease-related, or physiological manifestation of stress can render a group of fish useless for controlled experiments. Maintenance of fish under optimal conditions helps to ensure that the responses seen during the experiment reflect the treatments and are not artefacts of holding stress. Some of the principal causes of stress in fish are handling, mechanical damage,

dietary imbalances, diseases, parasitism, extremes or abnormal changes in light and temperature, and poor water quality.

The use of the media was limited to reduce salt nutrient discharge into the local wastewater system. Therefore, in some cases the experimental fish were kept under a recirculation system in which water quality rapidly deteriorated due to unforseen circumstances involving the holding tank water. The water quality chemistry in the experimental tanks was of a lower quality than that of the natural habitat. Despite this, the fish still showed a good appetite and had an exceptional growth rate pattern. However, it should be noted that adverse water quality might increase the lethal degree of the test salinity levels, which might in turn affect the measurement of salinity sensitivity.

Measurements of water quality chemistry in the experimental vessels are shown in relevant chapters. Results showed that the ammonium and nitrite concentrations were greater than those levels recommended for pond aquaculture (Boyd, 1990) and for recirculating systems (Forteath, 1990) (Table 2.2). It is known that ammonia and nitrite can alter the permeability of the gill membrane. However, the toxicity of both ammonia and nitrite can be reduced in a highly saline environment (Forteath, 1990) and these effects may affect the salinity tolerance in fish when tested at different salinity levels.

The total hardness test of the tap water used was significantly low. Thus, $CaCO_3$ and $NaHCO_3$ were added to the test media in order to maintain suitable conditions for the activity of the nitrifying bacteria in the biofilter system. Adding calcium salt to the culture media also benefits the fish's osmoregulation capability. Hunn (1985) stated that the effect of calcium on the permeability of gills is probably involved with the relationship between calcium and NaCl. Grizzle et al. (1992) claimed that newly transformed juveniles of striped bass (*Morone saxatilis*) were more sensitive to calcium concentrations than the larvae and older juveniles, but the reasons were unknown. Boyd (1990) has also stated that calcium is necessary for proper osmoregulation, and calcium concentrations tend to increase with increased salinity. Therefore, any variations of total hardness would also affect the salinity tolerance in

fish. However, only the supernatant of the solutions was used. Therefore only CO_3^{2+} and HCO_3^- were added to the media

2.3.5 Animal ethics

Studies were carried out at the Arthur Rylah Institute for Environmental Research, Department of Natural Resources and Environment, and the Snobs Creek fish hatchery, Marine and Freshwater Resources Institute, Victoria under the approval of the NRE Flora and Fauna Animal Ethics Committee, approval number AEC00/009 "Aspects of Sub-lethal Salinity on the Early Life Stage of an Australian Native Fish, Murray Cod", "Flora and Fauna Permit Number 10001211".

Table 2.2 Recommended water quality for aquatic organisms in recirculating systems (Forteath, 1990).

Parameter	**Concentration**
Ammonia (mg NH_3-N/L)	0.082
Total ammonia (mg/L)	0.5
Nitrite (mg NO_2-N/L)	0.5 in freshwater, 20-25 in seawater
Nitrate (mg NO_3-N/L)	< 100
pH	6.5-8.5 in freshwater, 7.8-8.2 in seawater
Alkalinity (mg/L $CaCO_3$)	20-200 (prefer above 100)
Hardness (mg/L $CaCO_3$)	> 50
Carbon dioxide (mg/L)	< 5
Chlorine (mg/L)	< 0.005 at pH 7, 25ºC

CHAPTER THREE

Salinity sensitivity of eggs and larvae of Murray cod and trout cod

3.1 Introduction

The small amount of information available on sensitivity of critical life stages of fishes to salinity indicates that larval stages may be more sensitive than adults stages. Fish eggs appear to be relatively tolerant of salinity increases (Hart et al., 1991).

Extruded eggs in the broad sense are the very first living form of fish to come in contact with the external environment. However, eggs have yet to be activated by water or insemination of male gametes to proceed to further development in the course of ontogeny (Alderdice, 1988). Therefore, any alteration of ionic concentrations that occurs within the normal environment may have effects on the viability of sperm and/or the developmental processes of eggs such as fertilisation and the development of larvae. This chapter aims to examine the effects of different salinities on hardening processes, fertilisation and development of embryos inside the eggs of Murray cod to determine their tolerance. The investigation was made by observing the development of embryos, and measuring fertilisation success in terms of hatching viability. The salinity tolerance of larvae was made by measuring the yolk utility of larvae. Yolk utilisation efficiency is an important index that demonstrates the course of development. Larvae have a better chance of surviving in the natural habitat if the yolk is utilized more efficiently. During a period of yolk resorption, the desirable/optimal environmental conditions for utilisation are important in that, as a result, larvae may be expected to be stronger, better swimmers, less susceptible to damage, and less liable to predation (Blaxter, 1988).

A number of studies have demonstrated in different ways the salinity tolerance limits of eggs and larvae. Some claimed that eggs are more tolerant than larvae and vice versa (Blaxter, 1969; Chervinski, 1984; Hart et al., 1991). Holliday (1969) stated that the response to salinity at the most susceptible stage of development would determine the survival of the embryo up to hatching, and high mortalities at specific stages of development have been recorded in a number of works. For example, Oliphan (1941, cited in Holliday, 1969) documented that hatching of three freshwater spawning species was better at 2.5 and 5 g/L than in freshwater. After hatch, the yolk-sac larvae of many teleost species can survive in a very wide range of salinities (Holliday, 1969). The ability to tolerate salinity of the larvae of some freshwater spawning species

begins soon after the yolk sac has been absorbed (Baggermann, 1960 cited in Holliday, 1969). Conte *et al*. (1966) reported that the salinity tolerances of steelhead trout (*Oncorhynchus mykiss*) varied with age. The effects of salinity on larval abnormalities were also recorded in saltwater (Holliday, 1969; Rosenthal & Alderdice, 1976; Santerre & May, 1977).

Throughout their life cycle, Murray cod occupy a variety of habitats in which different levels of salinity may occur. Murray cod usually spawn upstream (Codwatch, 1993; Koehn, 1995), after which the hatch is gradually transported downstream into a possible higher level of salinity. Thus, it is essential to know for conservation purpose the salinity sensitivity of both eggs and larvae. The salinity sensitivity of yolk larvae was measured in terms of lethal and threshold salinity.

3.2 Materials and methods

3.2.1 Test media

Water used as a control throughout all trials (about 0.01 g/L salinity) was obtained from a tributary of Snobs Creek in Victoria. The water which was pumped from the channel was fed through a series of disc filters (100 and 50 μm) and sterilised by ozone gas. The sterilised water was finally filtered by using a charcoal filter and kept in a concrete tank. The test media were prepared using a commercial sea salt (Pacific Sea Salt) and the pre-treated water as a diluent as described in Chapter 2. The test media were kept enclosed in 15-L plastic buckets with aeration.

Ammonium and nitrite concentrations of the test media in rearing jars were measured using the HACH DR/4000 U Spectrophotometer. Alkalinity of the solution was measured by using the H_2SO_4 digital titration method. pH was measured using the pH-Conductivity-Salinity Meter (WP-81) and the water temperature was measured by using an Aquarium Thermo Sensor. Conductivity was measured using the ACTIVON Model 301 Conductivity Meter.

3.2.2 Experimental organisms

The study was initially designed to use only Murray cod (*Maccullochella peelii peelii*). However, fresh gametes, i.e. eggs and sperm, were not available during the experiment. Thus, some components of the experiment utilised fresh gametes of trout cod (*M. macquariensis*) instead of Murray cod. However, the numbers of eggs used were limited as trout cod is a protected species. Therefore, there were no replicates when trout cod were utilised. Trout cod were used according to the evidence of their close relationship to Murray cod as described by MacDonald (1976), Kailola et al. (1993) and Douglas et al. (1995).

3.2.3 Incubation processes

Fertilised eggs were incubated in 200 mL plastic jars in selected salinity levels at densities of 20-25 eggs per 200 mL. The incubated jars were placed in a 100-L waterbath tray. The required water temperature was controlled by using a constant flow of preheated water. The positions of the incubating jars were rotated daily to ensure constant temperature. Water in the incubating jars was replaced daily and aerated through injection needles. During the hatching period, the eggs were bathed once with 2.5% formalin for 30 minutes. The water qualities were monitored in terms of pH, alkalinity, and ammonium and nitrite concentrations.

3.2.4 Rearing processes

Post-hatch larvae were reared in 200 mL plastic jars in selected salinity levels with various densities. The rearing jars were placed in a 100 L waterbath tray. The required water temperature was controlled by using a constant flow of preheated water. The positions of the incubating jars were rotated daily to ensure constant temperature. The culture media was replaced daily and aerated through injection needles. The water quality physico-chemistry was monitored in terms of pH, alkalinity, and ammonium and nitrite concentrations.

3.2.5 Data analysis

The lethal concentration (LC) was calculated using the Probit procedure as described in Chapter 2. The data of the yolk utilisation efficiency were analysed using the two-factors factorial analysis of variance by GLM procedure in SAS. The comparisons of the dry weight of the Murray cod larvae were made using Least Significant Difference (LSD). All tests were conducted with SAS Software. Yolk utilisation efficiency was calculated using equation 2.8.

The dehydration was calculated using equation 2.10.

3.3 Salinity sensitivity of cod eggs

3.3.1 Hatching viability of Murray cod eggs

Experimental protocol

The fertilised eggs of the Murray cod were obtained from the brood of fish spawned naturally in an earthen pond at Snobs Creek. The spawning process in the brood pond was inspected daily at approximately the same time in the morning. Therefore, assuming that the eggs used in this study were collected within 24 hours after spawning, the eggs were exposed to the test salinity levels within 30 hours post-fertilisation. When the eggs were collected, the salinity level in the brood pond was 0.013 g/L and the temperature was 18.5°C. A total of 440 eggs were deliberately selected so as to exclude the non-fertilised eggs. The eggs were disinfected and then randomly divided into 11 groups of 40 eggs – one group for each salinity level. The eggs in each salinity level were again divided into 4 groups.

- The eggs of groups one and two were used for measuring of percentage of hatch.
- The eggs in the third group were used for measuring of dehydration of the first day yolk-larvae.
- The eggs in the last group were used for pathological observation.

All eggs were bathed in a 2.5% formalin solution for 30 minutes, and transferred to containers to incubate in the test salinities of 0-10.0 g/L with an interval of 1.0 g/L. The salinity level criteria were designed according to the information obtained from the literature reviews. It has been documented that the LC50 of the eggs of some five species of Australian freshwater fishes is in a range of 5 to 17 g/L (Ryan & Davies, 1996). It also should be noted that this experiment was carried out followed the salinity tolerant trials of Murray cod fingerlings (chapter 5) which found that the fingerlings did not tolerate salinities higher than 10.0 g/L.

Pathological responses were investigated at day-6 during incubation when the eyes of the embrysos could be clearly seen beneath the eggshells. The eggshells were removed, and the 6-day old embryos were examined under the microscope.

Soon after hatching, the larvae in each salinity of the third group were divided into their groups, wrapped with aluminum foil and dried to a constant weight at 60°C. Each group of larvae was used as the replications (not a true replicate) for the statistical analysis of the body water contents. The numbers that hatched, hatching times and survival time of the larvae of the first and second group were recorded. The data relating to water quality in this trial are not available due to some technical difficulties.

Results

The study found that the hardened eggs when exposed within 30 h after spawning were highly tolerant to salinity. The eggs were found to hatch in all test salinities (0-10.0 g/L) (Table 3.1), within 7-8 days. The percentages hatching were similar among salinities of 0 to 5.0 g/L. All embryos incubated in salinities above 6.0 g/L showed deformation of notochord. The differences of the larvae dry weight were highly significant among test salinities ($p<0.0001$). The dry weights were significantly reduced in salinities higher than 5.0 g/L ($p<0.01$). When the larvae were reared in the incubating salinities, it was found that only the larvae hatched and reared in freshwater developed through the period of yolk absorption, i.e. survived more than 7 days.

Table 3.1 Percentages of hatch, hatching time, survival time, and dry weights of Murray cod transferred within 30 h after fertilisation to be incubated in different salinities. *a*, *b*, *c*, *d* and *e* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different from each other.

Salinity (g/L)	**% Hatch**	**Hatching time** (days)	**Survival time** (days)	**Dry weight** (%)
0	80	8	> 7	5.4±0.1 *a*
1.0	75	8	4	5.3±0.2 *a*
2.0	80	7	< 1	5.2±0.1 *a*
3.0	65	7	< 1	5.4±0.1 *a*
4.0	70	7	< 1	5.2±0.3 *a*
5.0	75	7	< 1	5.3±0.1 *a*
6.0	50	7	< 1	4.7±0.2 *b*
7.0	55	7	< 1	4.4±0.1 *c*
8.0	55	7	< 1	4.1±0.1 *d e*
9.0	60	7	< 1	4.3±0.1 *e*
10.0	65	7	< 1	4.5±0.0 *c d*

3.3.2 Hatching viability of trout cod eggs

Experiment protocols

a) Pre-test of the sperm viability

In this trial fresh gametes of Murray cod were not available. Thus, fresh eggs and sperm of trout cod were used instead off Murray cod. Fresh milt was stripped from a male trout cod, *Maccullochella macquariensis* (weight = 6,070 g, SL = 600 mm, TL = 705 mm), and stored in a 2.5 mL dry syringe at room temperature of 18.5°C. A small drop of milt was placed beside a drop of test saline water on a glass slide. A cover slide was placed on top of the milt and saline water allowed the sperm to disperse into the test water (Fig. 3.1). The motility of the sperm was observed under the microscope. The time when the cover glass was placed on top of the test samples was recorded as the starting time.

No moving sperm were observed in salinities higher than 4.0 g/L (Table 3.2). O'Brian and Ryan (1990) reported similarly, that .5 g/L was the salinity limiting sperm motility of trout cod. If sperm stayed intact in the storing syringe they remained active

for more than 12 h. This result was used merely as a guideline when eggs were fertilised in different salinities, and was not used for any other discussion regarding salinity sensitivity since the method utilised has yet to be justified. However, the sperm viability test is rapid and simple, and may provide a conservative indication of a species' salinity tolerance (Hart et al., 1991).

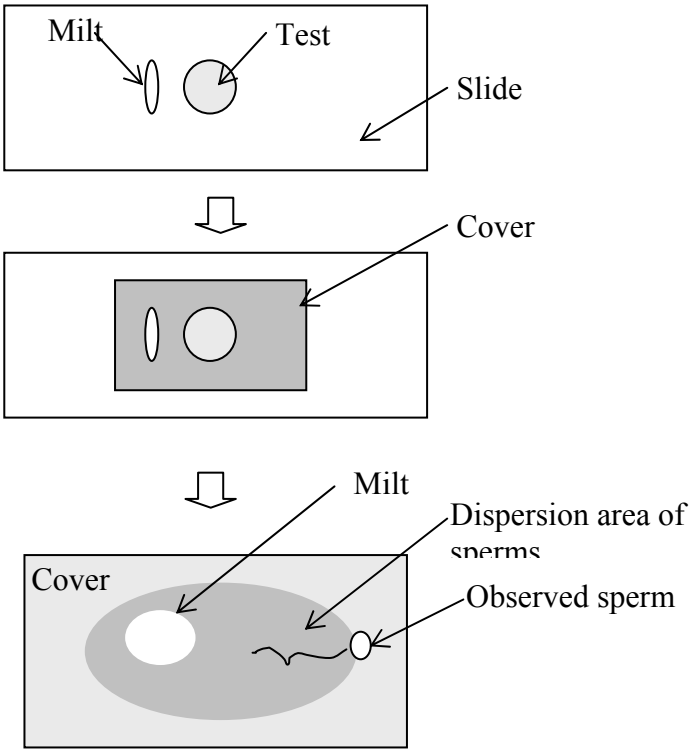

Figure 3.1 Observing the sperm viability of trout cod in different salinities.

Table 3.2 Observed motility of trout cod sperm in different salinities.

Salinity (g/L)	**Observed time** (min)	**pH of media**	**Alkalinity of media** (mg/L $CaCO_3$)
0	1.22±0.40	6.6	4.3
0.8	1.41±0.22	6.8	4.7
1.6	1.23±0.35	6.8	4.9
2.4	1.18±0.31	6.7	5.4
3.2	1.10±0.21	6.7	5.9
4.0	1.07±0.32	6.7	6.0
4.8	not observed	6.8	6.0
5.6	not observed	6.8	6.1
6.4	not observed	6.8	6.2
7.2	not observed	6.8	6.2
8.0	not observed	6.8	6.2

b) Pre-fertilisation exposure

In this study, trout cod was used as a substitute for the Murray cod. A male and female brood fish were used. The brood fish (one male of 6,070 g, and one female of 3,500 g) were induced to spawn using Chorionic Gonadatrophin hormone.

The milt was stripped, drawn and stored in a 2.5 mL dry syringe (Fig. 3.2). The eggs were also stripped and placed in a dry plastic jar. 220 eggs were then divided into 11 groups of 20 eggs – one group was used for each salinity level. Salinities between 0 and 8.0 g/L were utilised in this study. The concentrations of the test media were based on the results of the previous trial, which found that the trout cod sperm remained active in salinities up to 4.0 g/L. However, the highest concentration doubled those concentrations to ensure that mortality was certain to be observed in this trial.

The eggs were placed in the plastic jar and flooded with 200 mL of the test media. The eggs in the jars were then mixed with two drops of the male milt. The fertilisation was done randomly among the salinity levels resulting in the first and the last fertilisation being 1.6 minutes apart. The eggs and milt were used within 0.5 and 7 minutes respectively after stripping.

c) Post-fertilisation exposure

The experiment was carried out immediately following the previous trial. The stripped eggs of the trout cod were placed into the control water in a plastic jar and fertilised with a few drops of the milt (the eggs and the milt were used within 4 and 11 minutes respectively after stripping). The eggs were then transferred at 60 minutes after fertilisation to salinity levels between 0 and 8.0 g/L with an interval of 0.8 g/L. The fertilised eggs were incubated in the plastic jars at a density of 20 eggs per 200 mL. The water physico-chemistry was measured once during the incubation stage as shown in Table 3.3.

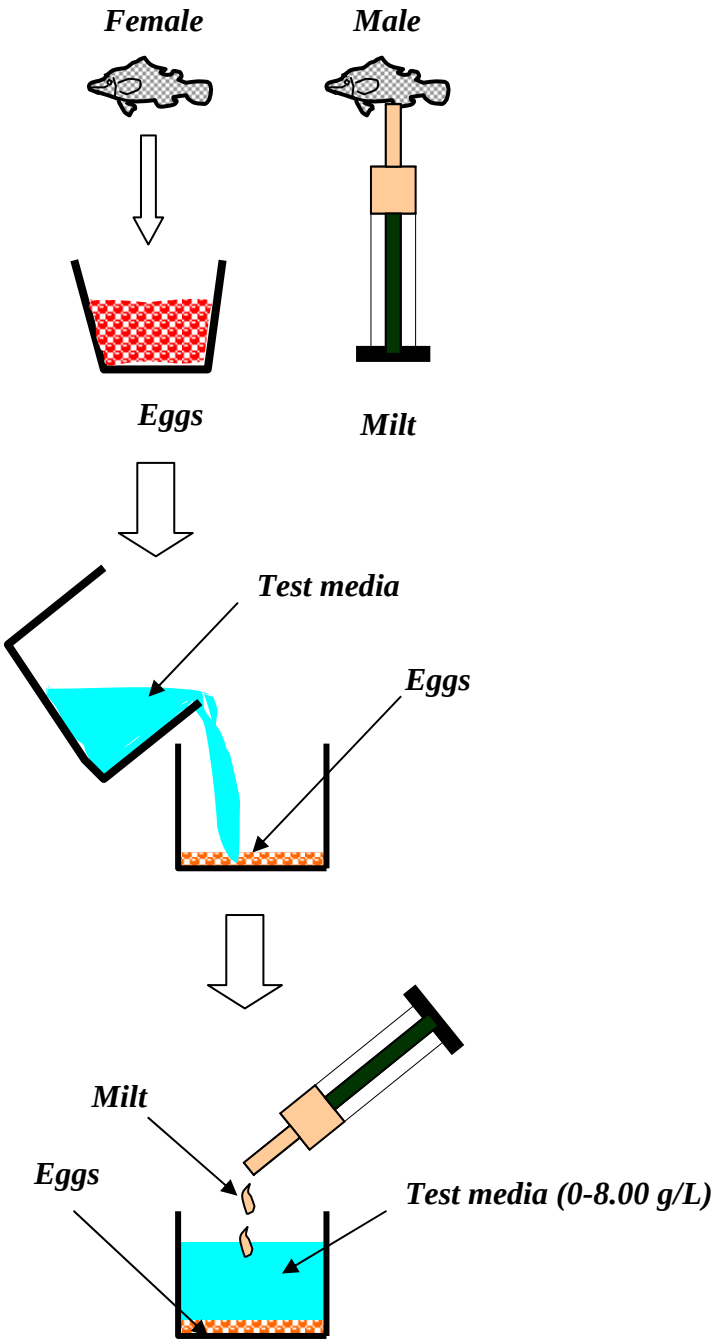

Figure 3.2 Fertilising of trout cod eggs in different salinities.

Results

A strong effect of salinity on egg hatching was observed. The results as shown in Table 3.4 indicated that no eggs were observed to hatch in any of the test salinity levels except for the control group. At 60 minutes post-fertilisation, 75% and 60% hatching was found in the control group and at 0.8 g/L, respectively.

Table 3.3 Water qualities in the trout cod eggs incubating jars.

Salinity (g/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2^--N)	**Alkalinity** (mg/L $CaCO_3$)	**pH**	**Temp.** (°C)
0	0.101	0.0010	4.8	6.77	20.8±0.8
0.8	0.179	0.0012	4.7	6.60	20.8±0.8
1.6	0.120	0.0021	4.9	6.53	20.8±0.8
2.4	0.086	0.0020	5.6	6.58	20.8±0.8
3.2	0.113	0.0023	5.9	6.57	20.8±0.8
4.0	0.145	0.0016	5.9	6.59	20.8±0.8
4.8	0.090	0.0019	6.1	6.61	20.8±0.8
5.6	0.085	0.0022	6.2	6.60	20.8±0.8
6.4	0.107	0.0014	6.2	6.61	20.8±0.8
7.2	0.111	0.0027	6.3	6.65	20.8±0.8
8.0	0.121	0.0017	6.5	6.71	20.8±0.8

Table 3.4 Hatching viability of trout cod eggs exposed to test salinity at the onset of and after fertilisation.

	% hatch	
Salinity (g/L)	**Pre-fertilisation**	**60 min Post-fertilisation**
0	40	75
0.8	0	60
1.6	0	0
2.4	0	0
3.2	0	0
4.0	0	0
4.8	0	0
5.6	0	0
6.4	0	0
7.2	0	0
8.0	0	0

3.4 Salinity sensitivity of cod larva

3.4.1 LC50 and threshold salinity of cod larvae

Experimental protocol

One-day-old yolk-larvae of trout cod were obtained from the mass production stock at the Snobs Creek hatchery. The eggs had been artificially fertilised and incubated in the control water. A total of 182 larvae were randomly divided into 13 groups of 14 larvae – one group for each salinity level.

One-day-old yolk-larvae of Murray cod were obtained from the mass production stock at the Snobs Creek hatchery. The eggs had been naturally spawned in an earthen brood pond, and incubated in control water. A total of 234 larvae were randomly divided into 13 groups of 18 larvae – one group for each salinity level. The larvae were then transferred to the nursing jars in the test salinities of 0-3.00 g/L with an interval of 0.25 g/L, at a density of 7 larvae per 200 mL. It had been found in the previous trials that larvae of Murray cod could not survive salinity of 2.00 g/L longer than one day; therefore, the highest concentration of the test media in this study was limited to 3.00 g/L.

The larvae were reared until they started swimming up the water column and searching for food, as a sign of the completion of yolk absorption. Mortalities were recorded daily and the water physico-chemistry (shown in Tables 3.5 and 3.6) was measured once during the development.

Results

The larvae of both trout cod and Murray cod started swimming and searching for food at day 12 of the experiment. Thus the final mortality rate was measured at 12 days of exposure. A strong effect of salinity on the survival of larvae of both species was observed. Only a marginally higher tolerance was observed in trout cod larvae.

Table 3.5 Water qualities in the trout cod larval rearing jars.

