METABOLISM AND CUTANEOUS EXCHANGE

IN AN

AMPHIBIOUS FISH

NEOCHANNA BURROWSIUS (PHILLIPPS).

A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Zoology in the University of Canterbury by A.S. Meredith

University of Canterbury

1985
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This thesis describes gas exchange, ion and water fluxes and nitrogen metabolism in the Canterbury mudfish, an amphibious fish, which was held in the laboratory in either water or air.

The Canterbury mudfish, Neochanna purrowsius, is a swamp dwelling species endemic to New Zealand, which is now rare as its habitats are being destroyed by man.

Passive and active exchanges in water or air were assessed using a number of methods, including measurement of gross fluxes, partitioning experiments and measurements of blood concentrations. The skin was found to be important for gas exchange and ammonia excretion in both water and in air. Ion and water fluxes through the skin were not unusually high. Indirect evidence points to a switch, on emersion, from protein to lipid catabolism. Even though the skin may be the site of more than half of the animals oxygen uptake, the gills and buccal epithelia were considered to be the primary gas exchange surfaces and by altering ventilation, the mudfish could compensate for reduced cutaneous exchange.

Histological investigations of the structure of the skin, gills and buccal epithelium did not reveal any remarkable features that might be associated with emersion, though the epidermis of the skin possessed large numbers of mucous cells.

The cutaneous exchanges, small body size, low metabolic rate and tolerance to fluctuating conditions were all considered important features of the overall emersion strategy. Physiological responses to emersion in mudfish more closely resemble those of intertidal marine amphibious fish species than those of tropical air-breathing species.

Emersion tolerance was found to be well developed in most members of the galaxiid family. This ability, associated with cutaneous gas exchange, is discussed in relation to the family's marine ancestry, climbing ability and terrestrial spawning.
CHAPTER 1.

INTRODUCTION

The Canterbury mudfish, Neochanna burrowsius (fig. 1) is a small freshwater fish endemic to the Canterbury region of New Zealand. It is one of three species of mudfish within the 13 species of the family Galaxiidae in New Zealand. This family is widespread around the southern hemisphere with species found in South America, South Africa, Australia and many offshore and oceanic islands. They are mostly small, elongate, scaleless fishes and occur in a wide variety of habitats in New Zealand.

The three mudfish species are cryptic, benthic and nocturnal with elongate, slender and tubular bodies, blunt heads, small eyes and pelvic fins either reduced or absent. Their body form reflects their distinctive habitat of swamps, creeks and drains which tend to dry up during summer. They are all able to breath air and survive out of water for several weeks; capabilities that are important in drought susceptible areas. However, all three species are now relatively rare and appear to be declining. Recently, the Canterbury and Black mudfishes were placed in the Red Data Book for endangered flora and fauna of New Zealand (Williams and Given, 1981), as recognition of the necessity of protection of them and their habitats. Although not listed, the Brown mudfish now appears similarly threatened.

The Canterbury mudfish was first described in 1926 (Phillipps, 1926) and named after a Mr Burrows on whose farm specimens were collected. Mention was frequently made of their 'aestivation' ability as they were often encountered during digging in the regions of dry water courses. Recent work has documented their rarity and decline, expressing concern at their disappearance from many areas (Skrzynski, 1968; Cadwallader, 1975a). The general ecology, biology, and prevalence of the
Figure 1: The Canterbury Mudfish

*Neochanna burrowsius* (Phillipps).

Specimen 110 mm total length.
Canterbury mudfish has been investigated and the present habitats described by Eldon (1978a; 1979a,b,c). The airbreathing and "aestivation" of fish has also been well documented (Eldon, 1979c). Since several of the habitats described contained high densities of mudfish, it became feasible to obtain reasonable numbers of fish to investigate their air breathing and terrestrial capabilities.

Air-breathing and emersion of fish have been popular topics of study in recent years (Johansen, 1970; Hughes, 1976; Kramer et al., 1978; Randall et al., 1981). Interest has centred largely on obtaining knowledge of novel airbreathing organs, and strategies of terrestrial survival, in a wide range of species. However, relating adaptations and strategies to evolutionary advancement has been popular as an alternative approach to studying principles of fish physiology. Studies have documented the abundance of fish species around the world which are either air-breathers, aestivating species, or that can be considered amphibious. A notable feature of these studies is the great diversity of adaptations and strategies that have arisen to cope with conditions that necessitate fish temporarily or frequently exploiting the aerial environment. These are found in both marine and freshwater environments with the majority occurring in tropical regions.

The Canterbury mudfish is potentially interesting as a local example of this widespread trend, and as a fish from a temperate rather than tropical region. It is even more imperative to study it at present because knowledge of its unique methods of exploiting the terrestrial environment may be lost if the decline in mudfish numbers is not reversed. Such philosophy stimulated this study of the physiology of the Canterbury mudfish in relation to its air-breathing and emersion, and interactions with environmental conditions that necessitate these behaviours.
The tenuous position of the mudfish restricts the level of the investigations to potentially harmless techniques so as to not further endanger the populations through overexploitation. Therefore, rather than concentrated analyses of certain features of the mudfish emersion physiology, a broader survey was undertaken of the gross physiological exchanges and their exchange surfaces, as they pertain to the aerial environment. This approach avoids sacrifice of large numbers of fish and allows small groups of fish to be repeatedly utilised. A broader range of knowledge of the adaptations and physiological mechanisms of mudfish may be more applicable, as such information may be of potential use in predictions or management of populations and habitats.

The tolerance and reaction of the mudfish to environmental hypoxia has been studied as a pilot to this investigation (Meredith, 1981). This study isolated hypoxia as a major factor in the native freshwater habitat that may stimulate movement onto land. It demonstrated that fish consistently leave the water under hypoxia and confirmed their survival potential out of water. It also cast doubt on the use of the term aestivation, as mudfish did not appear to enter a torpid state. Subsequently we found substantial cutaneous gas exchange in mudfish, a feature aiding survival in air (Meredith et al, 1982; see also as Appendix 1). A study of blood oxygen transport characteristics of mudfish has also indicated how little change or disruption may be necessary in the transition from land to water (Wells et al, 1984; see also as Appendix 2).