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Alkalinity** (mg/L $CaCO_3$)	**pH**	**Temp.** (°C)
0	0.121	0.0011	5.4	6.89	18.5±1.4
0.25	0.187	0.0023	5.8	6.78	18.4±1.3
0.50	0.132	0.0016	6.1	6.77	18.3±1.3
0.75	0.143	0.0021	5.6	6.79	18.5±1.4
1.00	0.123	0.0019	5.9	6.79	18.4±1.3
1.25	0.155	0.0025	5.8	6.79	18.1±1.2
1.50	0.101	0.0034	6.2	6.50	18.2±1.1
1.75	0.198	0.0026	6.8	6.63	18.1±1.0
2.00	0.139	0.0034	6.4	6.53	18.2±1.2
2.25	0.122	0.0017	6.6	6.67	18.5±1.3
2.50	-	-	-	-	-
2.75	-	-	-	-	-
3.00	-	-	-	-	-

Table 3.6 Water quality in the Murray cod larval rearing jars.

Salinity (g/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Alkalinity** (mg/L $CaCO_3$)	**pH**	**Temp.** (°C)
0	0.101	0.0010	4.8	6.77	20.5
0.25	0.179	0.0012	4.7	6.60	20.6
0.50	0.120	0.0021	4.9	6.53	20.7
0.75	0.086	0.0020	5.6	6.58	20.4
1.00	0.113	0.0023	5.9	6.57	20.5
1.25	0.145	0.0016	5.9	6.59	20.6
1.50	0.090	0.0019	6.1	6.61	20.7
1.75	0.085	0.0022	6.2	6.60	20.6
2.00	0.107	0.0014	6.2	6.61	20.5
2.25	0.111	0.0027	6.3	6.65	20.6

The results showed that all larvae of trout cod exposed to salinities above 0.75 g/L died within 12 days, before the yolk absorption was completed (Table 3.7).

All larvae of Murray cod exposed to salinity levels above 0.25 g/L died before the completion of yolk absorption (Table 3.8). The survival rates of both species were drastically reduced over the time of exposure (Figs 3.3 and 3.4).

Table 3.7 Observed mortality of larval trout cod directly exposed to the test salinities 24 h after hatch; 14 fish were used for each salinity level.

Salinity	**Day(s)**				
(g/L)	1	2	4	8	12
0	0	0	0	0	0
0.25	0	0	0	0	0
0.50	0	0	0	0	8
0.75	0	0	0	14	14
1.00	0	0	13	14	14
1.25	0	0	14	14	14
1.50	0	7	14	14	14
1.75	8	9	14	14	14
2.00	8	9	14	14	14
2.25	8	8	14	14	14
2.50	12	12	14	14	14
2.75	12	14	14	14	14
3.00	14	14	14	14	14

Table 3.8 Observed mortality of larval Murray cod direct exposed to the test salinities 24 h after hatch; 18 fish were used for each salinity level.

Salinity	**Day(s)**				
(g/L)	1	2	4	8	12
0	0	0	0	0	0
0.25	0	0	0	0	0
0.50	0	0	5	12	18
0.75	0	8	12	18	18
1.00	0	9	18	18	18
1.25	2	13	16	18	18
1.50	0	12	18	18	18
1.75	0	11	18	18	18
2.00	1	16	18	18	18
2.25	8	18	18	18	18
2.50	12	18	18	18	18
2.75	18	18	18	18	18
3.00	18	18	18	18	18

The estimated LC50s at day-12 were obtained at 0.50 g/L and 0.35 g/L in trout cod and Murray cod respectively (Table 3.9 and 3.10). The estimated threshold salinities (determining the no effect level, NEL) were 0.46 and 0.34 g/L in trout cod and Murray cod respectively.

Water quality in the incubating jars was at the acceptable level for fish in captivity (Forteath, 1990), but alkalinity was lower than the recommended level.

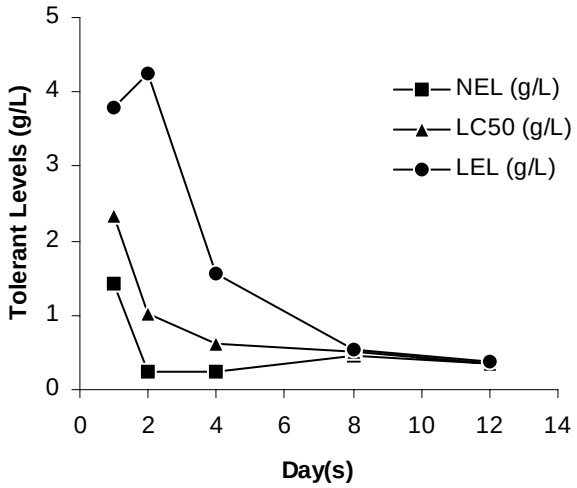

Figure 3.3 Salinity tolerance levels of trout cod larvae during the period of yolk absorption. The plots were made utilising the data from Table 3.6.

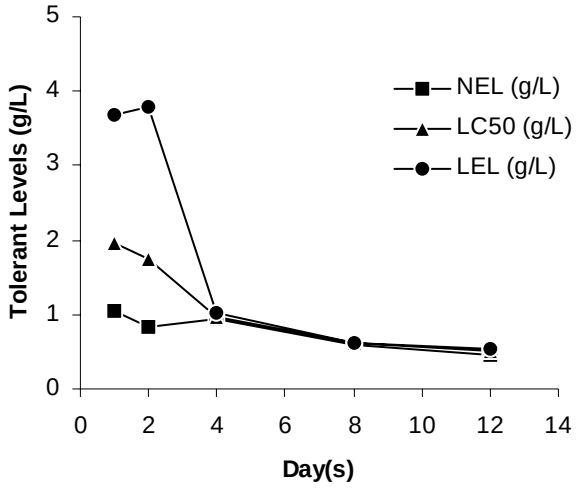

Figure 3.4 Salinity tolerance levels of Murray cod larvae during the period yolk absorption. The plots were made utilising the data from Table 3.7.

Table 3.9 Estimated tolerant concentrations of larval trout cod exposed to the test salinities 24 h after hatch. The NEL is the no effect level where 1% mortality is estimated, and LC99 is the effect level where 99% mortality is estimated using the Probit method.

Time (days)	NEL (g/L)	LC50 (g/L)	LC99 (g/L)	Reg. Coeff.	p
1	1.05	1.97	3.69	2.16	>0.05
2	0.82	1.76	3.79	1.99	>0.05
4	0.93	0.98	1.02	6.19	>0.05
8	0.60	0.62	0.63	4.55	>0.05
12	0.46	0.50	0.54	4.65	>0.05

Table 3.10 Estimated tolerance concentrations of the larval Murray cod exposed to the test salinities 24 h after hatch. The NEL is the no effect level where 1% mortality is estimated, and LC99 is the effect level where 99% mortality is estimated using the Probit method.

Time (days)	NEL (g/L)	LC50 (g/L)	LC99 (g/L)	Reg. Coeff.	p
1	1.43	2.33	3.79	2.63	<0.001
2	0.25	1.03	4.25	1.71	<0.05
4	0.25	0.63	1.55	4.55	>0.05
8	0.46	0.50	0.53	4.87	>0.05
12	0.34	0.35	0.37	4.88	>0.05

3.4.2 Yolk utilisation efficiency of Murray cod larvae

Experimental protocol

a) Exposed as egg

The fertilised eggs were collected within 24 h from an earthen brood pond following spawning. The eggs were cleaned with the control water and randomly divided into 3 groups of 20, 20 and 180 eggs.

The eggs of the first group were used to measure the dry weight of the whole egg. The eggs were dried to a constant weight at 60ºC. Eggs of the second group were used

to measure the dry weight of the egg shell. The egg contents were removed and the shells were dried to a constant weight at 60ºC. Dry weight of the egg cytoplasm was the weight of the whole egg less the shell weight.

Eggs of the last group were disinfected with a 2.5% formalin solution, and randomly divided into 6 groups of 30 eggs – one group for each salinity level. The eggs were then transferred for incubation at a density of 15 eggs per 200 mL in test salinities of 0-0.5 g/L with an interval of 0.1 g/L. The test concentrations were based on results of the previous trials which showed that the no effect levels (NEL) of trout cod and Murray cod were in the range 0.34-0.46 g/L.

After hatching, the larvae were further reared in the incubating salinities for 12 days during which time the yolk was almost completely absorbed. The larvae were rinsed with distilled water and dried to a constant weight at 60ºC.

b) Exposed as larva

The fertilised eggs were obtained from the same batch of eggs as used in the trial above. The eggs were disinfected with a 2.5% formalin solution and hatched in the control media. After hatch, the larvae were divided into 6 groups of 15 larvae – one group for each salinity level. The larvae were then transferred for development in the rearing jars with a density of 15 larvae per 200 mL in the test salinities of 0-0.50 g/L, with an interval of 0.10 g/L. The YE was calculated using equation 3.1. The water physico-chemistry in the rearing jars was measured once during the experiment as shown in Table 3.11.

Table 3.11 Water qualities in the rearing jars used for measuring the yolk utilisation efficiency of Murray cod larvae.

Salinity (g/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Alkalinity** (mg/L $CaCO_3$)	**pH**	**Temp.** (°C)
0	0.929	0.0073	13.8	6.76	17.6
0.10	0.720	0.0026	11.5	6.82	17.7
0.20	0.989	0.0053	10.8	6.74	17.6
0.30	0.685	0.0078	9.8	6.78	17.6
0.40	-	-	-	-	-
0.50	-	-	-	-	-

Results

Salinity within the tolerant range indicated that it did not affect the utilisation efficiency of yolk (Table 3.12). All test larvae of both trials died in salinities above 3.00 g/L. In the survival salinities, the differences of the utilisation efficiency were not significant both to the larvae exposed after hatch ($p = 0.9555$) and the larvae exposed as eggs ($p = 0.9132$). The overall averages of the YE were 105 and 104% when exposed before and after hatching respectively.

Water quality in the incubating jars was at an acceptable level for fish in captivity (Forteath, 1990), excepting that the ammonium concentration was higher than the recommended level.

Table 3.12 Yolk utilisation efficiency of larval Murray cod (12 days old) reared in different salinities (dry weight of whole egg = 4.11 mg/egg, dry weight of the egg shell = 0.67 mg/egg). *a* indicates significance of the differences ($p=0.05$), the figures followed by a similar alphabet letter are not significantly different from each other.

Salinity (g/L)	**Exposed as egg** (%)	**Exposed as larva** (%)
0	105±3, *a*	105±2, *a*
0.10	104±2, *a*	105±6, *a*
0.20	105±2, *a*	103±2, *a*
0.30	105±3, *a*	105±2, *a*
0.40	no survival	no survival
0.50	no survival	no survival

3.5 Discussion

Salinity sensitivity of eggs

The hardened eggs of Murray cod, following fertilisation, were found to be highly tolerant to salinity when transferred within 30 h to salinities up to 10.0 g/L. However, the hardened eggs of trout cod, when transferred within 1 h, showed susceptibility to very low salinity, i.e. they only hatched at a salinity of 0.8 g/L or less. If eggs of the two species were biologically compatible, the time between 1 and 30 h following fertilisation significantly affected the survival of eggs when they were incubated in different salinities. This study also found that transfer of trout cod eggs after

fertilisation significantly improved the survival of eggs compared to eggs fertilised directly in the different salinities.

The impacts of salinity on fertilisation of trout cod were determined when eggs were placed in a range of salinities (0-8.0 g/L), and mixed with male milt after 1-1.6 min. This short period of time ensured the viability of sperm, as it was found that the sperm remained active in the milt for more than 12 h if not exposed to air. Holliday (1969) also documented the successful fertilisation of a marine species when gametes were placed in a range of salinities and mixed after 1-2 min. The hatching percentage was used as the criterion of tolerance. The results showed that only those eggs fertilised in freshwater hatched. It is suggested that either eggs or sperm of trout cod did not tolerate salinity of 0.8 g/L. This study proposes that unsuccessful fertilisation might result from, at least,

- reduction in activity and viability of sperm (Westernhagen, 1988)
- inhibition of the sperm-activating substance function (Jobling, 1995)
- chemical changes in the eggs that causes them to release a developmental block or to trigger irreversible changes that would prevent fertilisation (Balon, 1985)
- inhibition of the imbibing process forming the perivitelline space (Zotin, 1965 cited in Holliday, 1969).

Westernhagen (1988) claims that a reduction of sperm activity results in a lowered fertilisation success rate. Although the viability of sperm was not investigated in this study, the sperm should remain active in the test media with a salinity level close to that of body fluid which was about 10 g/L (Brett, 1979). However, marginal changes of medium salinity levels may reduce sperm activity and viability. Jobling (1995) states that the sperm of many fish species do not become fully active until they have been shed and exposed to water. The differences in ionic and osmotic strengths between the seminal fluid and water seem to be the stimuli required for increased activity. In some species, the exposure of sperm to unfertilised eggs is required in order for the sperm to display maximal swimming activity.

How does salinity inhibit the function of the sperm-activating substance? Jobling (1995) also states that unfertilised eggs seem to release a substance that initiates the activation of the sperm. The contact of sperm with this activating substance leads to an increase in numbers of vigorously swimming sperm and the length of time they are motile. It is also possible that the sperm-activating substance is released from the micropyle and acts as an attractant to help guide the sperm to the micropyle in the outer egg membrane.

Exposure of trout cod eggs to different test salinity levels may have triggered changes that prevented the fertilisation rates in this study. Balon (1985) states that the process of fertilisation continues with an activation that releases the egg's developmental block and triggers irreversible changes. The changes comprise growth of the fertilisation cone, a cortical reaction, perivitelline space formation and bipolar differentiation. The fertilisation process then ends with the fusion of the male and female pronuclei. During natural mating, the gametes are released into the water where insemination coincides with the activation of the ovum by water contact (Brummett & Dumont, 1979). The cortical granules of the eggs release their contents by forming a perivitelline fluid and space. This cortical reaction is followed by rapid changes of external tension and density and a simultaneous continuity of meiosis. Along with the changes in ion concentrations, there is a subsequent formation of pronuclei. However, these developments can also be initiated by contact with water without the need for insemination (Balon, 1985). Zotin (1965, cited in Holliday, 1969) denoting that the perivitelline space is not formed in freshwater spawning species if the imbibing process is inhibited by the present of relatively small amount of NaCl.

When the trout cod eggs were fertilised in freshwater and transferred after 1 h to incubate in different salinities, the percentage hatchings were improved compared to the eggs fertilised in the same test salinities. Evidently the improvement of the salinity tolerance in the fertilised egg could have contributed to the egg case's strengthening after completion of the hardening process. Weisbart (1968) stated that despite the permeability of the hardened chorion to the medium, its presence afforded significant resistance to increased salinity. Weisbart (1968) found that survival times in different salinities of *Oncorhyncus* sp. embryos decreased when the chorion was removed.

Jobling (1995) points out that the hardened chorion helps to protect the embryo during the earliest stages of development. The study of O'Brien and Ryan (1999) found that trout cod eggs before hardening were able to hatch in salinities up to approximately 1.8 g/L, and in salinities up to approximately 8.7 g/L after hardening. Prolonged periods of the water hardening processes may help increase the egg case's strength and provide a more advanced stage of embryonic development, which then enhances the tolerance level.

There are also enzymes present in the egg membranes, which may help to protect the developing egg against bacterial and fungal attack. These enzymes within the egg membrane do not appear after the egg has been activated by fusion with the sperm. The outer membranes of the fertilised eggs of a number of fish species are known to possess enzymes that can degrade the polysaccharides within the cell wall and expose them to bacteria and fungi. Thus, enzymes within the egg membrane probably do have an important defensive role in protecting the embryo against invaders or pathogens; in addition the thick chorion and enlarged perivitelline space themselves help to protect the developing embryo against physical damage (Jobling 1995).

Although the role of hardened cases on osmotic and ionic permeability is not well defined, Heath (1987) reports that egg cases after having been hardened remain permeable to dissolved substances. Therefore, salinity at a certain level can still affect further development of the zygote and embryo. This present study also found dehydration in the post-hatched larvae.

The impact of salinity on water hardening has not been well defined. However, Black (1951, citied in Holliday, 1969) documented that a salinity of 3 g/L inhibited hardening of the chorion of *Oncorhyncus* sp. The hardening process may be inhibited in water containing strongly hydrated ions such as aluminum and zinc which compete with calcium for binding sites on the mucopolysaccharide to form the shell-like chorion (Heath, 1987). The role of sodium has not been mentioned. However, this study found that water hardening occurred at all test salinity levels (1-10 g/L). The freshwater hardened eggs were white in colour and very rigid when pressed with fingers. However, the eggs that hardened in salinity higher than 6.0 g/L appeared to be less opaque than those hardened in lower salinities. This suggests that sodium

chloride may inhibit or compete with calcium to bind with mucopolysaccharide to form a rigid egg case. Moreover the calcium-combined mucopolysaccharide is unlikely to be stable in salinity levels higher than 6.0 g/L as it eventually disintegrates with long exposure.

Sublethal effects of salinity to embryonic development

In the study with Murray cod eggs, results showed that eggs hatched in all test salinity levels 0-10.0 g/L when transferred 30 h after fertilisation. However, the post-hatched larvae died within 7 days in all test salinity levels. Guo et al. (1993) reported that the larvae of silver perch (*Bidyanus bidyanus*) were able to hatch at a salinity level of 9.00 g/L but all of the yolk-dependent larvae died within 4 days after hatching. Wal (1985) also found that the eggs of the catadromous fish Australian bass (*Macquaria novenmaculeata*) could hatch in low salinity, but no larvae survived. Morgan et al. (1992) reported that the egg hatchability of steelhead trout (*Oncorhynchus mykiss*) and fall Chinook salmon (*O. tshawytscha*) was high at salinity levels of 0-12.0 g/L, but the survival rate of newly hatched alevins was low and the fish were smaller in size at 12.0 g/L. They suggested that the normal development rate of both species was limited due to the level of salinity at which their metabolic rates was lowest.

The major sublethal effect on the development of the Murray cod embryo was the presentation of a distorted notochord. The degree of severity could be observed by the bending angle of the vertebra which increased with the increasing of salinity from 6.0 to 10.0 g/L. A possible suggestion here was that although the egg case was hardened to its full strength, salinity higher than 6.0 g/L would have impacts on embryonic development of Murray cod.

Abnormalities in the larval vertebra may also be attributed to other environmental factors during development. Rosenthal and Alderdice (1976) claim that severe morphological aberrations, e.g. distorted notochords, have been found in embryos whose normal environments have been altered by low oxygen levels and/or extreme salinity levels or temperature variations. Santerre and May (1977) also reported that for the moi (*Polydactylus sexfilis*) the percentages of morphologically abnormal

larvae were increased in several salinity levels at extreme temperature ranges within the moi's environment.

Other chronic effects on the developing embryo also have been reported. Holliday (1965, cited in Holliday, 1969) found shrinking of cells of the blastula cap in the embryos of *Gadus callarius* in increased salinity when hatched in saline water.

Salinity sensitivity of larvae

The tolerance limits of both larval species were much lower than the tolerance levels of the hardened eggs (0.8 g/L in trout cod eggs, and above 10.0 g/L in Murray cod eggs). However, Chervinski (1984) documented relatively high tolerance (10.00 g/L) in the post-larvae of channel catfish (*Ictalurus punctatus*).

Blaxter (1969) has also added that newly hatched larvae are vulnerable and most highly sensitive to changes in environmental conditions. However, Hart et al. (1991) have claimed that fish eggs have more tolerance than larvae. This present study suggests that this claim is relevant not only to hardened eggs, but also is applicable to eggs exposed before or during the stages of the fertilisation process. McCormick and Saunders (1987) have pointed out that whereas salmonid eggs cannot survive for more than a few days in seawater, the post-hatched alevin has an even lower survival rate, presumably due to the loss of chorion. They concluded that the salinity tolerance of Atlantic salmon (*Salmo salar*) alevin decreased as the water-impermeable vitelline membrane decreased in favour of a water-permeable epithelium in the gills. Holt and Banks (1989) also claim that the salinity ranges in which larvae can grow and develop is limited by a poorly developed osmoregulatory function and associated metabolic rates.

During the research of yolk utilisation in Murray cod larvae, results indicated that there were no significant differences in the yolk utilisation efficiency among groups of fish reared in salinity levels between 0 and 0.30 g/L. Moreover, larvae reared at salinity levels higher than 0.30 g/L eventually died during the experiment. This suggests that the tolerance limit of yolk larva was approximately 0.30 g/L, which is compatible to the threshold salinity of 0.34 g/L estimated in the salinity tolerance

trial. It can also be concluded that developments of the endogenous to exogenous stages are not affected by salinity within its tolerance range. Results also showed that the yolk utilisation efficiencies did not differ between the groups of larvae exposed before and after hatching.

Gunasekera et al. (1999) has stated that the yolk absorption efficiency in Murray cod larvae during embryogenesis can be attributed to the rate of breakdown of the yolk protein. The role of salinity on the rate of yolk protein utility was not mentioned in this study. However, in the marine fish species, i.e. milkfish (*Chanos chanos*), salinity affects both yolk absorption and the embryonic and larval growth stages (Swanson, 1996). Swanson, (1996) has found that milkfish larvae hatched in 20 g/L had larger yolk reserves but were smaller and grew more slowly than the larvae hatched at 35 and 50 g/L salinity levels. Salinity affected both the rate and the pattern of yolk utilisation, but these did not relate directly to the osmotic ingredient. Basically, low salinity levels retarded yolk absorption while high salinity levels reduced yolk utilisation efficiencies.

3.6 Summary

Trout cod and Murray cod were used in the investigations of salinity sensitivity of eggs and larvae. The effect of salinity during fertilisation was also studied. The experiments were limited to the stage where the larvae utilised their own food reserves in the yolk sac, which took a period of 13 days after hatching. The test media were prepared using a commercial sea salt and channel freshwater of 10 mg/L salinity. The findings are as follows:

Trout cod egg salinity sensitivity

1) There were no hatching successes when the eggs were fertilised at test salinity levels between 0.8 and 8.0 g/L, and only 40% hatched in freshwater.
2) 75% of eggs hatched in freshwater and 60% hatched in 0.8 g/L salinity respectively when they were transferred to the test salinity media 60 minutes after fertilisation.

Murray cod egg salinity sensitivity

1) Water hardened eggs hatched in all test salinity levels between 1.0 and 10.0 g/L when eggs were transferred approximately 30 h after fertilisation, but with a reduced hatching rate in high salinities.
2) There was a significant distortion of the notochords found in embryos when incubated in salinity levels higher than 6.0 g/L.
3) A significant reduction in dry weight was found in the larvae hatched from the eggs incubated in salinity levels higher than 5.0 g/L.

Trout cod larvae salinity sensitivity

1) The estimated LC50 at day12 of the yolk absorption period is 0.50 g/L.
2) The estimated threshold salinity at day 12 of the yolk absorption period is 0.46 g/L.
3) Salinity tolerances were reduced in the longer exposure times from 1 to 12 days.

Murray cod larvae salinity sensitivity

1) The estimated LC50 at day 12 of the yolk absorption period is 0.35 g/L.
2) The estimated threshold salinity at day 12 of the yolk absorption period is 0.34 g/L.
3) Salinity tolerances were reduced in the longer exposure times from 1 to 12 days.
4) The yolk utilisation efficiency does not differ among groups of larvae exposed to salinity levels beyond the threshold salinity (1.00 to 3.00 g/L).

The yolk utilisation efficiency does not differ among groups of larvae exposed before and after hatching.

CHAPTER FOUR

Salinity sensitivity of Murray cod fry

4.1 Introduction

Impacts of salinity on freshwater fish are compounded by various other factors, while salinity itself acts as a masking factor that may modify or prevent the success of environmental restoration actions. Controlling factors such as temperature and pH can also modify the impacts of salinity on fish. Limiting factors such as food can restrict the supply or removal of metabolites (Fry, 1971). In their natural habitat, Murray cod spawn in spring where water temperature ranges from 18 to 25°C (Lake, 1978; Codwatch, 1993) increasing gradually through to the end of the season. Temperature also influences growth and production of food organisms such as phytoplankton and zooplankton.

A number of studies have demonstrated that pH modifies the impacts of salinity on growth of fish and invertebrates such as Atlantic salmon, *Salmo salar* (Saunders et al., 1982), juvenile prawn, *Penaeus setiferus* (Martinez et al., 1998), tiger prawn, *Penaeus monodon* (Allan & Maguire, 1992), larvae of Chinese mitten-handed crab (Zhao & Jin, 2001), and freshwater prawn, *Macrobrachium rosenbergii* (Cheng & Chen, 2000).

A number of studies have demonstrated the impacts of temperature on growth and physiological changes in fish such as goldfish, *Carassius auratus auratus* (Catlett & Millich, 1976), brown trout, *Salmo trutta* (Elliott, 1981), rainbow trout, *Oncorhynchus mykiss* (Morgan et al., 2001), molly, *Poecilia sphenops* (Hernandez et al., 2002), Nile tilapia, *Oreochromis niloticus* (Atwood et al., 2003), and roach, *Rutilus rutilus* (Hardewig & Dijk, 2003). Some studies also report interactions between temperature and salinity on growth of fish such as sea bass, *Dicentrarchus labra* (Alliot et al., 1983), Arctic cisco, *Coregonus autumnalis* (Fechhelm et al., 1993), striped bass, *Morone saxatilis* (Harmon & Peterson, 1994), juvenile Nile tilapia, *Oreochromis niloticus* (Likongwe et al., 1996), Atlantic salmon, *Salmo salar* (Handeland et al., 1998), Japanese seaperch, *Lateolabrax japonicus* (Dai et al., 1998), juvenile mullet, *Mugil* sp. (Peterson et al., 2000), and striped bass, *Morone saxatilis* (Secor et al., 2000).

Payne et al. (1988) reported that temperatures in the range of 20-28°C, modified the growth of tilapia (*O. mossambicus*). They found that at 20°C growth rates declined with increasing salinity (6-14 g/L) but at 28°C there was a much improved growth rate at the highest salinity (14 g/L). Watanabe et al. (1993) also found that salinity modified the effects of temperature on the growth of the juvenile, sex-reversed male Florida red tilapia. At 0 g/L, feed consumption and growth reached a maximum at 27°C, while at 18 and 36 g/L, consumption and growth were highest at 32°C. The results suggest that, in freshwater, heating water to temperatures above 27°C is not justifiable while at 18 or 36 g/L, heating water to 32°C can maximise growth rates without lowering growth efficiency. Eddy (1981) states that growth in the environment where there is a variation in temperature and salinity levels is the net energy resulting from osmoregulation and metabolism. Elliott (1981) points to four responses with a continuous increase of temperature to the maximum tolerance level, namely, the rate of gastric evacuation, the energy loss of fish deprived of food, the standard metabolic rate and the maintenance of energy intake.

Impacts of food availability and starvation on salinity tolerance have been rarely defined. Generally fish should use less energy and have less energy expenditure in optimal salinity conditions when other factors are constant, and this should prolong their survival in starvation conditions. A study with the freshwater prawn (*Macrobrachium rosenbergii*) by Cooper and Heinen (1991) found that the starved larvae lived longer in their optimal salinities than other tested salinities. Water temperature was also found to have an impact on starvation. Brett et al. (1969 in Weatherley, 1972) claimed that starvation of fingerling sockeye salmon (*Oncorhynchus nerka*), in the optimal temperature that promoted growth, accelerated weight loss in the fish. However, this study expects to see the contribution of salinity to energy expenditure when food is insufficient.

This chapter aims to investigate the impacts of temperature, pH and food availability on the salinity tolerance of Murray cod fry. The measurements of salinity tolerance were carried out in terms of lethal, optimal and threshold salinities.

4.2 Materials and methods

4.2.1 Test media

Water used was drawn from the Melbourne main supply and subsequently directed through a high-pressure sand filter to remove particulate material and then through an activated carbon filter to remove some dissolved materials. Residual chlorine levels were monitored regularly to ensure they remained below 0.02 mg/L. Water then passed through a primary chiller unit into a 45,000 L capacity underground concrete tank for at least 48 hours for aging and settling any remaining particulates.

The test media were prepared by using Pacific Sea Salt (a commercial grade sea salt) and aged Melbourne main water as a diluent. The prepared media were kept in 50 L glass aquaria with lids on and without aeration. The media were prepared regularly. In order to obtain accurate concentrations of the rearing solution, the salinity was measured in terms of conductivity but presented as g/L TDS throughout the study. The culture media were gravity-fed into the rearing tanks. The ammonium and nitrite concentrations of the rearing media were measured every fourth day using the MERCK Photometer SQ 300. The water total hardness was measured by using the AquaMerck Total Hardness Test (1.084047 Gesantharte-Test). The pH was measured using a WTW Microprocessor pH meter. The conductivity was measured by using the Activon (Model 301) conductivity meter. The Oximeter (WTW Oxi 330) was used to measure dissolved oxygen within the water. The water temperature was measured using an Aquarium Thermo Sensor.

4.2.2 Experimental organisms

A batch of 2,000 Murray cod fry (0.021 g) was purchased from the Snobs Creek Hatchery in Victoria. The fish were obtained from a female brood stock that had spawned naturally in a brood pond. The yolk-absorbed larvae were reared in a hatchery for about 2 weeks, before being transferred to the experimental station of the Freshwater Ecology Laboratory at the Arthur Rylah Institute in Victoria. Before being used for a series of experiments the fish were acclimated to the experimental

conditions for 21 days in the laboratory, where the water temperature was constantly controlled at 20ºC. The fish were stocked in 50-L flow-through glass aquaria, fed twice a day with Instar-I *Artemia* nauplii. The fish were bathed weekly with sea salt at a concentration of 2 g/L for 30 mins and also bathed every second week with a fungicide chemical for aquarium fish to prevent white spotted disease and fungus infections.

4.2.3 Data analysis

The LC50 at 96 h in different temperatures and pH was calculated from the mortality number using the Probit method in SAS. The relation equations between salinity and growth rate were calculated using the regression analysis in the SPSS program. The freshwater growth rate (FRG), optimal salinity (OPS), threshold salinity (THS), and upper sublethal salinity (USLS) were calculated using the relationship between salinity and specific growth rate (SGR) obtained from the experiments. The two-factor factorial design was adopted when an interaction between salinity and temperature, and salinity and feeding regime were tested. The treatment means in terms of the SGR and relative weight gain (RWG) among test salinities were compared using a one-way analysis of variance (ANOVA), and paired comparisons were made using Least Significant Difference (LSD). The small or large percentage values (0-30% or 70-100%) that form a binomial distribution were arcsine-transformed before being used for comparison (Zar, 1984). The dehydration rate was calculated using equation (2.10).

4.3 Lethal salinity

4.3.1 Impacts of temperature on LC50

Experimental protocol

The experimental units comprised four different temperature sets: 15, 20, 25 and 30ºC. Temperature in the experimental vessels was controlled at the required levels by using a constant flow-through water bath. A total of 840 Murray cod fry (0.086g)

were divided into 4 groups of 210 fish – one group for each temperature level. The fish for each temperature set were then randomly divided into 14 groups of 15 fish – one group for each salinity level from 0 to 13.0 g/L (the concentrations utilised in this trial were pre-tested and it was found that the fry survived in concentrations up to 11.0 g/L). Another 210 fry of 0.029 g were also tested at 20ºC. The fish were kept in 4-L glass aquaria with a density of 1.25 fish/L and fed daily with the nauplii of *Artemia* at a density of 200 *Artemia*/L. The numbers of dead fish (mortality numbers) were counted every 24 h for a period of 96 h. The pH of the tested media was 6.7-7.1, and hardness was 34-40 mg/L $CaCO_3$.

Results

Temperature was found to modify salinity tolerances of fry fish. All of the fish died within 4 days in the test temperatures between 15 and 30ºC at salinity of 13 g/L (Table 4.1). The LC50 was estimated at 11.3, 12.0±0.5 (n=2), 11.4 and 7.0 g/L at temperatures 15, 20, 25 and 30ºC respectively (Table 4.2).

Table 4.1 Mortality numbers of Murray cod fry (0.086 g) exposed to different salinities at different temperatures.

Salinity (g/L)	**Mortality**				
	15ºC	**20ºC***	**20ºC**	**25ºC**	**30ºC**
0	1	0	0	0	1
1.0	0	0	1	0	0
2.0	0	0	0	0	0
3.0	0	0	0	1	0
4.0	0	0	1	1	0
5.0	0	0	0	0	1
6.0	0	0	0	0	2
7.0	1	0	0	0	6
8.0	2	1	0	0	13
9.0	0	0	0	0	15
10.0	10	3	0	0	15
11.0	1	0	2	3	15
12.0	14	9	11	14	15
13.0	15	15	15	15	15

* fry of 0.029 g

Table 4.2 LC50 at 96 h of Murray cod fry (0.086 g) exposed to different salinities at different temperatures, calculated from data in the Table 4.1 using the Probit method.

Temperature(ºC)	**LC50**(g/L)	**Reg. Coeff.**	**p**
15.0±0.4	11.3	0.65	<0.0001
20.0±0.6*	11.6	1.03	<0.05
20.0±0.9	12.3	1.08	>0.05
25.0±0.7	11.4	1.11	>0.05
30.0±0.5	7.0	1.17	>0.05

* fry of 0.029 g

4.3.2 Impacts of pH on LC50

Experimental protocol

The experiment units comprised four different pH values of 6.0, 7.0, 8.0 and 9.0. Sodium bicarbonate and sodium carbonate were added to the water to help maintain the hardness at 100-110 mg/L $CaCO_3$. pH of the media was adjusted twice a day using 0.1N-NaOH and 0.1N-HCl solutions. However, it was not possible to control the designated levels. The actual measured pH was 6.2±0.2, 7.1±0.2, 8.0±0.2 and 8.8±0.2.

A total of 840 Murray cod fry (0.092g) were divided into 4 groups of 210 fish – one group for each pH level. The fish for each pH level were then randomly divided into 14 groups of 15 fish – one group for each salinity level of 0 to 13.0 g/L. The fish were kept in 4-L glass aquaria with a density of 1.25 fish/L and fed daily with the nauplii of *Artemia* at a density of 200 *Artemia*/L. The mortality numbers were counted every 24 h for a period of 96 h. Water temperature of the test media was at 20.0±1.2ºC.

Results

pH was found to modified salinity tolerances of fry fish. All of the fish died within 4 days in the test pH between 6.2 and 8.8 at the salinity of 13.00 g/L (Table 4.3). The LC50 was estimated at 10.0, 11.3, 11.5 and 12.1 g/L at pH of 6.2, 7.1, 8.0 and 8.8 respectively (Table 4.4).

Table 4.3 Mortality numbers of Murray cod fry (0.092 g) exposed to different salinities at different pH.

Salinity (g/L)	Mortality			
	pH 6.2	pH 7.1	pH 8.0	pH 8.8
0	0	0	1	0
1.0	0	0	0	0
2.0	0	0	0	0
3.0	0	0	0	0
4.0	0	0	0	0
5.0	1	0	0	0
6.0	0	2	1	0
7.0	0	0	0	2
8.0	1	0	0	0
9.0	0	0	2	0
10.0	9	1	0	0
11.0	11	0	0	0
12.0	15	15	12	6
13.0	15	15	15	15

Table 4.4 LC50 at 96 h of Murray cod fry (0.092 g) exposed to different salinities at different pH, calculated from data in the Table 4.3 using the Probit method.

pH	LC50(g/L)	Reg. Coeff.	p
6.2	10.0	1.11	>0.05
7.1	11.3	1.11	<0.01
8.0	11.5	1.60	>0.05
8.8	12.1	1.04	<0.05

4.4 Sublethal salinity

4.4.1 Growth characteristics in increasing salinity

Experimental protocol

The objective of this experiment was to determine the relationship between growth and salinity. The parameters describing salinity sensitivity were to be estimated from the relationship between growth and salinity, as the growth could be measured either in terms of the specific growth rate (SGR) or the relative weight gain (RWG). The SGR and RWG may provide different estimated degrees of tolerance, in terms of optimal salinity (OPS), threshold salinity (THS) and upper sublethal salinity (USLS).

However, only the SGR, which is more frequently used among fish biologists, was used throughout this study to estimate the sensitivity of fish.

The highest salinity was limited to 10.0 g/L which was about 1 g/L less than the LC50 found in the previous lethal salinity trials. The highest concentration was designed in order to avoid causing the tested fish to suffer, thus complying with the animal ethical permit.

The experimental vessels were set up in a constant room temperature (approximately 20ºC). A total of 300 Murray cod fry with an average initial weight of 0.101g were used. The fish were randomly divided into 3 groups of 100 fish – one group for each replicate. 100 fish in each replicate were then divided into 10 groups of 10 fish – one group for each salinity level. The fry were reared in 4 L glass aquaria in the salinity levels of 0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 g/L at a density of 2.5 fish/L, and fed to satiation twice a day with Instar-I *Artemia* nauplii. After being reared for 30 days the numbers that survived were counted. The final weights of the fish were measured individually. The excess water on the body surface was removed using hand towel paper before measuring the body weight. The water quality physico-chemistry in the rearing tanks was measured regularly (Table 4.5).

Table 4.5 Water qualities in fry rearing tanks (20.0±0.2°C).

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness*** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)
0	1.13±0.19	0.04±0.01	54±12	7.23±0.26	8.34±0.06
2.0	1.20±0.24	0.04±0.01	57±2	7.28±0.01	8.31±0.10
3.0	1.37±0.24	0.05±0.02	62±5	7.35±0.12	8.34±0.10
4.0	1.26±0.14	0.04±0.01	53±4	7.37±0.11	8.32±0.09
5.0	1.81±0.37	0.05±0.01	60±6	7.32±0.21	8.35±0.09
6.0	1.04±0.43	0.04±0.02	69±8	7.29±0.04	8.32±0.18
7.0	1.39±0.56	0.03±0.03	74±5	7.39±0.12	8.34±0.11
8.0	1.78±0.67	0.03±0.02	79±8	7.30±0.09	8.35±0.26
9.0	1.23±0.31	0.04±0.01	80±2	7.35±0.22	8.56±0.17
10.0	1.12±0.39	<0.02	86±23	7.30±0.09	8.44±0.03

* measured from the storage tanks, not from the rearing tanks

Results

It was found that the salinity sensitivities estimated from the specific growth rate (SGR) were different from those estimated using the relative weight gain (RWG) (Table 4.6). The relation equations between salinity and growth rate (both SGR and RWG) were drawn by the cubic polynomial equations as shown in Fig. 4.1. The relationships were highly significant ($p<0.0001$). While the OPS and THS were marginally smaller, the USLS was relatively larger when calculated using the SGR-growth curve than when calculated using the RWG-growth curve. This suggested that different measurements of growth provided different estimated values of salinity sensitivity if the polynomial curve fitting was utilised.

Table 4.6 The estimated optimal salinity (OPS), threshold salinity (THS) and upper sublethal salinity (USLS) of Murray cod fry reared in different salinities for a period of 30 days, calculated using the relationship as shown in Fig. 4.1.

Growth (%/day)	**OPS** (g/L)	**THS** (g/L)	**USLS** (g/L)
SGR	6.4	8.7	11.2
RWG	6.5	9.0	10.8

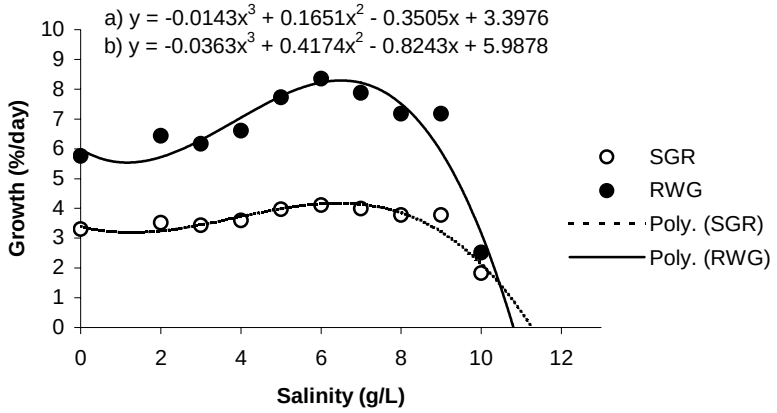

Figure 4.1 Salinity, specific growth rate (SGR) and relative weight gain (RWG) of Murray cod fry reared for 30 days in different salinities: a) correlation between SGR and salinity with R^2=0.8568; b) correlation between RWG and salinity with R^2=0.8374.

The SGR and RWG were significant among the tested salinities ($p<0.0001$) (Table 4.7). The significances were compatible in both the SGR and RWG at all test salinities. While fungal infection caused a high mortality of 30.0% in fish of the freshwater group, no infection was observed in fish reared in the saline media.

Ammonium concentrations in the rearing tanks were much higher than the threshold concentration recommended for fish stocking facilities (Forteath, 1990).

Table 4.7 Specific growth rate (SGR), relative weight gain (RWG) and mortality of Murray cod fry (initial weight of 0.101 g) reared in different salinities for 30 days at 20ºC. *a*, *b*, *c*, *d* and *e* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different from each other.

Salinity (g/L)	**Final weight** (g)	**SGR** (%/day)	**RWG** (%/day)	**Mortality** (%)
0	0.275±0.027	3.30±0.16 *a*	5.76±0.43 *a*	30.0
2.0	0.296±0.033	3.52±0.30 *ab*	6.44±0.92 *ab*	3.3
3.0	0.287±0.032	3.43±0.05 *ab*	6.17±0.21 *ab*	10.0
4.0	0.301±0.031	3.59±0.19 *abd*	6.61±0.51 *abd*	0.0
5.0	0.335±0.026	3.97±0.04 *cd*	7.73±0.15 *cd*	0.0
6.0	0.353±0.051	4.11±0.16 *c*	8.35±0.68 *c*	6.7
7.0	0.339±0.032	3.99±0.27 *cd*	7.88±0.78 *c*	0.0
8.0	0.318±0.035	3.78±0.26 *bcd*	7.18±0.81 *bcd*	3.3
9.0	0.318±0.030	3.78±0.22 *bcd*	7.18±0.58 *bcd*	0.0
10.0	0.177±0.017	1.83±0.15 *e*	2.52±0.32 *e*	33.3

4.4.2 Impacts of temperature on sublethal salinity

Experimental protocol

Facilities were limited for testing the salinity sensitivity of fry fish against different temperatures; therefore the 2 and 3 g/L test media were omitted in this experiment.

The experiment comprised three different temperature sets: 15, 20, and 25ºC. The temperature in the experimental vessels was controlled to selected levels by bathing the vessels in constant flow-through water units except for the unit of 20ºC, which was exposed to the constant room temperature. A total of 240 Murray cod fry of an average initial weight of 0.101g were used. The fry were randomly divided into 3 groups of 80 fish – each group for each temperature set. The 80 fish for each temperature were randomly divided into 8 groups of 10 fish – one group for each

salinity level. The fish were reared in 4-L glass aquaria in salinities of 0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 g/L at density of 2.5 fish/L, and fed to satiation twice a day with Instar-I *Artemia* nauplii. After being reared for 30 days the numbers that survived were counted. The final weights of the fish were measured individually. Each fish was used as replicate for statistical analysis (not a true replicate). The excess water on the body surface was removed using paper towel before measuring the body weight of the samples. The pooled samples each of 3 fish were then dried to a constant weight at 105ºC. The body water content was the subtraction of the dry weight from wet weight. The water quality physico-chemistry in the rearing tanks was measured regularly and shown in Table 4.8, 4.9 and 4.10.

Table 4.8 Water qualities in fry rearing tanks at 15°C (15.1±0.2°C).

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness*** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)
0	0.46±0.14	<0.02	52±12	7.39±0.05	8.50±0.33
4.0	0.73±0.28	<0.02	56±2	7.24±0.09	8.53±0.36
5.0	0.86±0.22	0.04±0.02	61±6	7.31±0.26	8.53±0.31
6.0	1.06±0.12	0.04±0.03	66±5	7.27±0.06	8.51±0.35
7.0	0.90±0.17	0.04±0.03	73±4	7.45±0.09	8.46±0.19
8.0	0.78±0.19	0.03±0.02	78±9	7.19±0.06	8.62±0.25
9.0	0.73±0.21	<0.02	82±2	7.35±0.03	8.82±0.13
10.0	0.57±0.32	<0.02	88±2	7.39±0.15	8.68±0.21

* measured from the storage tanks, not from the rearing tanks

Table 4.9 Water qualities in fry rearing tanks at 20°C (20.0±0.2°C).

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness*** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)
0	1.10±0.39	0.03±0.01	52±12	7.28±0.16	8.34±0.05
4.0	1.40±0.54	0.04±0.01	56±2	7.34±0.01	8.30±0.12
5.0	1.87±0.49	0.05±0.01	61±6	7.34±0.22	8.34±0.09
6.0	1.83±0.53	0.05±0.03	66±5	7.16±0.06	8.36±0.15
7.0	1.57±0.41	0.04±0.03	73±4	7.34±0.11	8.34±0.10
8.0	1.82±0.73	0.03±0.01	78±9	7.33±0.11	8.65±0.23
9.0	1.21±0.38	0.03±0.01	82±2	7.34±0.12	8.68±0.20
10.0	1.03±0.29	<0.02	88±2	7.27±0.08	8.54±0.02

* measured from the storage tanks, not from the rearing tanks

Table 4.10 Water qualities in fry rearing tanks at 25°C (25.0±0.1°C).

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness*** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)
0	2.04±0.73	0.03±0.01	52±12	7.36±0.05	8.25±0.11
4.0	1.92±0.72	0.04±0.01	56±2	7.30±0.07	8.22±0.22
5.0	2.18±0.78	0.05±0.01	61±6	7.43±0.27	8.24±0.23
6.0	2.01±0.87	0.04±0.03	66±5	7.19±0.06	8.21±0.23
7.0	1.91±0.07	0.04±0.03	73±4	7.38±0.05	8.24±0.23
8.0	2.26±0.92	0.04±0.02	78±9	7.27±0.09	8.47±0.46
9.0	2.05±0.76	0.03±0.01	82±2	7.39±0.07	8.52±0.46
10.0	1.27±0.34	<0.02	88±2	7.37±0.14	8.42±0.04

* measured from the storage tanks, not from the rearing tanks

Results

It was found that temperatures between 15 and 25ºC modified salinity sensitivity of Murray cod fry reared in different salinities between 0 and 10.0 g/L. The differences were significant among the temperatures ($p<0.0001$) and among tested salinities ($p<0.05$) (Table 4.11). There was no interaction between temperature and salinity ($p=0.4057$). While the SGR was relatively constant in salinities between 0 and 9.0 g/L at 15 and 25ºC, it was significantly high in 6.0 g/L at 20ºC, suggesting that this was the optimal salinity for growth.

Table 4.11 Specific growth rate (SGR) of Murray cod fry (initial weight of 0.101 g) reared for 30 days in different salinities at different temperatures; *a*, *b* and *c* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different from each other.

Salinity (g/L)	**SGR** (%/day)		
	15ºC	**20ºC**	**25ºC**
0	1.45±0.84 *a*	3.08±0.48 *a*	3.91±0.74 *a*
4.0	1.33±0.93 *a*	3.37±0.54 *a*	4.95±0.61 *b*
5.0	1.55±0.66 *a*	3.95±0.57 *a*	4.61±0.47 *b*
6.0	1.88±0.46 *a*	4.15±0.97 *b*	4.60±0.48 *b*
7.0	1.88±0.90 *a*	3.66±0.77 *a*	4.46±0.55 *ab*
8.0	1.65±0.87 *a*	3.41±0.48 *a*	4.49±0.61 *b*
9.0	1.22±0.76 *a*	3.46±0.82 *a*	3.84±0.58 *a*
10.0	0.18	2.02±0.70 *c*	0.34

When the sensitivity levels were estimated from the relation between SGR and salinity (Fig. 4.2) it was found that while the highest optimal salinity was observed at

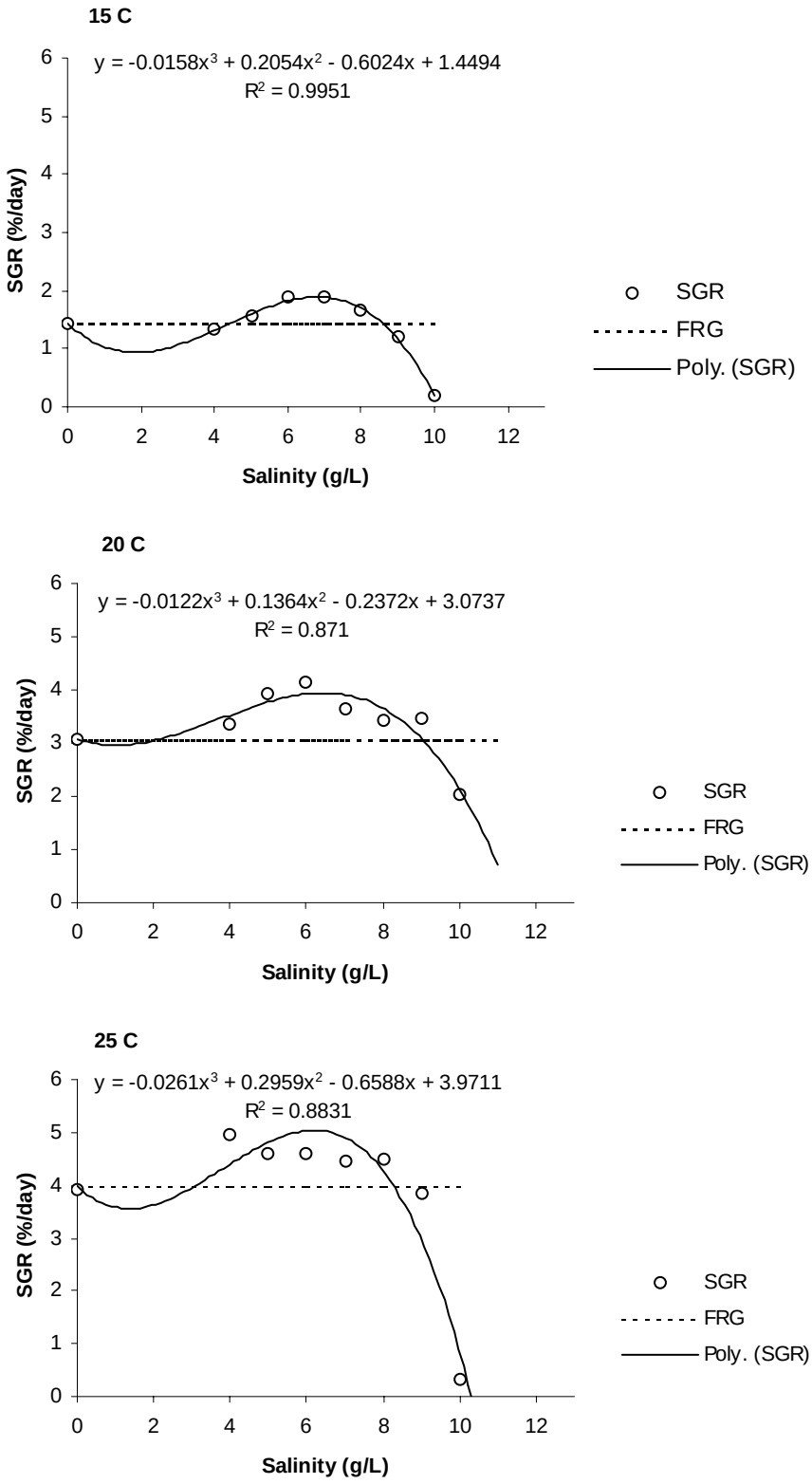

Figure 4.2 Specific growth rate (SGR) of Murray cod fry reared in different salinities at different temperatures for 30 days at 15, 20 and 25ºC. The relationships between SGR and salinity were drawn as the cubic polynomial regression. The relationships were significant ($p<0.05$ in all temperatures).