In describing the adaptations and physiological mechanisms that make up the overall strategy adopted by mudfish for terrestrial survival, many potential problems must be approached. These include: movement and orientation without the support of water; maintenance of respiratory exchange and metabolic rate in a different medium and without further
feeding; excretion of metabolic byproducts, especially the nitrogenous compounds of protein catabolism, and the maintenance of homeostatic equilibria in ion, water and acid-base balance. The effects of environmental variables such as temperature, water availability and microhabitat conditions are also considered. In answering this broad spectrum of questions, an appreciation of the overall strategy of terrestrial survival in mudfish can be gained.

The development of a strategy should be reconciled with the phylogenetic potential and environmental pressures exerted on the mudfish. These latter components may help to explain why certain strategies have developed and why these strategies may superficially resemble those of other groups. This can be extended to look at and estimate the terrestrial survival potential of other close relatives of the mudfish species.
CHAPTER II

GENERAL METHODOLOGY

2.1 STUDY AREAS AND COLLECTION OF FISH

The Canterbury mudfish has been described as rare (Cadwallader, 1975a) and as a vanishing species (Skrzynski, 1968), but has recently been found to be abundant at several restricted localities (Eldon, 1979a). Possibility of land development in many of these localities puts several populations at risk of extinction. Mudfish used in this study were caught from three of these sites (Fig. 2.1; also see Eldon, 1979a). Removals of mudfish from these sites were limited so as to not endanger the populations. Several mudfish from a previous study (Meredith, 1981), which had been reared in a private pond, were also used.

2.1.1 Washpen Stream, View Hill, Oxford (NZMS 1 S75, 510861).

This habitat consists of a broad area of partially drained swamp at the base of a range of foothills (Fig. 2.2). The area is undeveloped Maori land, lightly grazed by cattle and horses. Throughout most of the year water is confined to ditches and channels and arises from hillside runoff and small springs. Mudfish are sparsely distributed throughout most of the waterways but are densely congregated in only one channel that seldom flows and forms a chain of weed-choked pools (Fig. 2.2). Upland bullies (Gobiomorphus breviceps) and tadpoles of the frog Litoria raniformes coexist with the mudfish.

Fish were captured using dip and push nets. Nets were pushed under the dense aquatic vegetation and those fish disturbed in the weed descended and were caught in the nets beneath. Mudfish were caught regularly from this population and comprised the majority of the fish used in this study.
Figure 2.1. Map of Canterbury showing the locations of the three areas from where mudfish were collected.

2. Hororata River.
2.1.2 Hororata River floodplain (NZMS 1 S82, 475497)

This area comprises a series of sink holes, small springs, streams and ponded areas on the Hororata river floodplain (Fig. 2.3). The area is extensively farmed with sheep and cattle and most watercourses are used for stock water. Watercourses are filled with shingle, fallen willow branches, fine mud, silt and aquatic weeds (Fig. 2.4). Mudfish were found in all areas although adult fish were rarely encountered. Aestivating fish were occasionally found in sink holes and under fallen willow branches. Common and upland bullies (Gobiomorphus cotidianus and G. breviceps) coexist with the mudfish in the streams and impounded areas. Free swimming fry and small mudfish less than 60mm total length were caught with dip nets in the water column and in marginal aquatic vegetation. The small fish caught from this area were raised to adult size in the aquarium system.

2.1.3 Buchanans Stream, Willowbridge, Waimate (NZMS 1 S128, 677057).

The mudfish habitat at Waimate consists of a large impounded area filled by a series of springfed streams with the outlet constricted by a narrow concrete weir (Fig. 2.5). The area is designated a wildlife refuge by the Wildlife Service and is surrounded by farmland. Seasonal droughts have excluded large numbers of other fish species from this habitat but recent irrigation of surrounding farmland has prevented the drying up of the area and trout (Salmo trutta) are now well established in the habitat. The fate of the mudfish population is therefore uncertain.

This population was sampled once. Adult mudfish were captured in the aquatic vegetation around the margins of the pond and schooling fry captured by dip net in the open water.
Figure 2.2  Photograph of area of swamp around Washpen stream, Oxford showing one of several pools mudfish were captured from.

Figure 2.3  Area of farmland on Hororata river floodplain that contains springs, sink holes and water courses inhabited by mudfish.
Figure 2.4 Spring fed watercourse on Hororata river floodplain where juvenile mudfish were caught.

Figure 2.5 Imponded area at Buchanans stream, Waimate, from where adult mudfish and mudfish fry were captured.
2.2 MAINTENANCE OF FISH

Fish caught in the field were transported to the laboratory using white plastic buckets filled with fresh pondwater which was gassed with oxygen. Lids on the buckets maintained the high oxygen conditions. The fish were maintained in an air conditioned aquarium room for up to 2 years in 80 litre glass, or concrete and glass aquaria. Less than 20 fish were placed in each aquarium. The aquaria were supplied with fresh artesian water flowing at a rate of 1-2 litres per minute. Fine shingle and rocks were supplied as a substrate and shelter. The water temperature varied seasonally from 12-18°C but was constant diurnally. A constant 12 hour light cycle was maintained with artificial fluorescent lighting. Fish were fed twice weekly on live tubifex worms and weekly on either a commercial moist pellet preparation or freshly hatched Artemia larvae.

While the spawning periods of wild mudfish is late winter to spring (Eldon, 1979c), that of captive fish varied from spring to mid summer. This was possibly due to the lack of environmental cues such as day length and temperature fluctuations. Fish with swollen abdomens indicating advanced gonadal development were not used for experimentation.

2.3 TREATMENT OF DISEASE.

Diseased fish were seldom encountered in the natural environment. The occasional fish caught with haemorrhaging fins or damaged during capture usually died soon after capture or during transportation. A high incidence of a range of diseases was often seen after transportation of fish and during their acclimation to laboratory conditions. This was found to be more prevalent in the warmer summer and autumn months. Fish diagnosed or treated for disease were not used for
experimentation until 4 weeks after total recovery.

Medications used in the treatment of disease are listed in Table 2.1.

Table 2.1 Medications used for the treatment of disease in captive fish.

<table>
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<td>Chloramphenicol</td>
<td>B</td>
<td>10</td>
<td>Roche Pharm. Co., N.Z.</td>
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<td>Oxytetracycline</td>
<td>T</td>
<td>-</td>
<td>Sigma Chem. Co. USA.</td>
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<td>Sulphanilamide</td>
<td>B, T</td>
<td>200</td>
<td>B.D.H., UK.</td>
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<td>Furanace-P</td>
<td>B</td>
<td>0.1</td>
<td>Dainippon Pharm. Co., Japan.</td>
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<td>Formalin</td>
<td>B</td>
<td>50</td>
<td></td>
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<tr>
<td>Malachite green</td>
<td>B</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Methylene blue</td>
<td>B</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

*— after Stuart (1983).