15ºC, the highest threshold and upper sublethal salinities were obtained at 20ºC (Table 4.12). This suggested that a temperature of 20ºC enhanced salinity tolerances of fry fish.

Table 4.12 Estimated freshwater growth rate (FRG), optimal salinity (OPS), threshold salinity (THS), and upper sublethal salinity (USLS) of Murray cod fry reared for 30 days in different salinities at different temperatures.

Temperature (ºC)	**FRG** (SGR,%/day)	**OPS** (g/L)	**THS** (g/L)	**USLS** (g/L)
15	1.45	6.8	8.6	10.1
20	3.07	6.5	9.0	11.4
25	3.97	6.2	8.3	10.3

High mortalities were observed in salinities above 7.0 g/L at 15 and 25ºC. Only one fish survived at 10.0 g/L in 15 and 25º C (Table 4.13). Most of the fish survived at 20ºC throughout the period of the experiment. Skin erosion was observed in fish reared in 10.0 g/L. This suggested the sublethal effects at salinity of 10.0 g/L.

Table 4.13 Mortality percentages of Murray cod fry reared in different salinities at different temperatures.

Salinity (g/L)	**Mortality** (%)		
	15ºC	**20ºC**	**25ºC**
0	20	0	10
4.0	0	0	10
5.0	0	0	0
6.0	0	0	0
7.0	0	0	0
8.0	40	0	50
9.0	10	10	20
10.0	90	20	90

No impact of salinity between 0 and 10.0 g/L on dehydration was observed, though there were significant differences in body water content found among the test salinities ($p<0.0001$) and among the temperatures ($p<0.0001$) (Table 4.14). The differences were likely caused by the differences in body weight rather than salinity (Fig. 4.3).

Ammonium concentrations in the rearing tanks were much higher than the threshold concentration recommended for fish stocking facilities (Forteath, 1990), especially in the high temperatures.

Table 4.14 Body water content of Murray cod fry (initial weight of 0.101 g) reared for 30 days in different salinities at different temperatures; *a*, *b*, *c*, *d* and *e* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different from each other.

Salinity	**Body water content** (%)		
(g/L)	**15ºC**	**20ºC**	**25ºC**
0	81.7±0.5 *a*	79.6±0.1 *bc*	78.4±0.2 *a*
4.0	80.2±0.7 *bcd*	78.2±0.2 *e*	78.2±0.2 *a*
5.0	79.5±0.2 *cd*	78.9±0.4 *d*	78.1±0.3 *a*
6.0	79.4±0.1 *d*	79.3±0.1 *cd*	78.2±0.3 *a*
7.0	79.6±0.4 *cd*	78.9±0.1 *bc*	78.2±0.1 *a*
8.0	80.4±0.4 *bc*	78.8±0.2 *a*	78.4±0.2 *a*
9.0	80.6±0.4 *b*	79.0±0.5 *ab*	78.7±0.6 *a*
10.0	-	80.1±0.1 *c*	-

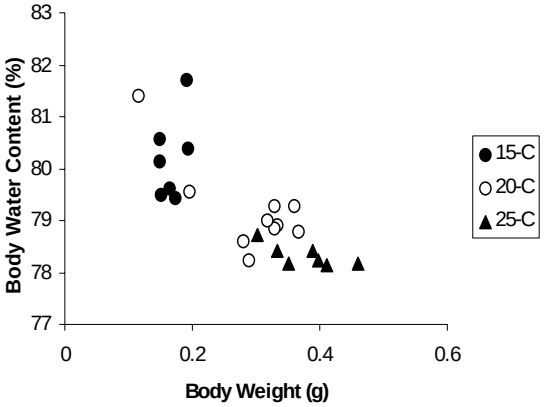

Figure 4.3 Body water content of Murray cod fry reared in different salinities at temperatures 15º, 20º and 25ºC, showing relation to the final body weight.

4.4.3 Impacts of starvation on sublethal salinity

Experimental protocol

The experiment comprised three different feeding regimes: daily feed (f1), every second day feed (f2), and every third day feed (f3). A total of 300 Murray cod fry of an average initial weight of 0.101 ± 0.011 were used. The fry were randomly divided into 3 groups of 100 fish – one group for each feeding regime. The 100 fish in each feeding regime were then divided into 10 groups of 10 fish – one group for each salinity level. The fish were reared for 30 days in 4-L glass aquaria in salinities of 0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 g/L with a density of 2.5 fish/L, and fed according to the experimental regimes three times a day with Instar-I *Artemia* nauplii. The last feed was given at about 4-5 pm with an amount just sufficient to be ingested within a few hours to ensure that there was no food left at the next feeding time. After being reared for 30 days all fish were sacrificed for measurement of weight gain. The mortality was determined. The final weights of the fish were measured individually, and each fish was used as a replicate for statistical analysis (not a true replicate). Before the samples were weighed, excess water on the body surface was removed using paper towel. The water physico-chemistry in the rearing tanks was measured regularly. The results were as shown in Tables 4.15, 4.16 and 4.17.

Table 4.15 Water qualities in fry rearing tanks when fish were fed daily (f1).

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness*** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)	**Temp.** (°C)
0	0.79±0.50	0.03±0.02	52±12	7.35±0.06	8.34±0.05	19.83±0.06
2.0	1.03±0.61	0.03±0.01	53±6	7.34±0.25	8.35±0.12	20.00±0.26
3.0	1.11±0.60	<0.02	55±7	7.25±0.04	8.34±0.10	19.97±0.12
4.0	1.14±0.56	0.04±0.02	56±2	7.29±0.06	8.42±0.19	19.57±0.49
5.0	1.27±0.67	0.04±0.02	61±6	7.34±0.25	8.41±0.12	19.90±0.10
6.0	1.52±0.57	0.05±0.03	66±5	7.25±0.04	8.31±0.13	19.90±0.10
7.0	1.72±0.95	0.03±0.02	73±4	7.37±0.07	8.38±0.09	19.87±0.06
8.0	1.01±0.88	0.03±0.02	78±9	7.25±0.05	8.54±0.36	19.97±0.06
9.0	1.08±0.74	0.03±0.02	82±2	7.34±0.05	8.58±0.35	19.93±0.12
10.0	0.97±0.55	0.03±0.02	88±2	7.35±0.15	8.52±0.27	19.97±0.21

* measured from the storage tanks, not from the rearing tanks

Table 4.16 Water qualities in fry rearing tanks when fish were fed every second day (f2).

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness*** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)	**Temp.** (°C)
0	0.49±0.46	<0.02	52±12	7.41±0.09	8.28±0.07	19.90±0.10
2.0	0.56±0.40	<0.02	53±6	7.39±0.24	8.32±0.11	19.90±0.16
3.0	0.51±0.43	<0.02	55±7	7.28±0.20	8.34±0.12	19.93±0.06
4.0	0.63±0.35	<0.02	56±2	7.39±0.09	8.29±0.13	19.90±0.10
5.0	0.56±0.40	<0.02	61±6	7.39±0.24	8.11±0.13	19.97±0.06
6.0	0.64±0.34	<0.02	66±5	7.28±0.20	8.28±0.15	19.98±0.12
7.0	0.56±0.38	<0.02	73±4	7.43±0.08	8.31±0.13	19.93±0.07
8.0	0.56±0.38	<0.02	78±9	7.36±0.15	8.24±0.34	19.99±0.06
9.0	0.55±0.41	<0.02	82±2	7.43±0.09	8.38±0.15	19.91±0.12
10.0	0.49±0.46	<0.02	88±2	7.36±0.14	8.49±0.31	19.90±0.21

* measured from the storage tanks, not from the rearing tanks

Table 4.17 Water qualities in fry rearing tanks when fish were every third day (f3).

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness*** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)	**Temp.** (°C)
0	0.21±0.02	<0.02	52±12	7.38±0.04	8.31±0.08	19.91±0.10
2.0	0.22±0.04	<0.02	53±6	7.36±0.22	8.35±0.14	20.03±0.25
3.0	0.26±0.05	<0.02	55±7	7.25±0.14	8.41±0.12	20.00±0.10
4.0	0.31±0.10	<0.02	56±2	7.36±0.03	8.22±0.23	19.60±0.53
5.0	0.33±0.07	<0.02	61±6	7.26±0.24	8.34±0.10	19.91±0.10
6.0	0.28±0.06	<0.02	66±5	7.36±0.20	8.31±0.13	19.90±0.12
7.0	0.31±0.08	<0.02	73±4	7.39±0.05	8.34±0.09	19.90±0.11
8.0	0.27±0.14	<0.02	78±9	7.33±0.10	8.57±0.31	19.97±0.07
9.0	0.26±0.17	<0.02	82±2	7.40±0.07	8.34±0.16	20.00±0.02
10.0	0.23±0.11	<0.02	88±2	7.33±0.09	8.52±0.27	20.00±0.20

* measured from the storage tanks, not from the rearing tanks

Results

It was found that starvation modified the salinity sensitivity of Murray cod fry reared in different salinities between 0 and 10.0 g/L. The differences were significant among the feeding regimes ($p<0.0001$) and among the salinity levels ($p<0.0001$) (Table 4.18). While the SGR was relatively constant in all test salinities when fish were fed every third day, the SGR was significantly lower in salinity of 10.0 g/L when fish were fed daily and every second day. This suggested that suspension of feeding for one and two days did not adjust the impacts of salinity on growth. The analyses of data also found no interaction between the salinity level and the feeding regime ($p=0.1447$).

Table 4.18 Specific growth rate (%/day) of Murray cod fry (initial weight of 0.101 g) reared for 30 days in different salinities with different feeding regimes, daily fed (f1), every second day fed (f2), and every third day fed (f3); *a*, *b* and *c* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different from each other.

Salinity	*Feeding regime*		
(g/L)	f1	f2	f3
0	3.34±0.80 *a*	1.93±0.34 *ac*	0.96±0.21 *a*
2.0	3.97±0.62 *ab*	2.76±0.88 *b*	1.97±0.58 *b*
3.0	3.48±0.87 *a*	2.67±0.59 *b*	1.56±0.65 *bc*
4.0	3.56±0.84 *a*	2.63±0.78 *b*	1.71±0.56 *b*
5.0	4.03±0.44 *ab*	2.57±0.89 *ab*	1.84±0.49 *b*
6.0	4.27±0.57 *b*	2.60±0.55 *ab*	1.57±0.51 *bc*
7.0	4.00±0.57 *ab*	2.81±0.66 *b*	1.57±0.55 *bc*
8.0	3.96±0.73 *ab*	2.76±0.62 *b*	1.49±0.64 *bc*
9.0	3.92±0.56 *ab*	2.53±0.52 *ab*	1.64±0.47 *bc*
10.0	1.83±0.47 *c*	1.36±0.41 *c*	1.04±0.44 *ac*

Starvation modified the optimal salinity for growth of fry fish. It was found that the while the best SGR was likely to be obtained at salinities between 5.0 and 7.0 g/L when fish were fed daily, the best SGR was obtained at salinities between 2.0 and 9.0 g/L when fish were fed every second day and every third day.

When the sensitivity levels were estimated from the relation between SGR and salinity (Fig. 4.4), it was found that while the optimal salinity was higher in fish fed daily than fed every second day, by contrast, the threshold and upper sublethal salinities were higher in fish fed every second day than in fish fed daily (Table 4.19). This suggested that starvation modified the salinity sensitivity of fry fish. The relationship between growth and salinity of fish fed every third day was unable to be used to estimate the salinity sensitivity of fish.

Table 4.19 Estimated freshwater growth rate (FRG), optimal salinity (OPS), threshold salinity (THS), and upper sublethal salinity (USLS) of Murray cod fry reared for 30 days in different salinities with different feeding regimes.

Feeding regimes	**FRG** (SGR,%/day)	**OPS** (g/L)	**THS** (g/L)	**USLS** (g/L)
Daily	3.52	6.4	8.7	11.3
Every second day	2.07	5.7	9.3	11.9
Every third day	1.06	not estimated	not estimated	not estimated

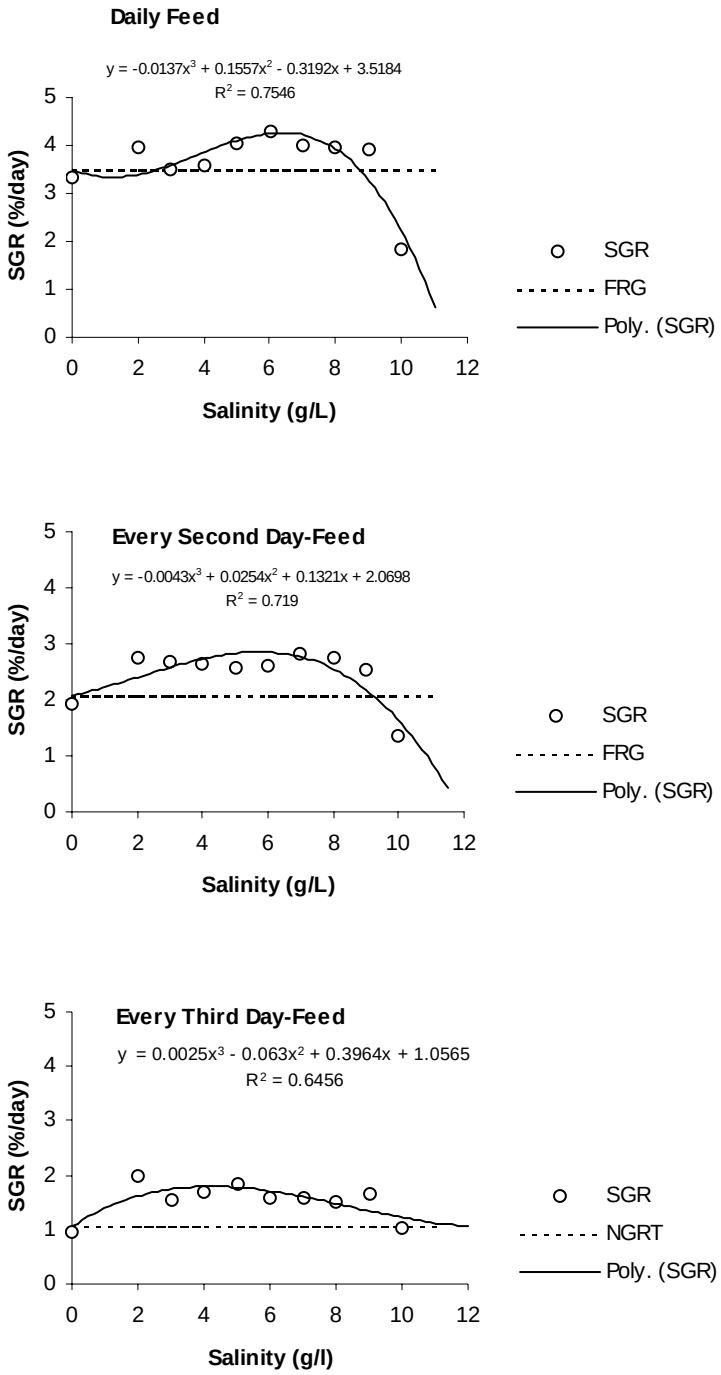

Figure 4.4 Specific growth rate (SGR) of Murray cod fry reared for 30 days in different salinities with different feeding regimes. The relationships between SGR and salinity were drawn as the cubic polynomial regression. The relationships at daily and every second day feed were significant ($p<0.05$). However, the relationship at every thrid day feed was not significant ($p>0.05$).

Though high mortality rates of 40% were found at the salinity of 10.0 g/L when feedings were suspended for one and two days (Table 4.20), weight gain was still observed. All fish survived at 10.0 g/L salinity when fed daily. The skin erosion syndrome was found in some fish that survived in 10.0 g/L. This suggested sublethal effects in salinity of 10.0 g/L.

Ammonium concentrations in the rearing tanks were much higher than the threshold concentration recommended for fish stocking facilities (Forteath, 1990), especially when fish were fed daily.

Table 4.20 Mortality percentages of Murray cod fry reared in different salinities with different feeding regimes.

Salinity	**Mortality** (%)		
(g/L)	**f1**	**f2**	**f3**
0	10	10	10
2.0	0	10	0
3.0	0	0	0
4.0	0	0	10
5.0	0	0	0
6.0	0	0	0
7.0	0	0	0
8.0	0	0	0
9.0	0	0	10
10.0	0	40	40

4.5 Discussion

Growth characteristics of Murray cod fry in different salinities

It was found that the growth rates of Murray cod fry reared in different salinities were compatible with the empirical salinity-growth model established in Chapter 2. The relationship between growth and salinity demonstrated the third degree polynomial function. The correlations were significant, with correlation coefficient (R^2) between 0.7962 and 0.9920. However, some growth curves showed a downward curve initially due to missing growth data at 2 and 3 g/L. The magnitudes of the growth curves varied according to the different levels of temperature and starvation. Temperature and starvation were also found to modify the impacts of salinity on growth of fry Murray cod. The impacts are discussed in later sections.

Effects of temperature on salinity sensitivity of Murray cod fry

During long-term exposure (30 days), the average growth rate in salinities between 4 and 9 g/L was better in the group of fish reared in a temperature of 25˚C than in fish reared at 20˚C and 15˚C, while the threshold salinity was greater at 20˚C than at 25˚C. This demonstrated that temperature adjusted the impacts of salinity on growth, resulting in modification of threshold salinity of Murray cod fry. Imsland et al. (2001) found the interaction of temperature and salinity on growth of juvenile turbot (*Scophthalmus maximus*) when reared in temperatures of 10 to 22ºC and salinities of 15, 25 and 33.5 g/L. Boeuf and Payan (2001) also claimed complex interactions between temperature and salinity on growth of fish. Elliott (1981) found that although the best growth of brown trout was obtained at 18ºC, the energy losses through the faeces and excretory products were also increased with temperature and therefore the energy available for growth was greatest at lower temperatures of about 15ºC. This temperature is therefore regarded as the optimum temperature. He suggested that the temperature of 18ºC supported the active metabolic rate (the rate of maximum energy intake and growth), but the optimum energy intake was obtained at a temperature of 15ºC (the value that produced the greatest growth for the least energy intake at each temperature). Therefore, this present study suggests that an optimal temperature of 20˚C rather than 25˚C determines the best threshold salinity sensitivity and the best growth in Murray cod fry.

This present study found that a temperature of 20˚C provided the highest LC50 and threshold salinity both in short and long-term exposures. Temperatures outside this range would alter growth, and therefore reduce the tolerance capability of Murray cod fry. Hardewig and Dijk (2003) stated that the reduction of growth rate outside the threshold temperature resulted from a reduction in the activities of digestive enzymes. Temperature tolerances also depend on the size (Atwood et al., 2003) and sexual differentiation (Hernandez et al., 2002) of the fish.

Different temperatures demonstrated the impacts on the amplitude and profile of salinity-growth curves, with curves flattened in 15ºC low temperature, expanded in 20ºC, and elevated but not expanded in 25ºC. The extension of the growth curve,

towards the end-point of growth in increasing salinity at 20ºC, explained the optimal temperature for the upper sublethal salinity.

This section concluded that the salinity-growth curves of Murray cod fry reared in different salinities and in different temperatures could be drawn by the cubic polynomial functions. Temperature modified the impacts of salinity on the tolerance capability of Murray cod fry. The optimal temperature to enhance salinity tolerances, i.e. LC50, threshold and upper sublethal salinities, was determined to be 20˚C. A temperature increment of approximately 5˚C from the optimal range enhanced growth in the tolerable range, but accelerated mortality in high salinity.

Effects of pH on salinity sensitivity of Murray cod fry

This study found that an increase in the pH of the test media from 6.2 to 8.8 enhanced salinity tolerance of fish fry. Parra and Yufera (2002) also found that larvae of marine fish such as gilthead seabream (*Sparus aurata*) could not tolerate pH lower than 4.82 within a period of 24 h.

McWilliams and Potts (1978) have pointed out that acid waters, particularly those below pH 4.5, are toxic to fish and can have marked effects upon the osmoregulatory processes. They found that the gills of brown trout were highly permeable to hydrogen ions at low environmental pH. This results in an inhibition of Na^+ influx in fish.

This study demonstrated that pH in the range of 6 to 9 modified the impacts of salinity on the median lethal tolerance of Murray cod fry. However, no errors or formal comparisons are made statistically. It also suggested that the lethal degree was enhanced in acid conditions more than in an alkaline environment.

Effects of starvation on salinity sensitivity of Murray cod fry

The feeding regimes were designed to simulate the starvation that fish might have encountered in their natural environment. It was found that the freshwater growth rate and optimal salinity were reduced when feeding ceased for one day, while salinity

tolerant thresholds were marginally increased. This demonstrated the modification of starvation on salinity sensitivity of Murray cod fry. It showed that in salinities lower than their threshold concentration, increasing the level of starvation in conjunction with increasing salinity reduced the optimal salinity for growth, but in contrast enhanced threshold salinity. This could be due to certain small amounts of sodium chloride concentration being utilised by fish, therefore extending the tolerance scales of the threshold level. Peters and Boyd (1972) also found that salinity compensated weight losses in starved hogchoker (*Trinectes maculatus*).

Qiu and Qin (1995) also found that the energy loss of starved juvenile carp (*Cyprinus carpio*) was reduced in the increasing salinity within their tolerance range when exposed to different salinities. Jobling (1995) states that though carbohydrates and lipids and proteins make up the major part of the ingested food diet of fish, it is the sodium ion that is important in food absorption into the epithelial cells of the intestinal walls (Jobling, 1995).

Saline environments were also claimed to enhance survival of aquatic animals in starving conditions. Cooper and Heinen (1991) reported that the larvae of freshwater prawn (*Macrobrachium rosenbergii*) lived longer in their optimal salinities than other tested salinities when feedings were suspended. It was concluded that the longer survival could be attributed to less energy expenditure to osmoregulate the salt-water balance in the optimal salinity (Cooper & Heinen, 1991).

The salinity-growth curves show that suspension of feeding affected the altitude and profile of the curves, with curves flattened when feedings were suspended for one and two days, but not otherwise resembling the curves obtained in the temperature trials. The curves indicated that the upper sublethal salinity did not differ among fish fed in different feeding regimes. This suggested that the suspension of feeding for one and two days did not adjust the upper tolerance limit of fish fry though weight gain was not observed.

This section concluded that starvation modified the impacts of salinity on the salinity tolerances of Murray cod fry. However, growth rates were reduced significantly in starving conditions when the fish were reared in different salinities. The suspension of

feedings for 1 day merely enhanced the threshold and upper sublethal salinities of Murray cod fry.

Mortality and sublethal effects

Most of the Murray cod fry died within 30 days when reared in a salinity level of 10.0 g/L. Mass mortalities were related to the combined effects of extreme salinity, temperature and lack of food rather than a single tested factor. Results showed that though fry fish grew best in freshwater at a temperature of 25.0ºC, fish could not survive in a salinity of 10.0 g/L at this temperature. This indicated that mortality was enhanced by increasing the temperature in a high salinity. The previous section also concluded that a temperature increment of approximately 5˚C from the optimal range enhanced growth in the tolerable salinity, but accelerated mortality in high salinity. In starvation conditions, it was found about half of the tested fish died within 30 days when reared in 10.0 g/L. This suggested that starvation significantly reduced chances of survival. Some of the survivors at the salinity of 10.0 g/L, both in the temperature and the starvation trials, demonstrated sublethal effects featuring skin erosion (see discussion in chapter 5). Fish also showed some degree of dehydration when reared in the salinity level of 10.0 g/L (see discussion in chapter 5). There were no quantitative evaluations on the impacts of the temperature and starvation on the salinity sublethal effects on Murray cod fry.

This section concluded that temperature and starvation had impacts on mortality rates and reduced the chances of survival in sublethal salinity.

4.6 Summary

pH, temperatures and starvation had significant effects on the growth and salinity tolerance of Murray cod fry. The salinity sensitivity of Murray cod fry can be summarised as follows:

1) The LC50 (96 h, pH 6.8) in different temperatures is 11.3, 12.0±0.5 (n = 2), 11.4 and 7.0 g/L at 15, 20, 25 and 30ºC respectively.

2) The LC50 (96 h, 20.0 ± 1.2ºC) in different pH is 10.0, 11.3, 11.5 and 12.1 g/L at the pH of 6.2, 7.1, 8.0 and 8.8 respectively.
3) The freshwater growth rates (as specific growth rate,%/day) at 15, 20 and 25ºC are 1.45, 3.24±0.23 (n=2) and 3.97 respectively.
4) The optimum salinities at 15, 20 and 25ºC are 6.8, 6.5±0.1 (*n* = 2) and 6.2 g/L respectively.
5) The threshold salinities at 15, 20 and 25ºC are 8.6, 8.9±0.2 (*n* = 2) and 8.3 g/L respectively.
6) The upper sublethal salinities at 15, 20 and 25ºC are 10.1, 11.8±0.6 (*n* = 2) and 10.3 g/L respectively.
7) At 20ºC, the LC50 (12.0 g/L) and upper sublethal salinity (11.8 g/L) are very similar.
8) The freshwater growth rates (as specific growth rate,%/day) when fed daily, every second day and every third day are 3.52, 2.07 and 1.06 respectively.
9) The optimum salinities when fed daily and every second day are 6.4 and 5.7 g/L respectively.
10) The threshold salinities when fed daily and every second day are 8.7 and 9.3 g/L respectively.
11) The upper sublethal salinities when fed daily and every second day are 11.3 and 11.9 g/L respectively.
12) The temperature levels between 15 and 25ºC have significant effects on growth rates among the tested salinities (0-10.0 g/L), but there is no interaction between temperature and salinity on the growth rate.
13) Starvation has a significant effect on the growth performance among the test salinities (0-10.0 g/L), but there is no interaction between starvation and salinity on the growth rate.
14) Signs of sublethal effects are the reduction of growth and skin erosion.
15) The skin corrosive syndrome is found in fish reared in salinity of 10.0 g/L.

CHAPTER FIVE

Salinity sensitivity of Murray cod fingerlings

5.1 Introduction

As a freshwater fish species, the Murray cod will expend extra energy in low salinity environments in order to absorb salt from the environment into its body and help compensate for the loss due to diffusion. Therefore, when all other factors are constant in an optimal salinity environment the need to absorb is reduced. This is expected to help increase the productive deposition of energy in the form of tissues, thus enhancing growth rate. However, as salinity increases, the extra energy required to regulate salt balance will alternately reduce growth rate. The required energy can also be measured in terms of oxygen consumption in the resting condition or the so-called standard metabolic rate. Increased salinity will increase oxygen consumption rates due to increase in energy expenditure (Sutanto & Peterson, 1996).

Fingerling Murray cod, at 26 mm, have been reported to grow in a saline farm pond where the salinities varied from about 0.6 to 5.7 g/L (Cadwallader & Gooley, 1985). Some freshwater fish are also found to tolerate relatively high salinity. Eddy (1981) has found that goldfish (*Carassius auratus*) and perch (*Perca fluviatilis*) are able to survive salinity of 15-16 g/L. The tested fish showed significantly increased plasma osmotic pressures together with higher drinking rates and signs of body dehydration rate together with ionic invasion into most tissues. In maintaining body salt balance freshwater fish regulate their ion concentrations, which are equivalent to the osmotic concentration of 260 to 330 mOsmol/kgH_2O (Jobling, 1995). If these concentrations remain unchanged when freshwater fish species are transferred into certain concentrations of saline water they are considered to be salinity adaptive fish. Fish species inhabiting estuaries exhibit varying degrees of euryhalinity, and most are capable of adapting to both freshwater and full-strength seawater. In addition, many of these euryhaline species are able to cope with a rapid fluctuation in salinity levels. This includes abrupt transfers from freshwater to seawater, and vice versa, though these abrupt changes in nature would not normally be experienced. The abrupt transfer of euryhaline teleosts between extreme saline environments leads to physiological changes that can be grouped into two stages, namely the adaptive period and the regulatory period. During the adaptive period there will be general changes in the plasma ionic and osmotic strength, but over time the plasma values will gradually be restored to original values. Thus, the direct transfer from freshwater to seawater

can result in transitory increases in both plasma osmolality and ionic concentration. In the regulatory period, the plasma ionic levels and osmolality are finely regulated as ionic homeostasis. Thus, during the regulatory period, which follows the adaptive period, fish can adapt to their new saline environment (Jobling, 1995). Generally a period of acclimation can influence tolerance to salt. The longer the fish species are acclimated to a particular salinity level regime, the more tolerant they become (Hart et al., 1991). However, for the true freshwater teleosts, such as silver perch (*Bidyanus bidyanus*), pre-acclimation for several days does not increase salinity tolerance outside its range (Guo et al., 1995).

There have been no studies on Australian species to test the different effects (if any) on growth due to constant compared with fluctuating salinity increases. Evidence is available showing that acclimated fish species are more tolerant of salinity than unacclimated individuals (Bailey & James, 2000).

This study investigated salinity tolerance of fingerling Murray cod in terms of lethal, optimal threshold, and upper sublethal salinities. Growth rates and oxygen consumption were measured to determine salinity tolerance and optimal salinity of fingerling Murray cod. The salinity adaptability in different modes of transfer was also investigated by measuring the blood osmolality.

5.2 Materials and methods

5.2.1 Test media

The test media used in this study were prepared by using Pacific Sea Salt, a commercial grade sea salt and aged Melbourne main water as a diluent as described in Chapter 2. Water used was from the Melbourne main supply and subsequently directed through a high pressure sand filter to remove particulate material and then through an activated carbon filter (Permutit) to remove some dissolved materials. Residual chlorine levels were monitored regularly to ensure they remained below 0.02 mg/L. Water then passed through a primary chiller unit into a 45,000 L capacity underground concrete tank for at least 48 hours, for ageing and settling of any remaining particulates. The test media were kept in 50-L glass aquaria with lids on and without aeration.

The water chemistry of the test media in the rearing jars was measured every fourth day by using a MERCK Photometer SQ 300. The water hardness was measured using the AquaMerck Total Hardness Test (1.084047 Gesantharte-Test). The pH was measured using the WTW Microprocessor pH meter. The water temperature was measured with an Aquarium Thermo Sensor digital thermometer. The conductivity was measured using a conductivity meter, Activon Model 301. The dissolved oxygen was measured by using an Oximeter, WTW Oxi 330.

5.2.2 Experimental organisms

The batch of Murray cod fingerlings (1.04 g) used in this study was purchased from a warmwater fish farm in New South Wales (Uarah Native Fish Farm). The fish were acclimated to the experimental conditions in a temperature-controlled room at 20ºC. The fish were kept in 50-L flow-through glass aquaria and fed twice a day with ox liver, and weaned with commercial trout pellets (Skretting Australia, A Nutreco Company; 51% crude protein, 16% crude lipid, 13.8% carbohydrate). The fish were bathed weekly with sea salt at 5 g/L for 30 minutes and also every second week with a fungicide chemical used for aquarium fish to help prevent white spot disease and fungal infections. The fish were graded into three different class sizes. After the fish accepted their artificial diet they were used for a series of experimental trials which started with the largest class size.

5.2.3 Data analysis

The LC50s at different periods of time were calculated from the logistic distribution using the Probit procedure. The freshwater growth rate and optimal, threshold and upper sublethal salinities were calculated using the salinity-growth model as described in Chapter 2 and Chapter 4.

The general linear model (GLM) was used in all cases. The means of the treatments were compared by a one-way analysis of variance (ANOVA) and the paired comparisons were made using Least Significant Difference (LSD). The small or large percentage values (0- 30% or 70-100% that form a binomial distribution) were arcsine-

transformed before comparison (Zar, 1984). The dehydration rate was calculated using the equation 2.10.

5.3 Lethal salinity

5.3.1 LC50

Experimental protocol

A total of 266 fingerlings (2.8-3.0 g) were randomly divided into 3 groups. Groups 1 and 2 comprised 85 fish, and group three comprised 68 fish. Fish in each group were divided into 19 groups – one group for each salinity. The fish were exposed to the test salinities between 0 and 18.0 g/L with an interval of 1.0 g/L, and kept unfed in 4 L glass aquaria for a period of 4 days. The concentrations of the test media were designated according to the growth observed in a salinity of 5.7 g/L (Cadwallader & Gooley, 1985). The concentrations used in this study were made up to 3 times higher than that salinity to ensure that mortality would be observed. Mortality was recorded daily. The water physico-chemistry was measured once at day 3 as shown in Table 5.1.

Table 5.1 Water quality in fingerling keeping tanks used for LC50 measurements. The water measurement was done once, at day 3.

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness** (mg/L $CaCO_3$)	**pH**	**Temp.** (°C)
0	0.67	0.03	47	7.45	20.1
1.0	0.78	0.04	47	7.32	20.0
2.0	0.94	0.03	47	7.34	20.0
3.0	0.66	<0.02	48	7.23	20.0
4.0	0.71	0.02	50	7.41	19.9
5.0	0.83	0.04	50	7.44	19.9
6.0	0.85	0.03	54	7.51	19.9
7.0	0.77	0.04	60	7.55	20.0
8.0	0.63	0.04	68	7.35	19.9
9.0	0.69	0.03	68	7.27	19.9
10.0	0.86	0.05	72	7.39	20.0
11.0	0.90	0.03	75	7.35	19.8
12.0	0.79	<0.02	76	7.45	19.9
13.0	0.88	<0.02	78	7.41	20.1
14.0	0.63	<0.02	77	7.50	20.0
15.0	0.74	0.03	80	7.48	20.0
16.0	0.77	<0.02	83	7.51	20.0
17.0	0.69	<0.02	84	7.38	19.9
18.0	0.78	<0.02	87	7.43	20.0

Results

Fingerlings showed tolerance to relatively high salinity (13.7-15.0 g/L). Fish started dying at 15 g/L after the first day and all fish died at 16.0 g/L within 4 days of exposure (Table 5.2). The LC50 was estimated 15.0, 14.4 and 13.7 g/L at 24, 48 and 96 h respectively (Table 5.3). The pH was 7.4±0.1 throughout, and the temperature was 20.0±0.1°C. The ammonium concentrations in the test media were relatively high compared to the threshold level recommended for fish stocking facilities (Forteath, 1990).

Table 5.2 Mortality numbers of Murray cod fingerlings (2.8-3.0 g) exposed unfed to different salinities for 4 days. The presented numbers were the total mortality of 3 replicates.

Salinity	**Mortality**		
(g/L)	**24-h**	**48-h**	**96-h**
0	0	0	0
1.0	0	0	0
2.0	0	0	0
3.0	0	0	0
4.0	0	0	0
5.0	0	0	0
6.0	0	0	0
7.0	0	0	0
8.0	0	0	0
9.0	0	0	0
10.0	0	0	0
11.0	0	0	0
12.0	0	0	0
13.0	0	0	0
14.0	0	6	9
15.0	7	10	12
16.0	14	14	14
17.0	14	14	14
18.0	14	14	14

Table 5.3 LC50 of fingerling Murray cod at room temperature (20.0±0.1°C).

Exposure (h)	**LC50** (g/L)	**Reg. Coeff.**	**p**
24	15.0±0.0	0.81	>0.05
48	14.4±0.4	0.84	>0.05
96	13.7±0.3	0.86	>0.05

5.3.2 Impacts of lethal salinity on osmoregulation

Experimental protocol

Murray cod fingerlings (with weights of 3.0-4.0 g) were randomly divided into 3 groups of 33 fish – one group for each replicate. 33 fish in each replicate were deliberately graded to ensure the homogeneity of different sizes, and divided into 11 groups of 3 fish – one group for each salinity level. The fish were kept unfed in 4 L glass aquaria while exposed to the salinities of 0, 2.0, 4.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0 and 14.0 g/L. The highest concentration of the test media was designed according to the LC50 of 13.7 g/L obtained from the previous trial. The measurements of blood osmolality and dehydration were made after 4 days of exposure.

To determine the blood osmolality, the fish were anaesthetised in a mixture of ambient test salinity and clove oil (20,000:1 in volume) for 2 mins and then dried with paper toweling. The blood sample (40 μL) of each individual fish was taken from the caudal peduncle and collected in a 1-mL plastic vial using a 500 μL micro syringe that had been rinsed with heparin (anti-coagulant solution). The blood osmolality was measured by using an osmometer (Osmomate 030).

To determine the dehydration of fish, fish were anaesthetised in a mixture of the ambient test salinity and clove oil (10,000:1 in volume). The sample fish were then dried with paper toweling to remove excess water from the body surface and the gill cavities. Then each individual fish was weighed immediately to avoid weight loss by evaporation. The sample was then placed onto a piece of previously weighed aluminum foil. The samples were then dried to a constant weight at 105ºC.

Results

Fingerling Murray cod showed that they were unable to regulate their blood osmotic concentration when the ambient salinity increased to about one-third of seawater in the short exposure time. The blood osmolality was significantly different among the tested salinities ($p<0.0001$) (Table 5.4). The osmolality significantly increased at salinities above 10.0 g/L and followed the osmolality measured in the test media (Fig. 5.1). The

results showed some steadiness in the osmolality readings in the salinities between 0 and 8.0 g/L. The blood osmolality at the iso-osmotic point was approximately 348 mOsmol/kgH_2O or equivalent to a salinity of 11.1 g/L. The osmotic concentration that caused death to 50% of the fish was observed at 444 mOsmol/kgH_2O.

Table 5.4 Blood osmolality and body water content of fingerling Murray cod exposed to different salinities for 96 h; *a*, *b, c, d* and *e* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different.

Salinity (g/L)	**Medium osmolality** (mOsmol/kgH_2O)	**Blood osmolality** (mOsmol/kgH_2O)	**Body water content** (%)
0	2±0	284±7 *a*	77.3±0.1 *a*
2.0	65±7	283±3 *a*	77.6±0.7 *a*
4.0	125±6	288±2 *a*	77.2±0.3 *a*
6.0	189±3	286±5 *a*	77.4±0.6 *a*
7.0	220±2	291±5 *a*	77.2±0.4 *a*
8.0	250±6	293±6 *ab*	76.7±0.6 *ab*
9.0	281±5	303±4 *b*	76.9±0.5 *ab*
10.0	312±1	320±6 *bc*	76.3±0.2 *bc*
11.0	345±4	348±8 *c*	75.8±0.9 *cd*
12.0	372±3	370±7 *c*	75.4±0.8 *d*
13.0	403±4	402±3 *d*	73.9±1.0 *e*
14.0	439±7	444±7 *e*	73.6±0.1 *e*

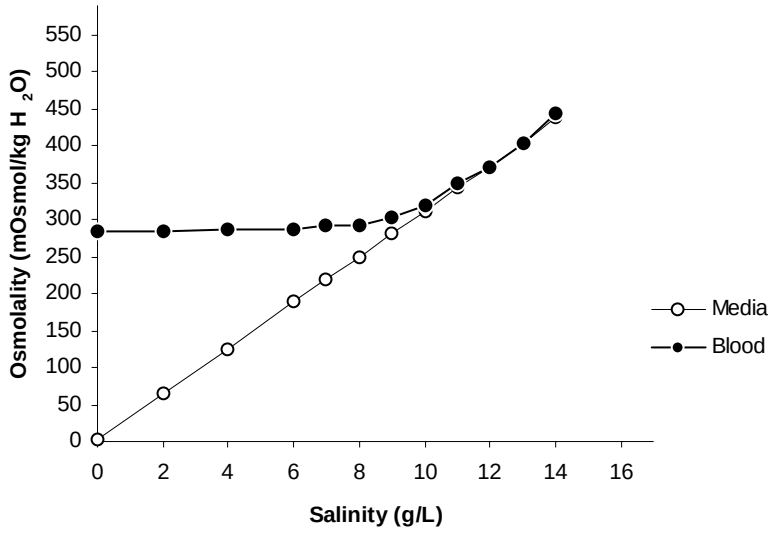

Figure 5.1 Blood osmolality of fingerling Murray cod exposed for a period of 4 days in different salinities.

Salinity was shown to cause dehydration only in extremely high salinities. The differences in body water content were significant among the salinities between 0 and 14.0 g/L ($p<0.0001$), and significantly decreased in salinities higher than 9.0 g/L (Table 5.4). 50% of fish died at 14.0 g/L when their water content was reduced from 77.3%, the level found in freshwater, to 73.6%.

5.4 Sublethal salinity

5.4.1 Impacts of sublethal salinity on growth

General experimental protocol

The highest concentration, 10.0 g/L, of the media used in this experiment was designed according to the results from the previous experiments, which found that fingerling Murray cod showed dehydration and significant increase in blood osmolality at 10.0 g/L. The concentration used was also designed to reduce suffering of the test fish, thus complying with the animal ethics regulations.

In all trials, fingerling Murray cod were fed once daily in the morning. Uneaten pellets were counted and removed in the evening. Before measurement, the fish were anaesthetised with clove oil (1:20,000). The body weights were measured in a water container of known weight.

5.4.1.1Growth characteristics in increasing salinity

Growth of fish species may be measured in terms of the relative weight gain or specific growth rate as mentioned in Chapter 4. This experiment also aimed to determine

- differences between specific growth rate and relative weight gain of fingerlings reared in different salinities
- relationship between length and weight of fingerlings reared in different salinities

- food conversion ratio, food ingestion rate and mortality of fingerlings reared in different salinities
- differences in the relationship between specific growth rate and salinity, and the relationship between relative weight gain and salinity
- salinity sensitivity parameters, i.e. optimal, threshold and upper sublethal salinities, estimated from the relative weight gain and specific growth rate.

Experimental protocol

A total of 150 fingerlings (2.01-3.32 g) were randomly divided into 5 groups of 30 fish – one group for each salinity. The 30 fish in each salinity group were randomly divided into 3 groups of 10 fish – one group for each replicate. Thus, each salinity was tested in 30 fish and three replicates. The fish were directly transferred to grow in the test media of 0, 2.0, 4.0, 6.0 and 8.0 g/L, in 20-L self-contained biofiltered glass aquaria. The total length and weight of each fish were measured every 20 days for a period of 62 days. The length-weight relationship at day-62 was drawn using the exponential function. The water physico-chemistry was monitored regularly; results are shown in Table 5.5.

Table 5.5 Water qualities in fingerling rearing tanks when directly transferred to the tested media.

Salinity (mg/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)	**Temp.** (°C)
0	0.28±0.29	1.00±0.58	45±20	7.27±0.14	8.46±0.30	20.43±0.63
2	0.20±0.26	0.95±0.54	52±23	7.46±0.11	8.52±0.29	20.20±0.75
4	0.29±0.19	0.94±0.07	61±22	7.62±0.09	8.58±0.13	20.28±0.75
6	0.20±0.16	0.60±0.73	69±26	7.54±0.11	8.60±0.19	20.18±0.85
8	0.30±0.36	0.32±0.41	74±26	7.53±0.04	8.68±0.11	20.05±0.72

Results

It was found that though the relative weight gain (RWG) was relatively larger than the specific growth rate (SGR), the differences among the test salinities (0-8.0 g/L) were compatible (Table 5.6). RWG, SGR, food conversion ratio (FCR) and ingestion rate were significantly poorer in the salinity of 8.0 g/L ($p<0.05$). High mortality was observed only at 8.0 g/L.

Table 5.6 Mean relative weight gains (RWG), specific growth rates (SGR), food conversion rates (FCR), ingestion rates and mortality of fingerling Murray cod when directly transferred to the test salinities for rearing. The means were calculated from the measurements at days 20, 30, 41, 51 and 62 of culture; *a* and *b* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different.

Salinity (g/L)	**W_{day-0}** (g)	**W_{day-62}** (g)	**RWG** (%/fish/day)	**SGR** (%)	**FCR**	**Ingestion** (%/day)	**Mortality** (%)
0	2.68±0.10	6.10±0.87	1.61±0.16 *a*	1.46±0.13 *a*	0.99±0.20 *a*	1.26±0.21 *a*	1±1
2	2.74±0.06	6.68±0.42	1.83±0.58 *a*	1.64±0.51 *a*	0.88±0.04 *a*	1.16±0.17 *a*	0
4	2.77±0.05	7.03±0.32	1.92±0.51 *a*	1.72±0.45 *a*	0.82±0.07 *a*	1.29±0.24 *a*	0
6	2.71±0.13	7.24±0.74	1.97±0.39 *a*	1.76±0.30 *a*	0.82±0.11 *a*	1.24±0.22 *a*	0
8	2.73±0.04	4.32±0.44	0.94±0.29 *b*	0.90±0.23 *b*	1.59±0.32 *b*	0.97±0.14 *b*	27±18

Salinity-growth curves, both drawn from the RWG and SGR (Fig. 5.2), were well fitted with the cubic polynomial function. Though RWG was relatively larger in value than SGR, the optimal, threshold and upper sublethal salinities were relatively similar when estimated from both RWG- and SGR-growth curves (Table 5.7).

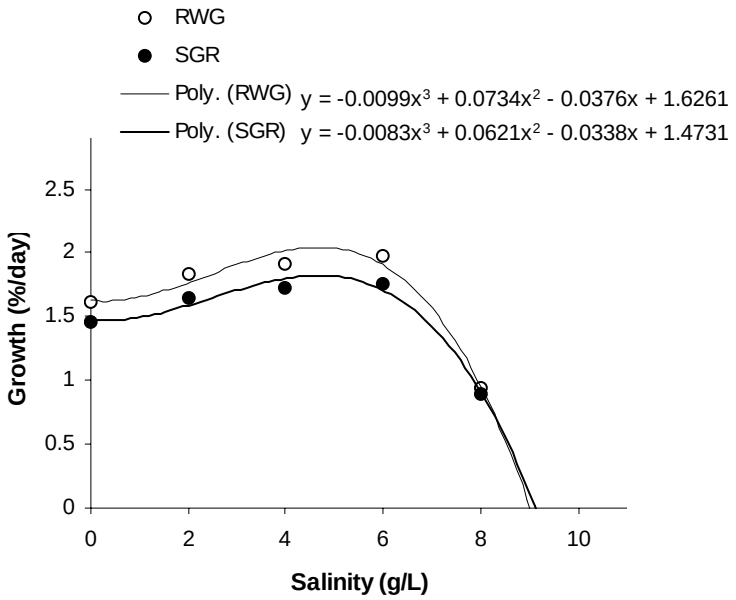

Figure 5.2 Relationship between salinity and growth of fingerling Murray cod reared in different salinities for a period of 62 days were drawn as the cubic polynomial regression. The correlation coefficient (R^2) between RWG and salinity is 0.9744 (p>0.05), and between SGR and salinity is 0.9752 (p>0.05).

Table 5.7 Freshwater growth rate (FRG), optimal salinity (OPS), threshold salinity (THS) and upper sublethal salinity (USLS) of fingerling Murray cod reared in different salinities for a period of 62 days.

Salinity (g/L)	**FRG** (%/day)	**OPS** (g/L)	**THS** (g/L)	**USLS** (g/L)
RWG	1.63	4.7	6.9	9.0
SGR	1.47	4.7	6.9	9.2

Salinity was found to have impacts on the length-weight ratio of fingerlings. It was found that the fish which grew in the salinity at 4.0 g/L had less weight at the same length than those reared in the other salinities (Table 5.8, Fig. 5.3). The relationship between length and weight of fingerlings could be drawn using the exponential function (Table 5.9).

Table 5.8 Length-weight ratio of fingerling Murray cod reared for 62 days in different salinities. The estimated weights used to calculate the ratio were obtained from the length-weight relationships in Table 5.9.