T= topical application; B= bathing solution (3 days).

Four main diseases or symptoms of diseases were encountered and treated in the laboratory.

(1) Whitespot

The protozoan parasite, *Ichthyopthirus multifilis*, was a frequent problem in the aquarium system at all times of the year. The source of the infection was thought to be the live food.

Fish and tanks were treated for whitespot by stopping the freshwater inflow and increasing aeration of the water. The water was also treated for 72 hours with formalin and malachite green to final concentrations of 50 and 0.05 mg/l respectively. Treatment was effective in removing the infection but was found to be lethal to residual live food and other fish species in the tanks. For fish species other than
mudfish or if combinations of fish species were present, tanks were treated with Furanace-P and malachite green to final concentrations of 0.1 and 0.05mg/l respectively for 5 days.

(2) Fungal Infection

Fungal infections of Saprolegnia sp were characterised by white fluffy out-growths on the skin surface. The infection was normally due to external damage to the fish surface caused by rough handling, abrasion or interactions with other fish, allowing the fungus to colonise the damaged areas. Fungus was also found to be a secondary problem in haemorrhaging and ulcerated fish.

Infected fish were removed from the tank and placed in a 3.0 mg/l solution of methylene blue for 72 hours. Oxytetracycline powder was applied to the infected area to control secondary infections. If the infection persisted for longer than 2 days then haemorrhagic areas became apparent and fish were seldom cured.

(3) Subcutaneous haemorrhage

Subcutaneous haemorrhaging was seen in stressed fish, at the base of the tail and fins and occasionally on the body surface. It was probably due to an internal bacterial infection of Pseudomonas spp or Aeromonas spp (Roberts, 1977). Fish were isolated in a 10 litre treatment tank and oxytetracycline applied to the infected areas daily. The fish were maintained in a 3.0 mg/l solution of methylene blue until recovery.

(4) Ulceration

Ulceration was characterised by pitted areas of broken skin with haemorrhagic margins. Ulcerated fish were isolated in a 10 litre treatment tank containing 10 mg/l chloramphenicol, and oxytetracycline powder was applied to the infected area. If fungal growths were also present, 3.0 mg/l methylene blue was added to the bath.
(5) Parasites

No internal parasites were identified from freshly collected specimens of the Canterbury mudfish (D. Blair, pers comm) and none have been previously recorded (Blair, 1984). Parasites have been described from the Brown mudfish (Eldon, 1978a; Blair, 1984), and Black mudfish (Blair, 1984).

2.4 FISH HANDLING AND ANAESTHESIA.

Aquarium fish were captured and handled using square mouthed nets, although direct handling was sometimes necessary.

Fish were anaesthetised prior to most experimentation to facilitate weighing and experimental manipulations. Two anaesthetics were used, allowing fish to be anaesthetised in air or in water, depending upon the experimental conditions.

(1) Anaesthesia in water

An aqueous benzocaine solution was prepared by dissolving 50mg of powdered benzocaine in 5ml of ethanol and then dissolving this in 500ml of tapwater. Fish were removed from the anaesthetic immediately after loss of orientation was observed (approximately 30 seconds).

(2) Anaesthesia in air

Ether anesthesia was produced by placing fish in a perforated plastic container suspended above a chamber containing ether-saturated cotton wool. Upon exposure to the ether atmosphere mudfish ventilated their gills very rapidly. Fish were removed soon after the initially rapid ventilatory movements ceased (approximately 15 seconds).

Anaesthetised fish were blotted dry for 10 seconds on soft tissue paper and weighed to the nearest mg on a Mettler PL200 top loading balance. No attempt was made to remove any water held in the mouth or
After use, experimentally manipulated fish were held in a separate aquarium for 3 weeks and were closely monitored for signs of damage or disease. Any fish with damage evident from experimental treatments were treated with oxytetracycline powder and kept in a 3.0 mg/l methylene blue solution for 48 hours, before being returned to the holding tank.

2.5 BLOOD SAMPLING AND PREPARATION

A technique was developed for obtaining adequate blood samples by non lethal cardiac puncture. This was a variation on the methods reviewed by Wingo and Muncy (1984).

Mudfish were lightly anaesthetised and placed ventral side up on damp filter paper. One ml insulin syringes (Monoject) with fixed 27 gauge needles were used. These had negligible dead space and enabled small volumes of blood to be handled. Syringes were flushed with an aqueous solution of anticoagulant immediately prior to use. The anticoagulant used was either lithium or ammonium heparinate depending upon whether the subsequent analysis was for nitrogenous or ionic constituents. The needle was inserted into the region of the ventricle and adjusted until a steady flow of blood could be generated by light suction. Cessation of heartbeat occasionally occurred on insertion of the needle. Blood could not be withdrawn until the heart beat resumed. Blood volumes of 20-50 µl were withdrawn from each fish. This represented 15-30% of total blood volume assuming a 5% blood volume.

Blood was transferred to heparinised microhaematocrit centrifuge tubes (Fisher, Pa. USA). Evaporative loss of plasma from the tubes was prevented by placing a drop of liquid paraffin in the tube above the sample. The bases of the tubes were sealed with plasticine (Sigillum, Modulohm I/S, Denmark) and centrifuged at 20,000 xG for 3 minutes. The tubes were broken at the cell/plasma interface and plasma extracted.
Plasma was handled using 5µl graduated capillary tubes (Accu-fill 90, Clay Adams, USA.) with mouth suction, or 25µl Drummond microdispensers (Drummond Co. USA.). Any sample showing a packed cell volume (PCV) lower than 15%, or that was greatly haemolysed, was discarded.

Oxytetracycline was applied to the puncture wound before fish were returned to water. On immersion, bled fish swam normally and showed no adverse effects of blood sampling. Fish were not resampled until at least 4 weeks after being bled.

2.6 PARTITIONING METHODS

Partitioned respirometry chambers were similar to those described by Meredith et al (1982); Fig. 2.6. Chambers were constructed from the barrels of 60ml and 35ml plastic syringes (Monoject). Rubber dams were constructed from the fingers of rubber surgical gloves, with the tips removed.

Each mudfish was lightly anaesthetised and a dam slipped over the fish's head to a position just caudal to the pectoral fins. Denture adhesive ('Super Polygrip'; Frank Stevens Ltd. Auckland) was used to cement the dam to the skin. The outer margin of the dam was folded over the end of the smaller syringe and the larger syringe slipped over the
to produce a tight seal. The mudfish was therefore held in place in the chamber with head and body in separate compartments. The denture adhesive ('Super Polygrip') was chosen as it did not contain any local anaesthetic agents, as found in most adhesive preparations.

For respirometry measurements, the ends of the chambers were sealed with a rubber bung containing an 18 gauge hypodermic needle as a sampling port and a 3ml syringe (Monoject) for volume compensation and mixing. Chamber gas samples were drawn from the needle through a three way tap into a 1ml insulin syringe. Mixing of the chamber atmosphere prior to sampling was achieved by alternately drawing air into the sampling and compensating syringes.

For the measurement of excreted metabolites, radioactive tracers and ions, the chambers were cemented onto 200ml plastic pottles which were either capped or left open to the air. Water volumes of 100–200 ml were placed in the pottles to cover the fish. Mixing in the pottles was achieved by aeration of the individual chambers.

Partitioning did not allow isolation of the exchanges of the skin surfaces from urine flow in the body compartment. Several unsuccessful attempts were made to separate these exchanges. A perspex chamber was constructed that allowed the body of a partitioned fish to be held on a stainless steel grill within a bath of liquid paraffin while the head remained in aerated water. Attempts were made to collect urine, that formed as an immiscible bubble at the urogenital pore, for measurement of urinary composition and flow rates. Urine spread as a thin layer across the hydrophilic body surface, and even when confined by a small ridge of petroleum jelly was not readily collectable. Cannulation of the urogenital pore with heat flared vinyl tubing was also unsuccessful as fish could not be adequately restrained to prevent the tubing becoming dislodged. Urinary cannulation of such small fish may also have caused
significant diuresis. Suturing or sealing of the urogenital opening with cyano-acrylate glues to prevent urinary flows was not attempted as this would have ultimately killed the fish. All of these methods were unsatisfactory as they either severely disrupted the physiological state of the fish or changed the external environment.

2.7 STANDARDISED PARAMETERS

Unless otherwise stated all experiments were carried out at 15°C, this temperature being in the midrange of normal environmental temperatures in mudfish habitats (Eldon, 1979a) and the mean aquarium holding temperature.

Sample sizes of fish for different experiments ranged from 6 - 16 and reflected the numbers of fish available in any size range. The variation in the sizes of mudfish captured necessitated the use of groups of fish of differing mean sizes. Where possible groups of comparably sized fish were selected for each experiment. These were normally fish of the size ranges 5-10 grams wet weight or 80-130mm total length (TL). Variation in both individual and group mean size was corrected using weight specific measurements although high variation within and between experiments was still obtained. No attempt was made to separate fish of different sexes or year-classes.

Wherever sacrifice of fish was necessary, the fish carcases were used for other analyses to limit further sacrifices.

2.8 STATISTICAL ANALYSES.

Pairwise T-tests and standard errors of the mean were calculated for all comparisons, (Sokal and Rohlf, 1973).
CHAPTER III

RESPIRATION AND METABOLISM

INTRODUCTION

The Canterbury mudfish is both a facultative air-breathing fish and an amphibious fish. Eldon (1979c) and Meredith (1981) have described air breathing in mudfish in conditions of poor water quality. They described how mudfish may leave the water, and Eldon (1978a, 1979c) recorded fish remaining out of water for several weeks. The occurrence of substantial cutaneous gas exchange has been reported in both air and water (Meredith et al., 1982) and this may partially explain the mechanisms allowing survival in air. However, responses to emersion and mechanisms of $O_2$ consumption, carbon dioxide excretion, metabolism, starvation physiology and levels of dormancy during emersion have remained largely unanswered. It is to these questions that this chapter is directed.

The respiratory physiology of air-breathing and amphibious fish has been extensively studied over the past 20 years (Hughes, 1976; Randall et al., 1981). Studies have shown that a number of diverse adaptations have arisen within groups to utilise the atmosphere as a supplementary breathing medium or as a complete terrestrial refuge. The range of adaptations and responses of amphibious fishes to emersion is matched only by the diversity of species that have evolved air-breathing and the conditions under which they have developed. Placing mudfish within the context of these studies may serve to explain the extent of their development of adaptations for survival in air.
MATERIALS AND METHODS.

3.2.1 RESPIRATION

3.2.1.1 Aquatic respiration

The pattern and frequency of ventilation of undisturbed mudfish was determined from visual observations of fish in perspex aquaria.

Oxygen consumption of aquatically respiring mudfish was measured in 500ml glass jars by a closed chamber method (Fig. 3.1). Oxygen tensions were monitored using a Beckman oxygen electrode, model 39553 and Fieldlab analyser, calibrated with air and deoxygenated water. The jar was emersed in a three litre water bath maintained at 15°C and aerated with compressed air. A magnetic flea directly below a steel grill was used to mix the water within the jar. Fish were placed in the jar above the steel grill and allowed to acclimate to the conditions for 30 minutes. After acclimation, aeration was stopped, all air bubbles removed and the O₂ electrode inserted in a rubber bung in the top of the jar. Decrease in oxygen tension was recorded on a Beckman chart recorder. Experiments were terminated when oxygen tensions reached 100 torr (1 torr = 0.133 kPa). Oxygen consumption was calculated from the linear decrease in oxygen tension and corrected to standard temperature and pressure (STPD).

3.2.1.2 Aerial Respiration

Methods of aerial ventilation were described from direct visual observations of undisturbed emersed mudfish. All observations were made in a temperature controlled room at 15°C, with the observer partially shielded from the fish by a cloth curtain.

Changes in ventilatory patterns were described for fish emersed on three different substrates: Dry tissue paper, moist tissue paper, and a thin layer of water that allowed some water to be taken into the mouth.
Figure 3.1 Diagram of apparatus for measurement of oxygen consumption of aquatically respiring mudfish.