Salinity (g/L)	**Length (L)** (mm)	**Estimated weight (EW)** (g)	**L/EW**
0	70	3.26	21
2	70	3.15	22
4	70	3.05	23
6	70	3.15	22
8	70	3.32	21

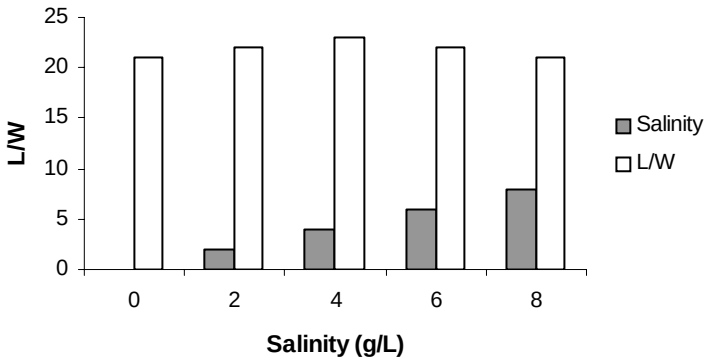

Figure 5.3 Length-weight ratios of fingerling Murray reared in different salinities for 62 days.

Table 5.9 Length-weight relationship of fingerling Murray cod reared for 62 days in different salinities.

Salinity (g/L)	No. of fish	Length (L) (mm)	Weight (W) (g)	L-W Relationship Function	R^2
0	29	83±6	6.84±1.16	$0.1118\ e^{0.0482L}$	0.9884
2	30	86±3	7.62±0.46	$0.1094\ e^{0.0480L}$	0.9913
4	30	90±2	8.03±0.42	$0.1075\ e^{0.0478L}$	0.9878
6	30	90±3	8.45±0.77	$0.1110\ e^{0.0478L}$	0.9902
8	22	77±3	4.84±0.48	$0.1089\ e^{0.0488L}$	0.9821

5.4.1.2 Growth in different modes of transfer

It has been claimed that periodic saline environments in Murray cod habitats have had impacts on the salinity tolerance of this species (Jackson & Pierce, 1992). Modes of transfer are also documented as having an effect on salinity tolerances of some freshwater fish such as *Tilapia nilotica* and a hybrid red *T. nilotica* (Wardoyo, 1991). However, in the case of an Australian species such as silver perch (*Bidyanus bidyanus*), pre-acclimation was found not to improve salinity tolerance (Guo et al., 1995).

This experiment aimed to determine the impacts of pre-acclimation on

- relationship between salinity and specific growth rate
- optimal, threshold and upper sublethal salinities
- pathological responses to sublethal salinities

a) Direct transfer

Experimental protocol

A total of 35 fingerlings of 12.12-23.08 g in weight were graded into 5 different class sizes, 12.00-13.00, 14.00-16.00, 17.00-20.00 and 21.00-23.08 g. The fish were directly transferred to be reared in the test salinities of 0, 2.0, 4.0, 6.0, 7.0, 8.0 and 9.0 g/L. Each salinity batch comprised 5 fish with different class sizes. Each fish was measured individually and utilised as a replicate. Therefore, there were five not-true replicates included in each salinity batch. The fish were reared in 20-L self-contained biofiltered glass aquaria. The length and the weight of each fish were measured every 30 days for a period of 60 days. The water physico-chemistry was monitored regularly; results are shown in Table 5.10.

Table 5.10 Water qualities in fingerling rearing tanks used for direct transfer.

Salinity (g/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness** (mg/L $CaCO_3$)	**pH**	**DO** (mg/L)	**Temp.** (°C)
0	0.98±0.69	1.70±0.58	56±17	7.37±0.16	8.16±0.14	20.03±0.23
2.0	1.14±0.26	1.11±0.50	60±12	7.24±0.11	8.42±0.18	20.00±0.34
4.0	1.22±0.33	1.16±0.51	63±12	7.33±0.12	8.46±0.21	20.20±0.08
6.0	1.04±0.46	1.15±0.54	67±13	7.44±0.21	8.32±0.19	20.00±0.35
7.0	1.29±0.39	1.24±0.57	69±12	7.32±0.12	8.38±0.23	20.21±0.05
8.0	1.20±0.78	1.61±0.73	73±18	7.44±0.21	8.41±0.17	20.08±0.15
9.0	1.36±0.46	1.31±0.61	78±21	7.33±0.24	8.28±0.12	20.07±0.42

b) Pre-acclimated in different salinities between 2.0 and 6.0 g/L

Experimental protocol

A total of 160 fingerlings of 2.01-3.32 g were divided into 4 groups of 40 fish. The 40 fish of each group were pre-acclimated for a period of 60 days in salinities of 0.0, 2.0, 4.0 and 6.0 g/L, namely control, p1, p2 and p3 respectively. The fish were reared in 50-L glass aquaria and fed daily. The acclimation media were exchanged daily. When the acclimation was completed, the fish were transferred to be reared in different salinities as follows (see also Table 5.11),

- 15 fish of the control group were reared in 0.0 g/L and used as the control for all trials.
- 30 fish of p1-group were graded into five different class sizes and transferred to be reared in test salinities of 2.0, 4.0, 6.0, 7.0, 8.0 and 9.0 g/L.
- 25 fish of p2-group were graded into five different class sizes and transferred to be reared in test salinities of 4.0, 6.0, 7.0, 8.0 and 9.0 g/L.
- 20 fish of p3-group were graded into five different class sizes and transferred to be reared in test salinities of 6.0, 7.0, 8.0 and 9.0 g/L.

The fish were reared in 20-L self-contained biofiltered glass aquaria for a period of 60 days. Individual fish were measured twice, at the start and at the end of the experiment, and used as not-true replicates for statistical analysis. Water physico-chemistry was measured regularly; results are shown in Tables 5.12, 5.13, and 5.14.

Table 5.11 Experimental designs for investigation of the impacts of pre-acclimation on salinity sensitivity of fingerlings Murray cod.

Mode of transfer	Pre-acclimatising salinity (60 days, g/L)	Growing salinity (60 days, g/L)	Fish/salinity
Control	0.0	0.0	15
p1	2.0	2.0, 4.0, 6.0, 7.0, 8.0,9.0	5
p2	4.0	4.0, 6.0, 7.0, 8.0, 9.0	5
p3	6.0	6.0, 7.0, 8.0, 9.0	5

Table 5.12 Water qualities in fingerling rearing tanks of the p1-group. The fish were reared in 2 g/L-media for 60 days and then transferred to these test media.

Salinity (g/L)	Ammonia (mg/L NH_3-N)	Nitrite (mg/L NO_2-N)	Hardness (mg/L $CaCO_3$)	pH	DO (mg/L)	Temp. (°C)
0	0.31±0.05	0.96±0.36	59±6	7.12±0.10	8.52±0.23	19.73±0.10
2.0	0.42±0.32	0.29±0.17	61±9	7.52±0.04	8.32±0.11	19.10±0.08
4.0	0.45±0.21	0.56±0.09	63±12	7.51±0.11	8.55±0.10	19.14±0.12
6.0	0.43±0.34	0.44±0.18	66±14	7.48±0.09	8.51±0.21	19.09±0.14
7.0	0.41±0.24	0.61±0.18	69±13	7.51±0.05	8.56±0.09	19.13±0.10
8.0	0.49±0.26	0.31±0.17	70±13	7.52±0.10	8.50±0.17	19.05±0.13
9.0	0.52±0.28	0.42±0.28	75±14	7.46±0.10	8.52±0.27	19.10±0.26

Table 5.13 Water qualities in fingerling rearing tanks of the p2-group. The fish were reared in 4 g/L-media for 60 days and then transferred to these test media.

Salinity (g/L)	Ammonia (mg/L NH_3-N)	Nitrite (mg/L NO_2-N)	Hardness (mg/L $CaCO_3$)	pH	DO (mg/L)	Temp. (°C)
0	0.31±0.05	0.96±0.36	59±6	7.12±0.10	8.52±0.23	19.73±0.10
4.0	0.56±0.31	0.32±0.17	62±10	7.48±0.09	8.40±0.14	19.05±0.10
6.0	0.49±0.21	0.53±0.18	66±11	7.50±0.09	8.44±0.16	19.10±0.10
7.0	0.39±0.26	0.53±0.26	68±12	7.53±0.06	8.64±0.17	19.10±0.08
8.0	0.51±0.21	0.29±0.18	71±14	7.46±0.16	8.44±0.18	19.08±0.10
9.0	0.48±0.24	0.32±0.25	73±10	7.49±0.09	8.44±0.26	19.20±0.16

Table 5.14 Water qualities in fingerling rearing tanks of the p3-group. The fish were reared in 6 g/L-media for 60 days and then transferred to these test media.

Salinity (g/L)	Ammonia (mg/L NH_3-N)	Nitrite (mg/L NO_2-N)	Hardness (mg/L $CaCO_3$)	pH	DO (mg/L)	Temp. (°C)
0	0.31±0.05	0.96±0.36	59±6	7.12±0.10	8.52±0.23	19.73±0.10
6.0	0.64±0.27	0.30±0.17	63±7	7.46±0.18	8.38±0.11	19.10±0.10
7.0	0.69±0.18	0.63±0.21	72±7	7.52±0.03	8.48±0.11	19.08±0.12
8.0	0.59±0.21	0.32±0.16	71±10	7.56±0.01	8.54±0.09	19.05±0.13
9.0	0.71±0.22	0.49±0.25	76±11	7.50±0.09	8.64±0.13	19.13±0.22

Results

Pre-acclimation was likely to affect the salinity sensitivity of fingerling Murray cod. The SGR was significantly different among the salinities of 0-9.0 g/L in all modes of transfer ($p<0.01$). The SGR significantly decreased in salinity of 9.0 g/L (Table 5.15). Mortality was only observed in salinities of 8.0 and 9.0 g/L (Table 5.16). The relationship between salinity and SGR was expressed by the cubic polynomial function (Fig. 5.4, 5.5, 5.6 and 5.7). The optimal salinity (OPS) increased from 4.5 to 5.0 g/L when fish were pre-acclimated in 2.0 g/L. The highest threshold salinity (THS) of 7.4 g/L was also observed in the group of fish pre-acclimated in 2.0 g/L. The highest upper sublethal salinity (USLS) of 9.9 g/L was obtained in the group of fish pre-acclimated in 4.0 g/L (Table 5.17). The differences between OPS, THS and USLS were only marginal. This suggested that pre-acclimatising fingerling Murray cod in salinities of 2.0 and 4.0 merely enhanced their salinity tolerances. Pre-acclimatising fish in 6.0 g/L for 60 days did not improve salinity tolerances.

The ammonium and nitrite concentrations were relatively high compared to those recommended for a recirculation system (Forteath, 1990).

Table 5.15 Mean specific growth rate (SGR) of fingerling Murray cod gradually transferred to be reared in different salinities with different modes of transfer. The letters *a*, *b* and *c* indicate significance of the differences ($p=0.05$); values followed by similar letters are not significantly different.

Salinity (g/L)	SGR (%/day)			
	Direct	**p1**	**p2**	**p3**
0	1.23±0.55 *a*	1.45±0.42 *a*	1.45±0.42 *ab*	1.45±0.42 *a*
2.0	1.35±0.03 *a*	1.83±0.48 *a*	-	-
4.0	1.39±0.02 *a*	1.59±0.12 *a*	1.59±0.33 *a*	-
6.0	1.43±0.14 *a*	1.82±0.11 *a*	1.62±0.16 *a*	1.60±0.41 *a*
7.0	1.14±0.06 *a*	1.49±0.21 *a*	1.04±0.02 *ac*	1.37±0.30 *a*
8.0	0.65±0.01 *b*	1.27±0.17 *a*	0.91±0.18 *bc*	0.61±0.48 *b*
9.0	0.11±0.03 *b*	0.43±0.21 *b*	0.51±0.35 *c*	0.15±0.23 *b*

Table 5.16 Mortalities of fingerling Murray cod reared in different salinities with different modes of transfer.

Salinity (g/L)	Mortality (%)			
	Direct	**p1**	**p2**	**p3**
0	0	0	0	0
2.0	0	0	-	-
4.0	0	0	0	-
6.0	0	0	0	0
7.0	0	0	0	0
8.0	20	0	0	0
9.0	20	20	20	20

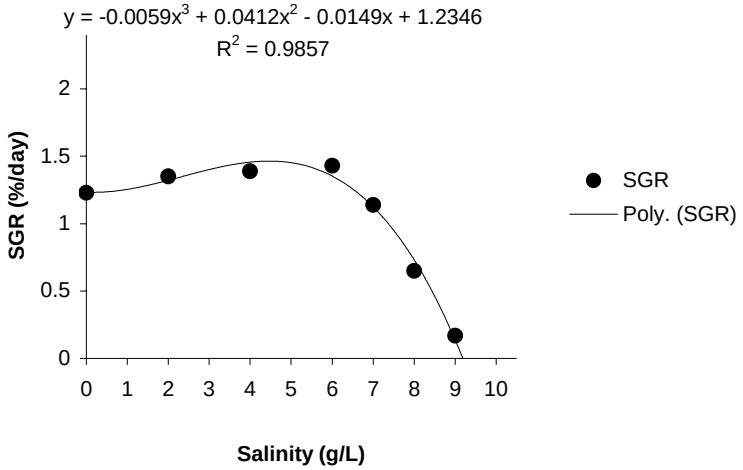

Figure 5.4 Relationship between salinity and specific growth rate (SGR) of fingerling Murray cod directly transferred to be reared in different salinities for 60 days ($p<0.05$).

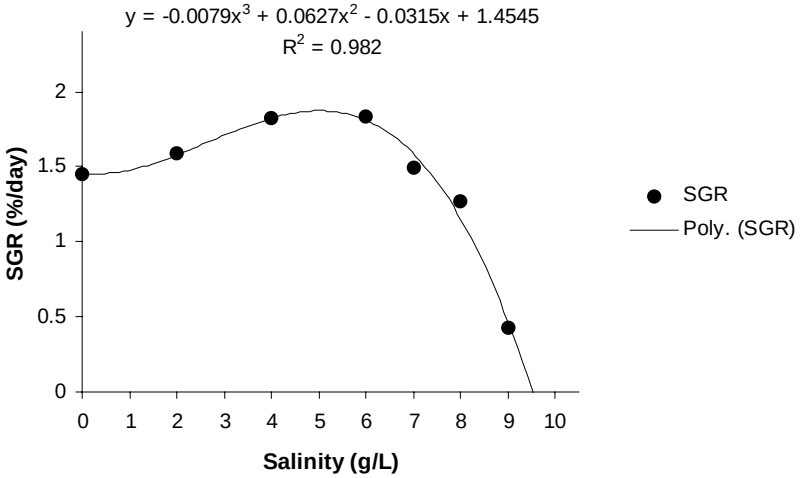

Figure 5.5 Relationship between salinity and specific growth rate (SGR) of fingerling Murray cod pre-acclimated in 2.0 g/L for 60 days and transferred to be reared in different salinities for 60 days ($p<0.05$).

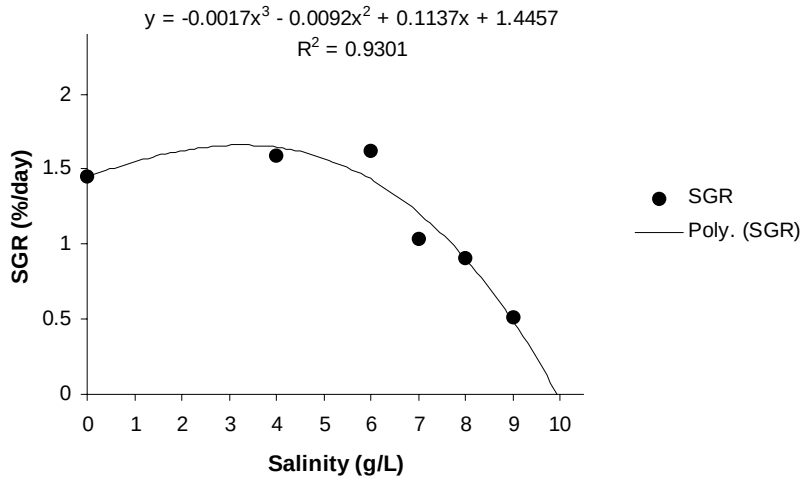

Figure 5.6 Relationship between salinity and specific growth rate (SGR) of fingerling Murray cod pre-acclimated in 4.0 g/L for 60 days and transferred to be reared in different salinities for 60 days ($p>0.05$).

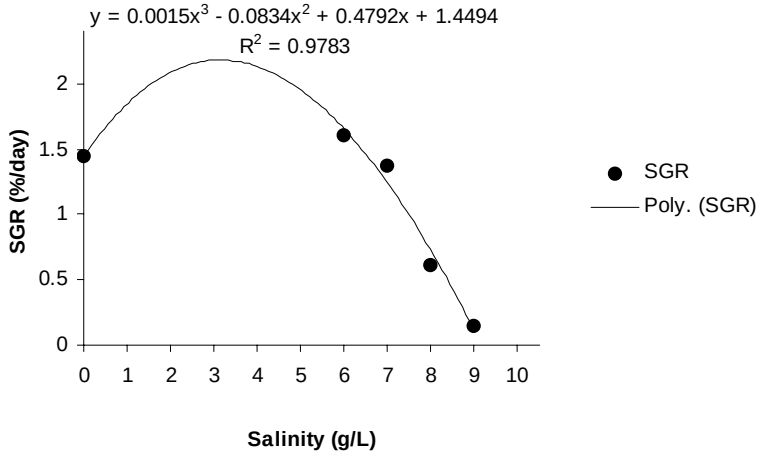

Figure 5.7 Relationship between salinity and specific growth rate (SGR) of fingerling Murray cod pre-acclimated in 6.0 g/L for 60 days and transferred to be reared in different salinities for 60 days ($p>0.05$).

Table 5.17 The estimated optimal salinity (OPS), threshold salinity (THS) and upper sublethal salinity (USLS) of fingerling Murray cod reared in different salinities with different modes of transfer.

Modes of transfer	**OPS** (g/L	**THS** (g/L)	**USLS** (g/L)
Direct	4.5	6.6	9.2
p1: pre-acclimation in 2 g/L	5.0	7.4	9.5
p2: pre-acclimation in 4 g/L	not estimated	5.9	9.9
p3: pre-acclimation in 6 g/L	not estimated	6.5	9.2

5.4.2 Impacts of sublethal salinity on osmoregulation

Experimental protocol

Murray cod fingerlings (2.82-3.09 g) were randomly divided into 11 groups of 20 fish – one group for each salinity level. The fish were reared in 50-L self-contained biofiltered glass aquaria, in test salinities of 0, 2.0, 4.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0 and 14.0 g/L. The fish were fed to satiation once daily. Measurements of the blood osmolality and dehydration were made twice at 1 day and 41 days after exposure. The water physico-chemistry was measured once at day-10 and the results are shown in Table 5.18.

Table 5.18 Water qualities in fingerling rearing tanks used for blood osmolality and body water content. The measurement was done once at day-10.

Salinity (g/L)	**Ammonia** (mg/L NH_3-N)	**Nitrite** (mg/L NO_2-N)	**Hardness** (mg/L $CaCO_3$)	**pH**	**Temp.** (°C)
0	0.56	0.54	50	7.33	20.1
2.0	0.54	0.42	51	7.54	20.1
4.0	0.41	0.36	52	7.56	20.1
6.0	0.37	0.51	54	7.57	20.1
7.0	0.37	0.48	59	7.59	20.0
8.0	0.41	0.46	67	7.45	20.0
9.0	0.50	0.61	68	7.33	20.1
10.0	0.30	0.68	70	7.41	20.0
11.0	0.29	0.53	76	7.35	20.0
12.0	0.28	0.57	75	7.38	20.0
13.0	0.33	0.35	78	7.52	20.1
14.0	0.27	0.71	80	7.34	20.1

Results

Periods of exposure between 1 and 41 days merely demonstrated the effect on the osmotic concentration of fingerling fish. The differences in the blood osmolality were highly significant among test salinities between 0 and 14.0 g/L at day-1 ($p<0.0001$) and 0 and 11.0 g/L at day-41 ($p<0.0001$) (Table 5.19). While the osmolality significantly increased in salinities higher than 9.0 g/L in fish exposed for a period of 1 day, a significant increase was observed in salinities higher than 6.0 g/L when fish were exposed for a period of 41 days. This suggested that a longer exposure of 41 days merely benefits the osmoregulation adaptability of fingerling Murray cod. There was no interaction between the salinity and exposure time between 1 and 41 days ($p=0.3384$).

Table 5.19 Blood osmolality of fingerling Murray cod reared for 1 day and 41 days in different salinities; *a*, *b, c, d* and *e* indicate significance of the differences (p=0.05). Values followed by similar letters are not significantly different.

Salinity (g/L)	**Medium osmolality** (mOsmol/kgH_2O)	**1 day** (mOsmol/kgH_2O)	**41 days** (mOsmol/kgH_2O)
0	1±1	282±6 *a*	280±8 *ab*
2.0	62±4	284±2 *a*	277±9 *a*
4.0	124±7	286±3 *a*	276±3 *a*
6.0	187±5	286±6 *a*	282±6 *abc*
7.0	219±2	290±4 *a*	288±4 *bc*
8.0	250±5	299±4 *ab*	290±4 *bc*
9.0	280±5	301±7 *ab*	292±7 *c*
10.0	312±4	318±4 *bc*	320±4 *d*
11.0	343±4	344±6 *c*	
12.0	375±7	376±5 *c*	
13.0	406±4	400±4 *d*	
14.0	438±6	340±8 *e*	

The osmolality of blood increased to the point where it was identical to the osmolality of the test media at 11.0 g/L after which it followed the osmolality of the media, i.e. the iso-osmotic line (Fig. 5.8). The fish did not survive after 38 days at salinity levels higher than 10.0 g/L.

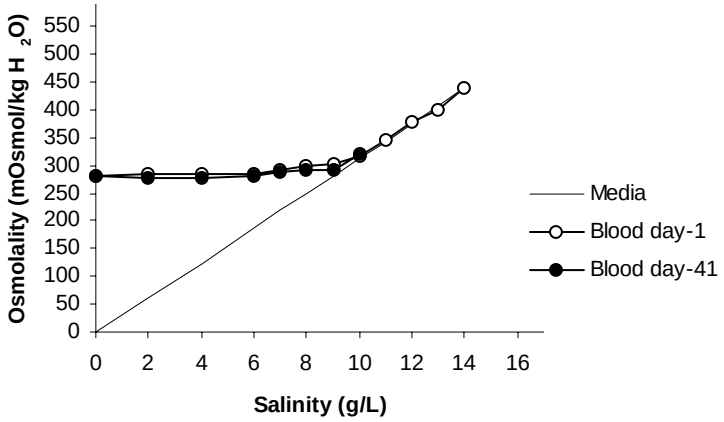

Figure 5.8 Blood osmolality of the fingerling Murray cod reared in different salinities for 1 day and 41 days.

Time of exposure between 1 and 41 days did not show an effect on dehydration in fingerling fish. The differences in body water content were significant among test salinities between 0 and 14.0 g/L at day-1 ($p<0.0001$) and between 0 and 11.0 g/L at day-41 ($p<0.01$) (Table 5.20, Fig. 5.9). Water contents were significantly reduced in salinities higher than 9.0 g/L both in 1 and 41 days. Dehydrations of 2.2 and 2.6% were observed at 10.0 g/L when exposed for periods of 1 day and 41 days respectively. This also suggested that a longer exposure of 41 days did not benefit osmoregulation adaptability of fingerling Murray cod.

Table 5.20 Body water contents of fingerling Murray cod reared in different salinities for 1day (2.82-3.75 g) and 41 days (3.98-7.01 g); *a*, *b*, *c*, *d* and *e* indicate differences at the confidence-level of 0.05. Values followed by similar letters are not significantly different.

Salinity (g/L)	Body water content (%)	
	1 day-exposure	41 day-exposure
0	77.3±0.1 *a*	76.8±0.6 *a*
2	76.8±0.7 *ab*	76.5±0.4 *ab*
4	76.7±0.3 *abc*	76.9±0.6 *a*
6	77.4±0.4 *a*	77.1±0.8 *a*
7	76.9±0.4 *a*	77.4±0.2 *a*
8	76.8±0.6 *ab*	76.4±0.9 *ab*
9	76.6±0.5 *ab*	76.8±1.2 *a*
10	75.6±0.2 *bcd*	75.4±0.5 *b*
11	75.3±1.2 *cd*	
12	74.8±1.4 *de*	
14	73.9±1.0 *e*	

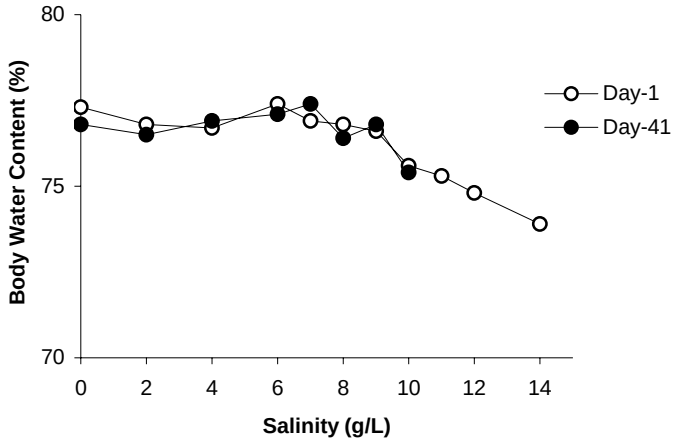

Figure 5.9 The body water contents of fingerling Murray cod reared in the different salinities for 1 day and 41 days.