M = Magnetic stirrer; F = Fieldlab analyser; C = Chart recorder; E = Oxygen electrode.
Frequencies of aerial ventilation were measured from immediately after emersion until the ventilation rate ceased to change over longer time periods. Ventilation frequency was also measured at 1, 2, 3, 7 and 28 days after emersion.

The oxygen consumption of mudfish (\( \dot{V}O_2 \)) was measured hourly over the first 10 hours after emersion and then at 1, 2, 3, 7 and 28 days. Oxygen consumption was measured using a Gilson differential respirometer with temperatures maintained at 15°C. A dark cloth was placed over the respirometer to decrease the illumination of the fish and to prevent visual disturbance. Carbon dioxide was absorbed using 100μl of 20% KOH soaked onto 1.5cm\(^2\) filter paper wicks placed in absorption wells in the necks of the vessels. Fish were placed on a moist circle of filter paper in either 30 or 70ml glass vessels depending upon the size of the fish. Vessels were immersed in the water bath and allowed to equilibrate for 30 minutes. Readings were taken over 30 minutes at 3 minute intervals. For measurement of oxygen consumption at times greater than 24 hours, fish were placed in perspex lunchboxes on damp cotton wool until 4 hours before measurements were to be taken. They were then transferred to the experimental flasks and maintained in a controlled temperature room for 2 hours before being transferred to the respirometer for a further two hours. This decreased the effects of handling stress and prevented the accumulation of excretory products and other possible sources of biological oxygen demand in the vessels.

Oxygen consumption was calculated from raw data using a BASIC program (Appendix 3) developed for use on an Apple IIE microcomputer. All results are expressed as weight specific oxygen consumption at STPD, \( \mu l \cdot g^{-1} \cdot h^{-1} \).

The dependance of aerial respiration rate on temperature was examined. Ventilatory frequency and oxygen consumption were measured as previously described for a group of fish after 24 hours of air exposure.
at 5, 15, and 25°C. This covered the normal range of environmental temperatures of the Canterbury mudfish (Eldon, 1979a). Temperature coefficients Q10 (Hill, 1976), were calculated over the temperature ranges 5-15 and 15-25°C.

The O₂ consumption, CO₂ excretion, and resulting respiratory quotients (RQ) of mudfish in air were measured using sealed chamber respirometers. The respirometers were made from 35 or 60ml syringe barrels (Monoject) and were equivalent to one half of the partitioned chambers described in section 2.6. 0.5ml volumes of air were removed from the chambers every 30 minutes. PO₂ and PCO₂ of the air samples were measured in an Instrumentation Laboratories Micro 13 Blood Gas Analyser calibrated at 15°C to gas mixtures of 0.5% CO₂ / 99.5% N₂ and 20%O₂ / 5%CO₂ / 75% N₂. Oxygen consumption (VO₂) and CO₂ excretion (VCO₂) rates were calculated from the linear decrease in O₂ and increase in CO₂ levels and corrected to STPD. All rates were calculated from a minimum of 4 analysed air samples. The respiratory rates of fish were thus measured every two hours, for the 12 hours following emersion.

Before experiments requiring injections or partitioning were begun, the effects on aerial respiration of anaesthesia, injections and partitioning were tested to gain an appreciation of the experimental effects of these manipulations. All respiration measurements were made by the sealed chamber method previously described, allowing 15 minutes for recovery from manipulations before measurements were begun (Section 2.6). Five treatments were used:

i) Fish handled (control).
ii) Fish handled and anaesthetised.
iii) Fish handled, anaesthetised and injected with saline.
iv) Fish handled, anaesthetised and partitioned.
v) Fish handled, anaesthetised, partitioned and injected.

Individuals were anaesthetised using 0.01% benzocaine and allowed to recover from the anaesthetic for 10 minutes before placement in respirometry chambers. Ten µl of Cortland saline (Wolf, 1963) was injected intraperitoneally using a 10µl Hamilton syringe (Hamilton Co., Reno. Nev. USA.). Partitioning was performed as in section 2.6. Treatments were compared by pairwise comparison of means by T-tests (Sokal and Rolhf, 1973) and a significance level of 0.05.

The dynamics of CO₂ excretion were investigated by measuring \( \dot{V}O₂, \dot{V}CO₂ \) and hence RQ in partitioned fish injected with preparations of carbonic anhydrase (CA) and acetazolamide (AZ), a specific inhibitor of carbonic anhydrase (Maren, 1967).

Carbonic anhydrase (EC.4.2.1.1) was obtained from Sigma Chemical Co., Mo, USA, as a dialysed and lyophilised preparation from bovine erythrocytes and was dissolved to a final concentration of 5000 WA units/100µl in Cortland saline. 10µl volumes of the CA solution were injected intraperitoneally into each fish. Acetazolamide, (N-(5-Sulphamoyl-1,3,4-thiadiazol-2-yl)- Acetamide), obtained as the dry powder from Sigma Chemical Co., USA, was dissolved to a final concentration of 10mg/ml in Cortland saline. 10µl volumes were injected intraperitoneally into each treatment fish. These levels are within the range of those used in other studies (Burggren and Haswell, 1979; Daxboeck and Heming, 1982; Randall et al, 1978). Both chemical solutions were freshly prepared before use. Where possible the same fish were used for control and enzyme injections with 3 weeks recovery between treatments. Experimental treatments and time intervals were:

(i) Fish injected upon emersion and measured over the first 4 hours of air exposure.

(a) Partitioned and saline injected
(b) Partitioned and CA injected
(c) Partitioned and AZ injected

(ii) Fish injected 4 hours after emersion and measured at 4–8 hours of air exposure.
(a) Partitioned and saline injected
(b) Partitioned and CA injected

(iii) Fish injected 8 hours after emersion and measured at 8–12 hours of air exposure.
(a) Partitioned and saline injected
(b) Partitioned and CA injected

The \( \dot{V}O_2 \), \( \dot{V}CO_2 \) and RQ values were compared between the different treatments and time intervals. Treatments were compared using pairwise T-tests of means.

3.2.2 METABOLISM

3.2.2.1 Weight Loss

The weight loss of fish maintained in air at 100% relative humidity (RH) was measured over 28 days. Fish were maintained on damp cotton wool in small plastic lunch boxes at 15°C in a 12 hour light cycle. Individual fish were removed at intervals, blotted dry for 10 seconds on tissue paper and weighed to the nearest mg on a Mettler PL2000 top loading balance. The cotton wool was replaced twice weekly to prevent build up of excretory products and microbial growth.

Weight loss was compared with a group of fasted fish which were held in an aerated aquarium for 28 days but not fed.

Mortality and weight loss were also noted for small groups of fish fasted or emersed for up to 85 days.
3.2.2.2 Fat metabolism

The contribution of fat metabolism to total metabolism was assessed in groups of 28 day air exposed, 28 day fasted and control fish. The fish were killed, weighed, the gonads and viscera exposed by a ventral incision and the carcass dried to a constant weight at 80°C. Fat content was measured by a simplification of the method of Folch et al (1957). Carcasses were immersed in a 2:1 solution of methanol and chloroform for 96 hours then removed and dried to a constant weight at 80°C. The change in dry tissue weight was related to the tissue fat content.

Several previously preserved fish were dissected, the gonads and associated viscera, excluding the liver, removed and the gonads analysed for lipid content as described above.

RESULTS

3.3.1 RESPIRATION

3.3.1.1 Aquatic respiration

The mudfish appeared to use both buccal and opercular pumps to sequentially pump water through the mouth and across the gills. The amplitude of the ventilatory movements varied from barely detectable movements in five of the twelve fish to visible sequential distention of both buccal and opercular cavities in the other seven fish. Undisturbed mudfish often underwent apnoeic pauses of 5-60 seconds between periods of ventilation. The frequency of aquatic ventilation of quiescent mudfish, excluding apnoeic pauses, varied from 32-65 breaths per minute ($\overline{x} = 51 \pm 9$ (n=12)).

Aquatic oxygen consumption was relatively low and constant in all individuals. Mean oxygen consumption was $54.2 \pm 9.8$ (n=9).
3.3.1.2 Aerial respiration

Emersed fish, breathing air, inhaled via the mouth using the buccal pump to inflate their buccal and opercular cavities. They closed their mouth to hold the chambers inflated. Fish initially lay on their ventral surface with their bodies bent to maintain themselves in a dorsal stance while air-breathing.

Inspiratory movements included ventral and lateral distention of the floor of the buccal cavity and lateral distention of the opercular flaps. Opercular flaps either retained a marginal contact with the body wall or a mucus covered bubble of air emerged from the distended caudal end of the opercular chamber. Exhalation was either by the mouth which was audible as a double gasp, allowing both exhalation and inhalation phases, or via the caudal margin of the opercular chamber so that both exhalation and inhalation were sequential. In the second pattern inhalation occurred 0.5-2 seconds after exhalation.

There was no evidence that air moved internally any further than the buccopharyngeal or opercular chambers. Copious mucus secretion from the buccal and branchial surfaces appeared to accompany air breathing. For the first 30 minutes after emersion the muscles within the opercula twitched at a frequency of 30-40 per minute in most individuals.

Ventilatory movements of mudfish removed from water, varied depending upon water availability. If a thin water layer remained and could be used to moisten the gills and buccal cavity, then a mixture of air and water were pumped through the buccal and opercular cavities at a similar frequency to aquatic ventilation (30-40 breaths per minute). When free water was not available the ventilation frequency was very much reduced and the buccal and opercular chambers were held inflated for 0.25-3.0 minute intervals (Fig. 3.2). Fish maintained on dry tissue paper were more unsettled and ventilated more frequently and erratically than fish on a moist surface.
Figure 3.2 shows the changes in the frequency of ventilatory movements after emersion and over increasing periods out of water. Ventilatory rates decreased rapidly after emersion, reaching stable rates after 3 hours and then a lower rate by 24 hours. These rates were variable between fish but the mean ventilatory rate did not change significantly after 24 hours.

Fish moved around the chambers during emersion periods, being found in different positions in the chambers between subsequent days. Over emersion periods exceeding 24 hours, approximately 40% of fish at any one time would lie on their dorsal surface with their ventral surface exposed (Fig. 3.3). They would change this position frequently but the orientation of the fish did not appear to effect the ventilation frequency or pattern.

![Graph of aerial ventilation rate over increasing periods after emersion.](image)
Figure 3.4 Graph of aerial oxygen consumption of mudfish at 15°C over increasing periods of emersion.

Fish Weights 5.63 ± 0.67 (n=16).

Figure 3.5 Graph of the stable aerial ventilation frequency after 24 hours emersion at different temperatures.

Q10 (5-15°C) = 18.0 ; Q10 (15-25°C) = 5.7
Figure 3.3 Photograph of emersed mudfish showing the ventral exposed stance. Note the mucoid bubbles of air emerging from the inflated opercula.

The pattern of aerial $O_2$ consumption (measured by Gilson respirometry) following emersion of mudfish (Fig. 3.4), showed a transitory increase in the oxygen consumption over the first 4-6 hours, from 50 to $70 \mu l O_2 \, g^{-1} \, hr^{-1}$. This was followed by a decrease over the next 3 hours to a level similar to the initial aquatic respiration rate. Over longer time periods of up to 28 days the oxygen consumption did not significantly change from this stable rate. Oxygen consumption did not fall below 85% of the standard aquatic rate at any stage.

The ventilation rate of emersed mudfish at different temperatures (Fig. 3.5) shows that ventilation rate increased rapidly with increasing temperature. The Q10 values for ventilation rate were 18.0 and 5.67 respectively for the 5-15 and 15-25°C temperature ranges. These indicate that ventilation frequency is highly dependant on temperature.
Oxygen Consumption
μl O₂ g⁻¹ hr⁻¹

Aerial Ventilation Rate.
Breaths per minute.

Temperature (°C)

0 5 15 25
The changes in aerial oxygen consumption with changes in temperature are shown in Figure 3.6. The $O_2$ consumption rose with increases in temperature over the range 5-25°C. $Q_{10}$ values of 1.75 and 1.78 were obtained for the ranges 5-15°C and 15-25°C respectively. Thermal sensitivity was therefore constant over this range.

Figure 3.6 Graph of aerial oxygen consumption of mudfish after being emersed for 24 hours at three temperatures.

Respiratory Temperature Coefficients:

$Q_{10} (5-15°C) = 1.75$ ;  $Q_{10} (15-25°C) = 1.78$

The graph of $\dot{V}O_2$ and $\dot{V}CO_2$ over increasing emersion periods (Fig. 3.7) shows linear increases in both parameters. However, the increase in $\dot{V}O_2$ is slight and not significant from a slope of zero (Regtest; $p>0.05$). In contrast to results obtained with Gilson respirometry, (Fig.3.4), $\dot{V}O_2$ did not fall with time. Thus the fish may require increased settling time in the latter experiment respirometers.