5.4.3 Impacts of sublethal salinity on pathological responses

Experimental protocol

The abnormalities that may be encountered were observed in fish reared in the salinity tolerance trials. The characteristics of physical change were categorised and the numbers of fish that showed these abnormalities were recorded.

Results

Pathological responses were found in fingerlings reared in salinities where the reduction in growth was observed. The symptoms were characterised as:

- skin erosion
- abnormal-eye
- emaciation and melanism

The corrosive skin syndrome was the most common symptom that occurred in the fish reared in salinities higher than 7.0 g/L (Table 5.21). The earliest case appeared within 10 days after exposure, and the severity increased with long exposure (Fig. 5.10). Some of the severely affected fish survived through the period of experiment (60-62 days). Abnormality of the eyes was found in the form of cataracts, exophthalmos, and loss of one or both eyes. The wound caused by the loss of an eye healed in fish reared in the saline media, and these fish survived through the period of the experiment. Fish reared in a salinity of 10.0 g/L also showed signs of emaciation associated with melanism.

Table 5.21 Pathological responses found in fingerling Murray cod reared in different salinities for 60 days or 62 days.

Salinity (g/L)	**No. of fish used**	**% pathological responses**		
		Skin erosion	**Eye abnormality**	**Emaciation**
0	50	0	4	0
2.0	40	0	0	0
4.0	45	0	0	0
6.0	50	0	6	0
7.0	20	0	10	0
8.0	50	30	10	0
9.0	50	40	20	8

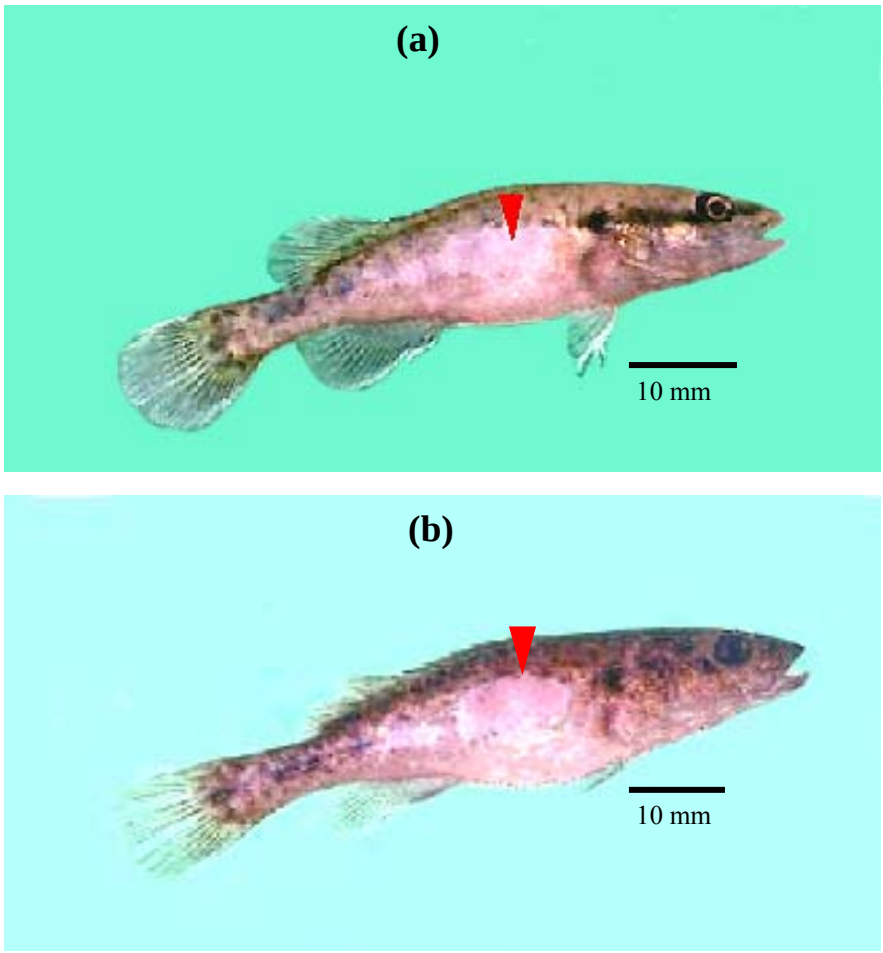

Figure 5.10 Corrosive skin syndrome in fingerling Murray cod observed after rearing for, (a) 10 days and (b) 20 days in salinity of 8.0 g/L.

5.4.4 Impacts of sublethal salinity on oxygen consumption

Experimental protocol

There were two trials of oxygen consumption rates, including immediate exposure and long-term exposure.

In the immediate exposure trial, Murray cod fingerlings weighing 13.02-53.23 g were graded into three different class sizes. The fish of each class size were divided into 9 groups – each group used for each salinity level. There were eight levels of salinity: 0, 2.0, 4.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 g/L, and a control group in freshwater. Therefore, there were three replicates of three different class-size fish at each salinity level. The oxygen consumption rate measurement comprised acclimatising the fish to the test media in the respiratory chamber for 30 minutes in 90% dissolved oxygen saturation water (about 8.19 mg/L at 20ºC) with a flow rate of 246 L/min. The measurements were then made immediately after the flow rate had been switched to 0.49 L/h continuously for 30-50 minutes.

In the long-term exposure trial, Murray cod fingerlings were initially reared for 60-70 days in the test media of 0, 2, 4, 6, 8 and 10 g/L. Fish weighing 7-8 g from each salinity level were selected – 3 fish from each salinity level were used for oxygen consumption measurements. The oxygen consumption measurement comprised acclimatising the fish to the test media in the respiratory chamber for 30 minutes in 90% dissolved oxygen saturation water (about 8.19 mg/L at 20ºC) with a flow rate of 246 L/min. The measurements were then made immediately after the flow rate had been switched to 0.49 L/h continuously for 30-50 minutes.

Results

Time of exposure was found not to affect the oxygen consumption rate in different salinities. Salinities higher than 8.0 g/L were shown to have an impact on the oxygen consumption rate of fingerling Murray cod. The consumption rates were significantly different in fish directly exposed to salinities between 0 and 11.0 g/L ($p<0.0001$) (Table 5.22), and were different in fish exposed for a period of 60-70 days to the salinities between 0 and 10.0 g/L ($p<0.001$) (Table 5.23). The consumption rates were

significantly increased in salinities above 8.0 g/L, both in directly exposed fish and in long-term exposed fish.

When the relationship between the consumption rate and body weight of the fish used was drawn (Fig. 5.11), it was found that the differences in body weight obscured the significance of the consumption rates in the direct exposure (the second column in Table 5.17). However, the differences were found to be significant when the consumption rates were calibrated to the body weight (the last column in Table 5.22).

Table 5.22 Oxygen consumption of fingerling Murray cod directly exposed to different salinities. *a*, *b* and *c* indicate significance of the differences (p=0.05); values followed by similar letters are not significantly different from each other.

Salinity (g/L)	Measured Consumption (c) (mg O_2/g/h)	Body weight (W) (g)	Relationship of Consumption-weight (function)	($R^{2)}$	Weight corrected consumption (c) (mg O_2/g/h)
0	0.298±0.100 *a*	28.62±12.05	$c=3.5608\ W^{-0.7694}$	0.8805	0.260±0.007 *a*
2	0.270±0.094 *a*	34.13±16.31	$c=1.7199\ W^{-0.5508}$	0.9999	0.264±0.005 *ab*
4	0.278±0.072 *a*	31.07±13.52	$c=1.5815\ W^{-0.5253}$	0.9748	0.265±0.005 *ab*
6	0.276±0.038 *a*	29.33±10.67	$c=1.0949\ W^{-0.4156}$	0.9683	0.266±0.004 *ab*
7	0.266±0.108 *a*	36.41±16.93	$c=2.5918\ W^{-0.6639}$	0.9829	0.271±0.006 *b*
8	0.308±0.110 *a*	29.51±15.34	$c=3.4675\ W^{-0.7517}$	0.9987	0.269±0.007 *ab*
9	0.344±0.068 *a*	24.54± 7.13	$c=3.9982\ W^{-0.7779}$	0.9921	0.284±0.007 *c*
10	0.287±0.096 *a*	35.70±16.17	$c=2.4834\ W^{-0.6263}$	0.9705	0.295±0.006 *d*
11	0.317±0.057 *a*	27.19±14.42	$c=1.1012\ W^{-0.3914}$	0.9974	0.291±0.004 *cd*

Table 5.23 Oxygen consumption of fingerling Murray cod reared in different salinities for 60-70 days. *a*, *b* and *c* indicate significance of the differences (p=0.05); values followed by similar letters are not significantly different from each other.

Salinity (g/L)	Consumption (mg O_2/g/h)	Weight (g)	Temperature (ºC)
0	0.631±0.009 *a*	7.2±0.6	20.9±0.1
2	0.600±0.012 *a*	7.5±0.2	21.4±0.0
4	0.574±0.067 *a*	7.7±0.7	21.1±0.1
6	0.596±0.054 *a*	7.9±0.9	21.4±0.0
8	0.617±0.047 *a*	7.6±0.9	21.6±0.0
9	0.714±0.034 *b*	7.7±0.5	21.2±0.2
10	1.302±0.045*	3.1±0.1	21.3±0.1

* not included in the statistical analysis because of the great difference in body weight

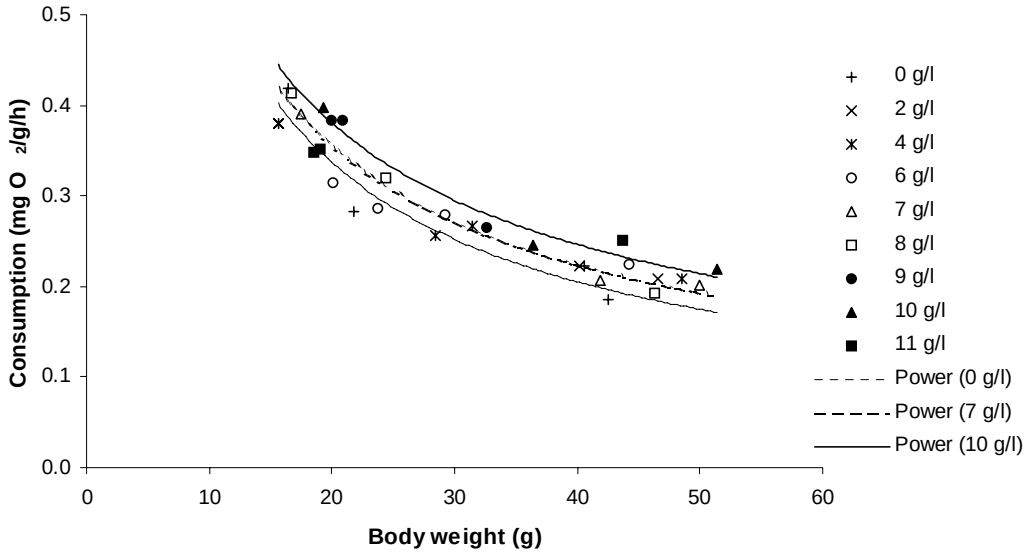

Figure 5.11 Oxygen consumption of fingerling Murray cod immediately exposed to different salinities; the differences are significant between the data illustrated with the open marks (0, 2, 4, 6, 7, and 8 g/L) and the data with the closed marks (9, 10 and 11 g/L) ($p<0.05$).

5.5 Discussion

Salinity sensitivity of fingerling Murray cod

In short-term exposure experiment, by estimating the LC50, it was found that half of the fingerlings tested were able to tolerate salinity levels up to 13.7 g/L for 4 days at about 20ºC. Ryan and Davies (1996) also documented the LC50 of Murray cod at 13.2 g/L when directly transferred; however, the length of time of exposure and the size of fish were not mentioned.

The overall growth results of Murray cod fingerlings were compatible with the salinity-growth model of freshwater fish species reared in different salinities as proposed in this study (see details in chapter 2). The relationship between growth and salinity was described by the characteristics of the cubic polynomial function.

The characteristics of growth rate could be described as follows:

- Growth rate immediately increases with increasing salinity from freshwater. This suggests growth is enhanced by salinity.

This finding is not a surprise because mild salinity has been known to enhance growth performances of fish (e.g., Dendrinos & Thorpe, 1985; Abud, 1992; Guo et al., 1993; Qiu & Qin, 1995; Kibria et al., 1999). The effects of salinity on growth performance may be a result of the changing rates of osmoregulation (Qiu & Qin, 1995). In their natural habitat freshwater species such as Murray cod will expend a certain amount of energy on osmoregulation processes in order to compensate for salt loss due to diffusion. Providing a small amount of salt in their habitat helps to compensate for energy expenditure and consequently promotes growth.

- From freshwater, the growth rate continues to elevate, peaking at salinities between 4.5 and 5.0 g/L and then falling off. This suggests an optimal salinity for growth.

Studies by Febry and Lutz (1987) and Potts (1954) have suggested that energy expenditure rates are lowest in an iso-osmotic environment, which should result in optimal performance. However, it has been found that the iso-osmotic concentration of fingerling Murray cod (11.0-11.1 g/L) is much higher than the optimal salinity (4.5-5.0 g/L) where maximal growth rates were observed in this study. Studies on growth trials on teleost fishes done by Kumaragura and Kamalam (1991), Morgan and Iwama (1991) and Lambert *et al.* (1994) have also failed to show these relationships of best growth and iso-osmotic environment. They state that the optimal salinity levels for growth appear to vary according to each species, their life stage, and seasons.

This study also found that the optimal salinity merely improved when the fingerling Murray cod were pre-acclimated in a salinity of 2.0 g/L for a period of 60 days. Konstantinov and Martynova (1990, 1993) claimed that the periodic fluctuations in salinity within the species tolerance limits accelerated the growth rate of juvenile fish. Konstantinov and Martynova (1990, 1993) stated that the enhancement of the growth rate was explained by the reaction of fish to an alteration of salinity levels associated with the adaptations to fluctuations in the tonicity of the medium. However, this reaction was attributed to the functional activation of osmoregulatory organs rather than the specific effect of sodium chloride. Results showed that the same acceleration

of the growth rates was also obtained in fish periodically dipped in a hyposulphite and soda solution in comparison to table and sea salt solutions. Their study concluded that periodic deviations of salinity from the optimal static level could accelerate the growth rates of juveniles provided that the fluctuations of the ions of the medium do not exceed the tolerance limits of the fish.

- Beyond the optimal salinity, the growth rate starts decreasing and falls below the freshwater water growth rate in salinities between 5.9 and 7.4 g/L. This point is very important as it determines the threshold salinity for growth of Murray cod. Pre-acclimatising fish in salinity of 2.0 g/L showed an improvement in this threshold tolerance.

Hart et al. (1991) have claimed that the period of acclimatisation can influence tolerance to salt in fish species. Generally, the longer the fish species has been acclimatised to a particular salinity level regime, the more tolerant it becomes. A number of studies demonstrated that low salinity pre-acclimation enhanced the growth rate of fish such as the fry of *Tilapia* sp. (Ye et al., 1990) and silver perch fingerlings (Guo et al., 1995). Ye et al. (1990) found that fry of *Tilapia* sp. demonstrated better growth rates in some levels of salinity but they were not able to adapt to rapid changes.

However, some of the studies claimed that pre-acclimation did not enhance salinity adaptability of the true-freshwater species such as silver perch (Guo et al., 1995). Guo et al. (1995) found that the pre-acclimatising of juvenile silver perch (*Bidyanus bidyanus*) to 12 g/L for seven days resulted in only a marginally better survival at higher salinities. A study by Chervinski (1984) showed that young freshwater catfish (*Clarius lazera*) pre-acclimated in 7.80 g/L and 9.75 g/L for 7 days as well as the direct transferred fish did not survive salinities higher than 9.8 g/L. Chervinski (1984) states that there are two types of freshwater fish – the so-called primary freshwater fish (migrating wholly in freshwater) and secondary freshwater fish (experience salinity for part of their life history). The primary freshwater species such as Claridae and Cyprinidae are only able tolerate low salinity levels below 9.75 g/L, whereas secondary freshwater fishes, such as Cichlidae, Cyprinodontidae and Poecilidae are

able to survive higher salinity levels through gradual adaptation due to a more developed osmoregulatory capability.

This study suggests that fingerlings of Murray cod will not adapt to salinity higher than the threshold of 7.4 g/L. This finding is particularly significant because no literature reviews describing the threshold salinity of Murray cod fingerlings have been published to date.

- Beyond the threshold salinity, growth continues falling and ceases at salinities between 9.2 and 9.9 g/L. This suggests the salinity tolerance limit for growth, the so-called upper sublethal salinity. However, mortality rates were reduced when the fish were gradually transferred or when there was a fluctuation in the test salinity levels. However, 20% mortality was observed in a salinity about 1-2 g/L lower than the upper sublethal salinity.

The upper tolerance limit of fingerling Murray cod in this study was identical to the tolerant limit of 9.75 g/L in the primary freshwater fish described by Chervinski (1984). This also suggested that fingerling Murray cod are true-freshwater species and will not grow if reared in salinities higher than 9.9 g/L.

- Growth rates also show correlation to the food conversion rates. The best growth rates are likely obtained between 2.0 and 6.0 g/L salinity where the lowest food conversion of 0.82-0.88 is also observed. This indicates a suitable salinity for growth of fingerling Murray cod. The ingestion rates were apparently lower in salinity between 8.0 and 9.0 g/L. This suggests a salinity stress level in fish.

Qiu and Qin (1995) found that the effects of salinity on food consumption in juvenile common carp (*Cyprinus carpio*) were significant where the maximum consumption rates at 3, 5 and 7 g/L were greater than in the control, and that there was a significant decrease at 9 g/L. Woo and Kelly (1955) suggest that the optimal salinity will cause a reduction in the metabolic rate of osmoregulation and a re-organisation of metabolism which will allow protein sparing in favour of a shift towards preferential utilisation of

carbohydrates and lipids. Ye et al. (1990) also suggest that the reduction in growth rate may be explained partly by the decreased food intake of the stressed fish affected by salinity. The salt causes a clear additional effect itself, because extra energy is required by the fish to enable it to survive under unsuitable conditions. De Boeck et al. (2000) also state that fish are likely to ingest less food at unsuitable salinity levels. This present study suggests that the significant differences in the growth rate performance of fingerling Murray cod are not attributed to the food conversion rate. The decrease in feeding efficiency strongly suggests physiological impairment of fish (Jarvis, 2001). Boeuf and Payan (2001) have claimed that better growth is not systematically correlated to a lower standard metabolic rate. They added though that many studies have claimed the energy budget of 20% to more than 50% is dedicated to osmoregulation, their study indicates that the osmotic cost is not as high (roughly 10%) as this.

Sublethal effects on fingerling Murray cod

Apart from reductions in the growth rate, the impacts of sublethal salinities in Murray cod fingerlings were also observed as pathology responses. Increase in the blood osmotic concentration was observed in fingerlings reared in salinities above their threshold salinity. However, dehydration was only observed in fish reared in salinities higher than their upper sublethal salinity. This suggests that the measurement of dehydration is not sufficient to detect early stages of sublethal responses. The pathological responses may also be associated with poor water quality and therefore may produce experimental errors. Such sublethal effects can be used for descriptive (qualitative) interpretation only.

The pathological responses were found in the form of corroded skin, abnormal-eye and emaciation, and were more likely to be encountered in fish reared in salinities higher than their threshold level.

The signs of corrosive skin syndrome in the fingerlings began with up to five skin lesions present on individual fishes, and these were distributed primarily on the lateral body surfaces. The earliest lesions of focal epidermal erosions 1 to 2 mm in diameter were associated with hyperemia and hemorrhage. These lesions later appeared to

progress laterally and more deeply into the dermis. In advanced cases, the ulcers extended deep into the underlying musculature. The symptoms were similar to the ulcerative dermal necrosis (UDN) found in migratory salmonids when they entered freshwater for spawning as described by Roberts and Shepherd (1986). Roberts and Shepherd (1986) also stated that, during the period of transfer from freshwater to seawater, if salmon smolts go into osmoregulatory failure, the fish do not shoal properly and do not feed. Chronic skin problems frequently occurred under poor husbandry conditions, such as eroded tails and fins, and if these fish continued to live they became increasingly emaciated and undernourished, and were usually dark in colour and lethargic.

Tashiro and Iwatsuki (1996) also reported skin erosion in young Japanese centropomid fish (*Lates japonicus*) when they were reared at salinity levels of 28-30 g/L.

Callinan (1988) has described the ulcerative skin diseases of Australian native fish species as unknown or of uncertain etiology where the skin lesions differ in appearance within and between fish species. Roberts and Shepherd (1986) also have stated that signs of muscular tetany in fish that were exposed to high salinity levels may be evident as a rippling along the flanks and an increased permeability of the cornea, often resulting in severe cataract and blindness due to lens oedema.

There was no publication cited in relation to salinity and emaciation and exophthalmos.
However, Munday (1988) has explained that exophthalmos is sometimes associated with emaciation caused by *Flavobacterium* spp.

Osmoregulation and salinity adaptability in fingerling Murray cod

Blood osmolality of Murray cod fingerlings exposed to different salinities was measured to investigate salinity adaptability. It was found that when exposed for 1, 4 and 41 days, the blood osmolality of the fish tested increased significantly in salinity levels higher than 9.0 g/L in both trials. Half of the tested fish died within 4 days at salinity of 14.0 g/L. The fish in the 41-days trial eventually died at the salinity of 10.0

g/L. In all trials the blood osmolality reached a point at approximately 11 g/L where it approximated the osmolality of the tested media. After 11.0 g/L the blood osmolality followed the iso-osmotic line.

A number of studies also demonstrated significant increases of blood osmolality when freshwater fish such as goldfish and perch (Eddy, 1981) and silver perch (Guo et al., 1995) were placed in saline water. Guo et al. (1995) have also found that the plasma osmolality of juvenile silver perch (*Bidyanus bidyanus*, 6-7 cm) exposed to different salinity levels rose to follow the iso-osmotic line in further increases of salinity higher than 9 g/L. This phenomenon demonstrates that true-freshwater fish, probably including fingerling Murray cod, are not capable of adapting to salinity higher than 9 g/L.

The study showed that 50% of fingerling Murray cod died within 4 days when their blood osmolality went up to 444 mOsm/kg H_2O. There were no significant differences in the blood of fish reared in salinities of 0 to 6.0 g/L for a period of 41 days. On the other hand, this suggests that fingerling Murray cod are able to maintain steady plasma osmolality in salinities within 0 to 6.0 g/L, in contrast to the secondary freshwater fish, such as coastal largemouth bass (*Micropterus salmoides*) (Susanto and Peterson, 1996) that are able to maintain their steady plasma osmolality in salinities within 0 to 8.0 g/L. This also suggests that fingerling Murray cod are not salinity adaptable fish.

Freshwater teleosts are normally able to maintain their blood plasma osmolality within a narrow range of 260-330 mOsmol/kgH_2O (Jobling, 1995). The failure to maintain these levels for prolonged periods results in death. The transition from freshwater to seawater requires a reversal from net ion influx to a net ion efflux that is regulated primarily by the gill, but can also involve the kidney, gut and urinary bladder. In most teleosts this reversal is initiated by exposure to a hyper-osmotic environment. For a variety of euryhaline teleosts, the gill's Na^+, K^+-ATPase activity increases after transfer from freshwater to seawater. Ionic and electrical gradients generated by this enzyme are central to the current models of branchial ion fluxes (McCormick and Saunders, 1987).