The slope of $\dot{V}CO_2$ is much greater than that of $\dot{V}O_2$ and slowly increased to approach the $\dot{V}O_2$ values. The increase in $\dot{V}CO_2$ was most
marked over the first four hours of emersion.

The graph of the RQ over increasing emersion periods shows an increase in RQ from 0.6 to 0.81 by twelve hours (Fig. 3.8). Since an RQ value of 0.8 may indicate respiratory equilibrium, fish may not be in respiratory equilibrium until at least 10 hours after emersion.

The effects of different experimental manipulations on the respiratory parameters of mudfish are shown in Table 3.1. Individual variation between fish was high resulting in large standard errors. None of the parameters differed significantly between treatments showing that none of the manipulations or combinations of manipulations markedly affected experimental fish more than the controls.

Over the first 4 hours of air exposure, (Table 3.2a), 40% of $\dot{V}O_2$ and 45% of $\dot{V}CO_2$ occurred via the skin. The RQ value was 0.63, well below respiratory equilibrium. An injection of CA had no significant effect on any respiratory parameters relative to the saline injected fish. The injection of AZ, however, affected several of the $\dot{V}O_2$, $\dot{V}CO_2$, and RQ values. The $\dot{V}O_2$ from the head chamber was significantly increased ($p<0.05$) and the $\dot{V}CO_2$ from the body compartment was significantly decreased ($p<0.05$). The total $\dot{V}O_2$ was significantly increased ($p<0.01$) and the RQ values from both chambers and in total were significantly reduced ($p<0.01, p<0.05, p<0.01$).

Table 3.2b shows an increased total $\dot{V}O_2$ compared to that of the shorter emersion period ($p<0.05$). The effect of an injection of CA over this period was a slight but insignificant increase in $\dot{V}O_2$ and an almost 50% increase in $\dot{V}CO_2$ from both compartments and in total ($p<0.01$). Correspondingly, all three RQ values were significantly increased ($p<0.05$). Overall, there was an increased excretion of $CO_2$ from all surfaces.
Figure 3.7 Graph of oxygen consumption and carbon dioxide excretion of eight mudfish during a twelve hour emersion period at 15°C. 
Fish Weight 4.74 ± 0.27 (n=8) 
Regression lines: \( Y = a + b \cdot X \) 
\( VO_2 = 90.4 + 1.39 \cdot t \) \( r = 0.85 \) 
\( VCO_2 = 52.3 + 2.84 \cdot t \) \( r = 0.88 \)

Figure 3.8 Graph of the respiratory quotients (RQ) calculated from the linear regressions of \( VO_2 \) and \( VCO_2 \) of eight mudfish over twelve hours emersion. Weight 4.74 ± 0.27 (n=8) 
Regression line: \( RQ = 0.589 + 0.0185 \cdot t \) \( r = 0.99 \) 
Time to RQ=0.8 is 11.42 hours.
Table 3.1 Oxygen consumption and CO₂ excretion of mudfish during the first four hours in air, subjected to different stresses and combinations of stresses. Units: μl(gas) g⁻¹ hr⁻¹.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>( \dot{V}_{O_2} )</th>
<th>( \dot{V}_{CO_2} )</th>
<th>RQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>48.1 ±4.1</td>
<td>17.9 ±2.3</td>
<td>0.37</td>
</tr>
<tr>
<td>Anaesthetised</td>
<td>8</td>
<td>44.0 ±4.4</td>
<td>18.2 ±2.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Anaesthetised and injected with saline</td>
<td>8</td>
<td>46.1 ±4.1</td>
<td>17.5 ±2.5</td>
<td>0.38</td>
</tr>
<tr>
<td>Anaesthetised and partitioned</td>
<td>6</td>
<td>49.9 ±4.0</td>
<td>23.1 ±2.2</td>
<td>0.47</td>
</tr>
<tr>
<td>Anaesthetised, partitioned and injected with saline</td>
<td>6</td>
<td>42.5 ±4.0</td>
<td>21.7 ±2.3</td>
<td>0.51</td>
</tr>
</tbody>
</table>

* None significantly different.

Table 3.2c shows that the respiratory quotient for the whole fish was still well below 0.8 at 8-12 hours after emersion. None of the parameters were significantly changed relative to the 4 hour emersed fish. This result differs from the previous results (Fig.3.7) where RQ had significantly increased over this period and therefore reflects a different settling period. Partitioned mudfish were therefore not achieving respiratory balance within 12 hours of emersion. With the injection of CA, the \( \dot{V}_{CO_2} \) and RQ of the compartments and in total were significantly increased relative to the control (p<0.05). The \( \dot{V}_{O_2} \) of the body and in total also increased significantly (p<0.05). The \( \dot{V}_{CO_2} \) and RQ of both compartments and in total were slightly but not significantly increased relative to the 4-8 hour CA injected fish.

Overall, both groups of fish injected with CA showed increased CO₂ excretion beyond four hours of emersion, while fish injected with AZ decreased CO₂ excretion over the first four hours of emersion. These changes occurred via both the head and body regions.
Table 3.2a,b,c. Oxygen consumption and carbon dioxide excretion of partitioned emersed mudfish, emersed over 4, 8 and 12 hours and injected with carbonic anhydrase, acetazolamide or saline.
<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>Weight (g)</th>
<th>HEAD</th>
<th>BODY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \dot{V}O_2 )</td>
<td>( \dot{V}CO_2 )</td>
<td>RQ</td>
</tr>
<tr>
<td>a 0-4 hr Emersion</td>
<td></td>
<td></td>
<td>( \pm )</td>
<td>( \pm )</td>
<td>( \pm )</td>
</tr>
<tr>
<td>saline injection</td>
<td>6</td>
<td>8.20</td>
<td>±.60</td>
<td>24.6</td>
<td>±3.3</td>
</tr>
<tr>
<td>CA injection</td>
<td>6</td>
<td>8.20</td>
<td>±.60</td>
<td>27.9</td>
<td>±4.2</td>
</tr>
<tr>
<td>AZ injection</td>
<td>6</td>
<td>8.20</td>
<td>±.60</td>
<td>39.5*</td>
<td>±5.4</td>
</tr>
<tr>
<td>b 4-8 hr Emersion</td>
<td></td>
<td></td>
<td>( \pm )</td>
<td>( \pm )</td>
<td>( \pm )</td>
</tr>
<tr>
<td>saline injection</td>
<td>6</td>
<td>8.12</td>
<td>±.60</td>
<td>30.7</td>
<td>±3.2</td>
</tr>
<tr>
<td>CA injection</td>
<td>6</td>
<td>8.04</td>
<td>±.52</td>
<td>38.5</td>
<td>±3.1</td>
</tr>
<tr>
<td>c 8-12 hr Emersion</td>
<td></td>
<td></td>
<td>( \pm )</td>
<td>( \pm )</td>
<td>( \pm )</td>
</tr>
<tr>
<td>saline injection</td>
<td>6</td>
<td>7.80</td>
<td>±.55</td>
<td>28.2</td>
<td>±2.9</td>
</tr>
<tr>
<td>CA injection</td>
<td>6</td>
<td>8.00</td>
<td>±.63</td>
<td>36.8</td>
<td>±3.3</td>
</tr>
</tbody>
</table>