Utida and Hirano (1973) state that the key structure that actually enhances salinity adaptability is the apical pit that is located on the surface of the chloride cell. The apical pit can be regenerated and degenerated within a short period of time after euryhaline fish are transferred from seawater and freshwater and vice versa. In the investigation of salinity adaptation among eels (*Anguilla japanica*) Utida and Hirano (1973) found that when they were reared in freshwater, two types of differentiating cells were observed in the basal part of the gill platelet, which have larger nuclei than other epithelial cells, namely A-cells and B-cells. After transferring the eel from freshwater to seawater, the number of A-cell increased markedly during the first week whereas B-cells increased only within the initial three days of adaptation and thereafter decreased. They have suggested that the B-cell is a transitional stage of the typical chloride cell. Furthermore, after being transferred to seawater, the apical pit appears in both A- and B-cells within 24 h. The proximal ends of these cells grow to reach the blood vessel to enable the function of ionic regulation. These changes suggest that the proliferation of chloride cells can take place only three days after being transferred and the chloride cell starts functioning within 24 h after regeneration. Tilapia (*Orechromis aureus*) and *O. niloticus* are also demonstrated to possess the chloride generation capability equivalent to seawater teleosts (Avella et al., 1993).

Changes in the gill structures of Murray cod have not been investigated in this study. However, studies with other Australian freshwater fish species, i.e. silver perch which are quite similar in their salinity tolerance, have found significant changes in the gill structures when they have been acclimated to saline water (Guo et al., 1995). The study found that juvenile silver perch when subjected to 12 g/L of salt for seven days presented with chloride cells with a more developed tubular system than those in the control fish . The length of the injunctions between the chloride cells and the accessory cells was significantly shorter in the fish adapted to 12 g/L than in the control. However, pre-acclimation of fish at 12 g/L for 7 days resulted in only a marginally better survival rate at higher salinities, which was similar to the results of gradual transfer trials of fingerling Murray cod. The results indicated a salinity adaptability of the silver perch in a narrow range even though the regeneration of chloride cells was observed. It suggests the possibility of regeneration of chloride cells in fingerling Murray cod in saline habitats.

Impacts of salinity on oxygen consumption of fingerling Murray cod

A number of studies found relationships between oxygen consumption rate and salinity in freshwater fish such as silver carp, *Hypophthalmicthys molitrix* (Von Oertzen, 1985) and common carp, *Cyprinus carpio* (Qiu & Qin, 1995). Susanto and Peterson (1996) have pointed out that the increase in oxygen consumption rates of freshwater species in salinity levels between 0 and 16 g/L is due to the increased energy expenditure.

This present study also found that the impact of salinities higher than 8.0 g/L was to increase the oxygen consumption rates of fingerling Murray cod. It was likely that fingerlings consumed less dissolved oxygen in a salinity of 4.0 g/L when exposed for a period of 41 days. Qiu and Qin (1995) also reported the lowest oxygen consumption rate at 4 g/L in the juvenile common carp (*Cyprinus carpio*); as well as this study there are the studies of Von Oertzen (1985) with juvenile silver carp (*Hypophthalmichthys moltrix*). However, the minimal oxygen consumption of fingerling Murray cod was not demonstrated at the iso-osmotic concentration as described by Farmer and Beamish (1969), Nordlie and Leffler (1975), Hettler (1976), and Abud (1992).

McCormick and Saunders (1987) suggest that the direct measurement of oxygen consumption rates in salinity adaptation is difficult to assess because of the relatively high individual variations, and the dependence on temperature and size (often requiring the use of regressions that can obscure the data). The measurement can also be affected by various levels of activity, and differences in the response to handling stress or confinement. Plaut (1999) also claims that insufficient sensitivity in the measurement of the oxygen consumption rate is due to the reactions of fish to the chronically unfavorable environmental conditions.

5.6 Summary

Fingerlings showed relatively high salinity tolerance. However, the fish showed they were unable to regulate their blood osmotic concentration when the ambient salinity increased to about one-third of seawater in the short exposure time. Dehydration was

also observed. In the longer exposure, fish showed a slightly better adaptability by maintaining a steadier blood osmolality. Pre-acclimation was also likely to affect the salinity sensitivity of fingerling Murray cod. However, the longer exposure time was found not to adjust the oxygen consumption rate in different salinities. There were pathological responses found in fingerlings reared in salinities where the reduction in growth was observed. The optimal, threshold and sublethal salinities of fingerlings may be estimated from RWG or SGR.

The salinity sensitivity of Murray cod fingerlings can be summarised as follows,

1) The LC50 (96 h, 19.8-20.1ºC) is 13.7±0.3 g/L.
2) The average optimal salinity when directly transferred is 4.6±0.1 g/L (n=2).
3) The average threshold salinity when directly transferred is 6.8±0.1 g/L (n=2).
4) The average threshold salinity when transferred followed pre-acclimation in 0.2, 0.4, 0.6 g/L for a period of 60 days is 6.6± 0.5 g/L (n=3).
5) The average upper sublethal salinity when directly transferred is 9.2±0.0 g/L (n=2).
6) The average upper sublethal salinity when transferred following pre-acclimation in 0.2, 0.4, 0.6 g/L for a period of 60 days is 9.5± 0.2 g/L (n=3).
7) Pre-acclimatising in 2.0-4.0 g/L is likely to enhance salinity tolerances.
8) The blood osmolality at the iso-osmotic point is 344-348 mOsmol/kgH_2O or equivalent to salinity of 11.0-11.1 g/L.
9) The blood osmolality at LC50 (96 h) is 444 mOsmol/kgH_2O or equivalent to salinity of 14.2 g/L.
10) The dehydration rate at LC50 (96 h) is 4.8%.
11) Pathological responses at sublethal salinity showed in the form of skin erosion, abnormal eye and emaciation.
12) Skin corrosive syndrome is found in fish reared in salinity levels over 7 g/L.
13) Oxygen consumption rates are significantly increased in salinities higher than 8.0 g/L.
14) Exposure for periods of 1 day and 41 days does not modify oxygen consumption rates in different salinities.

CHAPTER SIX

Summary and conclusion

6.1 Introduction

The objectives of this chapter are to

- summarise the results of this study
- describe the relationship between salinity and specific growth rates of fry and fingerlings of Murray cod
- compare the salinity tolerances of eggs, larvae, fry and fingerlings of Murray cod
- discuss the environmental factors in cod habitats that may adjust the salinity tolerances of Murray cod during development
- testify to the hypothesis on the declining of Murray cod population
- testify to the threshold salinity for a freshwater ecosystem as recommended by the Salinity Audit using information obtained from this study
- conclude the results of this study and suggest future studies

6.2 Summary

The impacts of salinity on the fertilisation of trout cod were determined when eggs were placed in a range of salinities (0 to 8.0 g/L), and mixed with male milt after 1-1.6 min. The percentage hatch was used as the criterion of tolerance. The results showed that either eggs or sperm of trout cod did not tolerate a salinity of 0.8 g/L.

The median lethal concentration (LC50) was measured for the larvae, fry and fingerlings. Murray cod larvae showed marginally higher tolerance to salinity than trout cod larvae when exposed for a period of 12 days, during the utilising of the yolk sac. Fingerlings of Murray cod tolerated salinity approximately 3 g/L higher than fry, and about 8 g/L higher than larvae (Table 6.1). LC50 for fry in different temperatures and pH also were measured. Fry demonstrated tolerance to higher salinity in pH 8.8 than in pH 8.0, 7.1 and 6.2, and tolerated higher salinity in temperatures between 15 and 20ºC than in 25ºC and 30ºC, when exposed for a period of 4 days.

Table 6.1 Summary of LC50, optimal salinity (OPS), threshold salinity (THS) and upper sublethal salinity (USLS) of eggs and larvae of trout cod and Murray cod, and of fry and fingerlings of Murray cod.

Developmental stage	LC50 at 96 h (g/L)	OPS (g/L)	THS (g/L)	USLS (g/L)
Trout cod				
Egg: during fertilisation	-	-	40% hatch in 0.0 g/L	-
Egg: 1 h after fertilizing	-	-	60% hatch in 0.8 g/L	-
Larva	0.50 (at 12 days)	-	0.46 (at 12 days)	
Murray cod				
Egg: 24 h after fertilizing	-	-	>10.0 g/L	-
Larva	0.35 (at 12 days)	-	0.34 (at 12 days)	-
Fry (0.20-0.45 g)				
- pH 6.2	10.0	-	-	-
- pH 7.1	11.3	-	-	-
- pH 8.0	11.5	-	-	-
- pH 8.8	12.1	-	-	-
- 15ºC	11.3	6.8	8.6	10.1
- 20ºC	12.0	6.5	8.9	11.8
- 25ºC	11.4	6.2	8.3	10.3
- 30ºC	7.0	-	-	-
- fed daily	-	6.4	8.7	11.3
- fed every second day	-	5.7	9.3	11.9
- fed every third day	-	-	-	-
Fingerling (2.01-53.34 g)				
- short-term exposure	13.7	-	-	
- direct transfer (2 trial)	-	4.6	6.8	9.2
- acclimation 60 days in 2 g/L	-	5.0	7.4	9.5
- acclimation 60 days in 4 g/L	-	-	5.9	9.9
- acclimation 60 days in 6 g/L	-	-	6.5	9.2

The threshold salinity for fry and fingerlings determined the lowest observed effect level. Growth performance was measured for periods of 30 days with fry and 60-62 days with fingerlings. The threshold salinity for larvae determined the 1% mortality figure during the period of yolk absorption. The threshold salinity was marginally higher for trout cod larvae than for Murray cod larvae (Table 6.1). The threshold salinity for fry at 20ºC was approximately 2 g/L higher the overall threshold salinity for fingerlings, and about 26 times higher than those of larvae (Table 6.1). The threshold salinity for fry was also investigated at three different levels of temperature

and three different levels of food availability. Fry were more tolerant at the threshold level in a temperature of 20ºC than in 15 and 25ºC, and surprisingly were more tolerant when feeding every 2 days than daily.

The optimal salinity determined the maximal specific growth rate when the fry were reared in different salinities. The optimal salinity for growth of Murray cod fry at 20ºC was approximately 2 times or 3 g/L higher than that of fingerling fish. The optimal salinity of fry fish reduced in temperatures higher than 15ºC, and also decreased when feeding was suspended for one and two days. Pre-acclimating in 2.0 g/L marginally increased salinity tolerances of fingerling fish.

The relationship between salinity and specific growth rates of fry and fingerlings of Murray cod was demonstrated by the cubic polynomial function as follows,

- The overall average salinity-growth curve of Murray cod fry at room temperature (approximately 20ºC) (Fig. 6.1) is determined as

$$y = -0.0120\,x^3 + 0.1675\,x^2 - 0.5316\,x + 3.7425\ .$$

- The overall average salinity-growth curve of fingerling Murray cod at room temperature (approximately 20ºC) (Fig. 6.2) is determined as

$$y = -0.0058x^3 + 0.0527x^2 + 0.0703x + 1.3703\ ,$$

where y is growth rate, i.e. specific growth rate or relative weight gain, x is salinity, and a, b, c and d are constants.

Oxygen consumption was also measured in fingerlings, and was shown to increase in all salinities (0-11 g/L) when directly transferred. The oxygen consumption increased in salinities outside 4 g/L when fingerlings were acclimated in the test salinities for a period of 60-70 days.

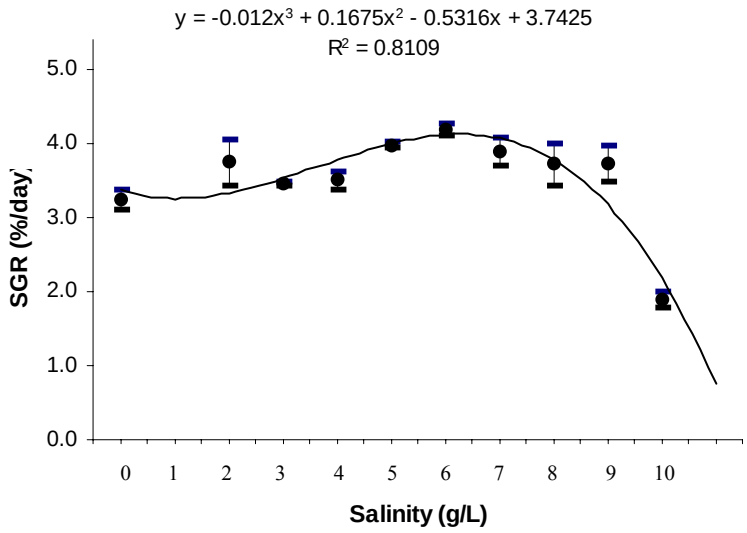

Figure 6.1 The overall relationship between salinity and specific growth rate of Murray cod fry reared for a period of 30 days in different salinities at room temperature (approximately 20ºC).

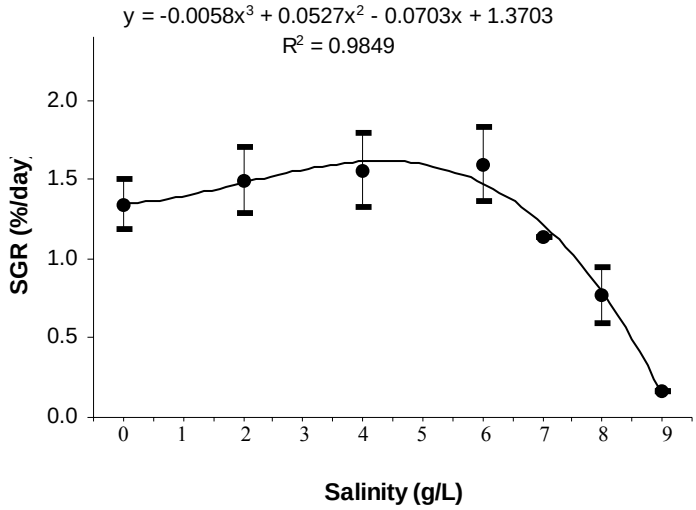

Figure 6.2 The overall relationship between salinity and specific growth rate of fingerling Murray cod reared for a period of 60-62 days in different salinities at room temperature (approximately 20ºC).

Blood osmolality of fingerlings increased to significance in salinities higher than 9 g/L or at 2.7 g/L higher than the threshold salinity. Dehydration was observed in a salinity of 10 g/L both in fingerlings and fry fish. Sublethal effects were found in salinities higher than 6 g/L in embryos, 7 g/L in fingerlings, and at 10 g/L in fry fish.

6.3 Discussion

Some studies, such as Hart et al. (1991), claimed that fish eggs were more tolerant to increasing salt concentration than larvae, while the suggestions by Blaxter (1969) and Chervinski (1984) were different. However, this study showed that degrees of tolerance depended on how and in which stages the effects were measured. Murray cod eggs after completion of water hardening processes showed tolerance to very high salinity, even higher than 9 g/L, that is close to the upper sublethal level of fry and fingerling fish. Nevertheless, after hatch the yolk-sac larvae eventually died within 4 days at 1.0 g/L. The larvae hatched in freshwater also died in 0.5 g/L when transferred to different salinities. This suggested that post exposure after hatch did not enhance salinity tolerances of yolk-sac larvae.

The impacts of salinity on fertilsation were also investigated utilizing trout cod eggs instead of Murray cod eggs. With the very limited observed data, this test suggested that fertilisation was unlikely to take place even in salinity as low as 0.80 g/L. It was likely that the tolerance concentrations for sperm, developing eggs and yolk-sac larvae corresponded. To obtain more details, it is suggested that there be a future study with Murray cod gametes.

The salinity tolerances of Murray cod were also claimed to be attributed to their marine-type ancestors, or related to the native stocks having been exposed to episodic high environmental salinity in their habitats (Jackson & Pierce, 1992). MacDonald (1976) also stated that Murray cod presumably colonized Australian freshwater from marine origins. However, this study showed that fingerlings possessed very limited salinity adaptability through pre-acclimation. Generally, well-adapted species possess the ability to generate the apical pit located on the surface of the chloride cells in their gills, as found in the euryhaline fish (Utida & Hirano, 1973). Kinne (1962, cited in Holliday, 1969) noted that the adjustments to salinity by fish were nongenetic, thus were not transmitted to the next generation.

To understand more about salinity adaptation in the developing stages of Murray cod, future studies of the changes in gill structure are suggested. Furthermore, future studies should also utilise salt with similar components to the saline waters in cod

habitats. Saline groundwaters in south-eastern Australia generally contain less potassium ion than is found in seawater or sea salt (Fielder et al., 2001; Ingram et al., 2002), It was documented that the effects of saline groundwater can be reduced when growing Australian snapper, *Pagrus auratus* by fortifying water with a similar concentration of KCl (Fielder et al., 2001). A number of studies (e.g., Eddy, 1981; Ellis et al., 1989) also noted the role of potassium ions in the regulation of salt balance in fishes (see chapter 1).

The results of this present study also showed that salinity tolerance limits (0.34-0.46 g/L) of larvae of Murray cod and trout cod were lower than the present salinity reported in some of the major rivers of the Murray-Darling River system, especially those of the Bogan, Macquarie, Castlereagh and Namoi rivers (Table 6.2). Nielsen and Hillman (2000) have also stated that salinities between 0.50 and 1.0 g/L would most likely be encountered in Australian rivers and wetlands. This suggested that cod larvae would not survive if spawned in such rivers. However, the spawning season general coincides with floods and high flows (Lake, 1967; Codwatch, 1993) that should dilute the concentration of salt during development of larvae. This suggested that the increased salinity from the past to present should not directly contribute to the decline of the stock recruitment of Murray cod for the present.

Table 6.2 Present and possible future salinities in some major rivers in the Murray-Darling Basin in New South Wales (modified from MDBC, 1999). The data were converted from the original figures which presented conductivity, using the conversion factor of 1 *TDS* (mg/L) = 0.60 x *EC* (µS/cm).

River valley	Average river salinity (g/L)	
	Year 1998	Year 2100
Lachlan	0.32	0.88
Bogan	0.43	1.40
Macquarie	0.37	1.26
Castlereagh	0.38	0.73
Namoi	0.40	0.93
Gwydir	0.34	0.44

The threshold concentration level (6.8 g/L) for fingerlings of Murray cod was also found to be lower than the salinity reported in some of their habitats. Nielsen and Hillman (2000) have documented salinities between 1.5 and 10.0 g/L in billabongs associated with rivers during low flow. Though it has been claimed that juveniles of

Murray cod do not use billabongs, there are documented reported catches of Murray cod in such a habitat (Cadwallader & Lawrence, 1990 cited in Humphries et al., 1999). This demonstrated the possibility of fingerlings being trapped and eventually dying in saline ponds.

A pH lower than 7 strongly enhanced the lethal degree of saline water for Murray cod fry. The floodplains in the Murray-Darling basin are said to have high concentration of tannins (Humphries et al. (1999), which generally reduces the pH of water. This suggested a reduction in chances of survival if Murray cod fry utilise such a habitat during development.

Temperatures in the range 15 to 25ºC, which generally exist in cod habitats, were also shown to adjust the tolerance range of Murray cod fry. During the period when Murray cod are developing from fry to fingerling, the temperature in the Murray-Darling Rivers is reported to be in the range of 12 to 30ºC (Humphries et al., 1999). This study suggested that fry fish would grow better and tolerate higher salinity in mid spring when the temperature was close to 20ºC.

The main purpose of this study was not to make an assumption of the relationship between the biology of trout cod and Murray cod and their environment. However, the present study implied that a threshold salinity between 0.34 and 0.46 g/L can be considered a safe level for cod habitat. This threshold level is significantly lower than the threshold salinity of 1.00 g/L (approximately 1,500 EC) accepted for freshwater ecosystems as recommended by the salinity Audit (MDBC, 1999).

However, the result obtained from laboratory conditions is unsound information upon which to apply to habitat management, unless the compounding factors associated with the environment are tested. Further work should focus on the spawning success of Murray cod in saline brood ponds as well as the sensitivity of larvae to saline groundwater. Monitoring of salinity in the natural spawning grounds especially during the spawning season will also provide essential information for fish stock management in the Murray-Darling Basin.

6.4 Conclusion

Fish are generally good indicators of the health of riverine and wetland systems. However, the ability of fish to acclimate to salinity makes them poor indicators of the threat of gradual salinity increases. Additionally, the salt sensitivities of fish can vary considerably from one catchment to the next. Ideally, laboratory and field based sensitivity trials should be conducted for fish found in each catchment of interest (Clunie et al., 2002).

While some work has recently been initiated, additional information on the lethal and sublethal impacts of salinity on Murray cod, in terms of growth, and metabolic capabilities was investigated in this study. This study concludes that the yolk-larvae life-stage in Murray cod is more sensitive to elevated salinity than are post-hardened eggs, fry and fingerlings. There is an optimum salinity level for maximum growth rate of fry and fingerlings. Salinity levels above or below the optimum result in a reduced growth rate. Further research in their natural habitats is needed to confirm whether or not this is a general occurrence. Laboratory-determined salinity sensitivities are not directly transferable to Murray cod survivorship in the field. The maintenance and interrogation of the species occurrence and water quality databases will provide a very necessary check of maximum salinity levels for Murray cod occurrence. Records of the distribution of viable populations would be advantageous to the collective knowledge of Murray cod salinity sensitivity.

This study has collected some essential scientific evidence of the impacts of salinity on the development of Murray cod. As the successful management of Murray-Darling freshwater ecosystems requires an understanding of the impacts of salinity on their inhabitants, the information obtained from this study should enable better management of the river systems for both this species and its close relations and more threatened species such as trout cod, Mary River cod and eastern freshwater cod. However, future studies taking into account conditions simulating the natural environment are essential.

If salinity in cod habitats reaches over 0.34 g/L this study would predict a significant impact on Murray cod recruitment. Murray cod showed characteristics of true freshwater fish, which possess very little capacity for salinity adaptation. While the

fry and fingerlings showed tolerance to moderate salinity, which was higher than salinity levels occurring in their habitats, the eggs and larvae were very sensitive to increasing salinity. The salinities currently experienced in some major rivers of the Murray-Darling Basin may have significant effects on Murray Cod recruitment, if not on the spawning process, at least on the development of the eggs and larvae.

This study found that trout cod eggs were very sensitive to increasing salinity unless they had been fertilsed and hardened. The eggs will not be fertilised even in a very low salinity. Causes of unsuccessful fertilisation are not known but seem to be due to the viability either of sperm or eggs when in contact with saline water. Hatching viability was enhanced if the eggs were exposed to saline water after the completion of hardening. Hardened eggshells, though suffering corrosion in high salinity, effectively protected the embryo during development in saline waters. Accompanying the corrosion of the eggshell, deformities of the notochord were also found in developing embryos. Salinity sensitivity of trout cod and Murray cod larvae were similar.

Fry and fingerlings of Murray cod were found to tolerate relatively high salinity. However, these high tolerances did not demonstrate salinity adaptability as it was found that merely improvement of tolerances was observed when fingerlings were pre-acclimated in low salinities before transfer to the higher salinities. The longer exposures were also found to marginally improve the osmoregulation capacity to maintain their normal blood osmotic concentration, but was not found to improve the consumption rates of oxygen. This suggests that fingerlings of Murray cod are not salinity-adaptable fish but that they are capable of tolerating high salinity if they cannot disperse.

The range of temperatures existing in natural habitats, especially during the developmental stages, may strongly adjust frys' or fingerlings' salinity tolerances. Low pH also enhances the lethal degree of saline water in developing fish. Though suspension of feeding on the alternate days did not reduce salinity tolerances of fry fish, a significant reduction in growth was observed. This would reduce chances of survival in the natural environment.

Measuring growth and blood osmolality is an effective method for investigating salinity tolerances in fingerling Murray cod. However, changes in the oxygen consumption rate were only observed in fish exposed to salinities close to the upper sublethal level. Dehydration was observed only in the salinities at the upper sublethal level and higher. Growth rate was a likely method to use to determine salinity tolerance over long exposure, as it well demonstrated some characteristics of a freshwater species such as fingerling Murray cod.

This study lacked information on changes of gill structure in fingerling Murray cod when pre-acclimated or reared in different salinities. This is essential in any further study as slightly changes may be observed in the course of adaptation even though they are not evident in growth or in osmoregulation. The advantage of studies in gill structure is that changes, i.e. generation of chloride cells, can be observed within short periods of time (Utida and Hirano, 1973). Such changes can eliminate the effects of handling stress due to long-term captivity.

The results from this study are merely a guideline if applied to studies of salinity sensitivity in the natural environment of Murray cod. The overall water qualities, including ammonium and nitrite concentrations in the experimental environment, were different from those existing in natural habitats. Poorer water qualities would enhance lethal levels obtained in this study. Ammonia, nitrite (Forteath, 1990) and cadmium are less toxic in saline waters than in freshwater (Eisler, 1971; Engel & Fowler, 1979; McLeese et al., 1987). Moreover, the saline groundwater present in Murray cod habitats may present a different degree of lethality to Murray cod fry. It is suggested that future studies use water taken from cod habitats or nearby groundwater.

Future studies may be focused on salinity impacts on the course of spawning in habitats similar to the natural spawning ground. Small broodponds in warm water fish hatcheries such as the ones of the Marine and Freshwater Resources Institute (Snobs Creek) are suitable for salinity impact trials. Though the study may be costly, the experiments could be done by adding sea salt to a concentration of the broodpond water several months prior to spawning season to acclimatise the fish to the environment of the ponds.

7. References

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