\* = p < 0.05; ** = p < 0.01
3.3.2 METABOLISM

3.3.2.1 Metabolic Weight Loss

The patterns of metabolic weight loss of air exposed and fasted fish were similar over a 28 day fasting period (Fig. 3.9). Loss rates were initially rapid but became stable and linear beyond three days of fasting. The rates of weight loss were significantly different between the two groups \(p<0.01\) with fasted fish losing weight more rapidly than emersed fish.

![Graph of percent wet body weight lost during fasting or emersion of mudfish over 28 days.](image)

**Figure 3.9** Graph of percent wet body weight lost during fasting or emersion of mudfish over 28 days.

Linear Regression (Excluding \(t=0\)).

- Fasted fish \(\ast\) Loss \(\% = 3.91 + 0.42 \times \) \(r = 0.997 \) \(n=8\)
- Emersed fish \(o\) Loss \(\% = 2.71 + 0.30 \times \) \(r = 0.993 \) \(n=9\)
Over an 85 day period, fasted fish showed a 27.5% weight loss which was only 76% of the loss predicted from the 28 day loss rate (Table 3.3). Mudfish showed no mortality over this period of fasting. Emersed fish showed a 40% mortality over an 85 day emersion period. Weight loss during this emersion period was 22.1%, or 86% of that predicted from 28 day loss rates (Table 3.3). Therefore, in both groups weight loss rates decreased over longer fasting or emersion periods.

Table 3.3 Weight loss rates of starved and emersed mudfish calculated from mean losses of groups of fish.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight n (g)</th>
<th>Total loss %</th>
<th>Weight loss % per day</th>
<th>Est. loss % at 6 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted fish 28 days</td>
<td>5.46 ± 0.79</td>
<td>11.8 ± 1.0</td>
<td>0.42</td>
<td>76.6%</td>
</tr>
<tr>
<td>Fasted fish 85 days</td>
<td>5.13 ± 0.51</td>
<td>27.5 ± 1.7</td>
<td>0.32</td>
<td>58.4%</td>
</tr>
<tr>
<td>Emersed fish 28 days</td>
<td>4.86 ± 0.62</td>
<td>8.4* ± 0.9</td>
<td>0.30</td>
<td>55.3%</td>
</tr>
<tr>
<td>Emersed fish 85 days</td>
<td>6.19 ± 1.08</td>
<td>22.1* ± 1.6</td>
<td>0.26</td>
<td>47.5%</td>
</tr>
</tbody>
</table>

§ Reduced by mortality.  * p<0.01.

3.3.2.2 Lipid levels

The lipid contents of groups of aquatic, starved and emersed fish were variable between fish but were not significantly different between groups (Table 3.4). Fat levels were therefore not significantly changed by air exposure or fasting in the experimental groups.

Gonads of several preserved fish showed lipid contents of 35% compared to whole body levels of 17% (Table 3.4). Therefore, gonads and viscera may be significant lipid storage sites in mudfish.
Table 3.4 Total lipid contents of starved and emersed mudfish.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>% lipid content (dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Aquatic, fed)</td>
<td>10</td>
<td>17.0 ± 3.8</td>
</tr>
<tr>
<td>Fasted, 28 days</td>
<td>7</td>
<td>16.0 ± 3.9 NS</td>
</tr>
<tr>
<td>Emersed, 28 days</td>
<td>6</td>
<td>15.3 ± 2.6 NS</td>
</tr>
</tbody>
</table>

DISCUSSION

3.4.1 RESPIRATION

Aquatic ventilatory pumping mechanisms are essentially similar in all fishes, except for the active, ram ventilating species (Shelton, 1970). They differ only in the relative importance and development of the buccal and opercular pumps. Shelton described at least four types of aquatic pumping strategies relating gill resistances and differential pump pressures. Without pressure recordings, the type of ventilation cannot be determined, in mudfish, but it appears that both buccal and opercular pumps make a significant contribution to ventilation.

Mudfish do not appear to show any peculiarities in aquatic breathing patterns that might indicate structural changes for air breathing. The apnoeic pauses indicate a low oxygen requirement of undisturbed fish. Apnoeic pauses have been described for other sluggish species (Forster, 1981). They may also have a low gill \( \text{O}_2 \) uptake requirement due to the supplementary cutaneous \( \text{O}_2 \) uptake (Meredith et al., 1982) and therefore may not suffer the degree of blood deoxygenation during apnoea, as is seen in other periodically apnoeic species (Forster, 1981).
The aquatic oxygen consumption is below the average for 34 temperate freshwater species, listed in Altman and Dittmer (1974), and similar to several small *Etheostoma* species (Ultsch et al., 1978).

The ventilatory movements of mudfish in air indicate a distinct preference for mudfish to pump water or a mixture of water and air through the mouth and gills if any water is available. This reflects the primary aquatic mode of life of the fish. Many tropical fish may utilise O$_2$ rich surface films when in hypoxic water rather than breath air (Kramer and Mehegan, 1981). However, mudfish do not do this and resort instead to true air-breathing.

Lack of a free water layer, and the higher O$_2$ capacitance of air, inhibits the aquatic ventilatory drive soon after emersion and ventilation frequency drops as the oral and opercular chambers are inflated. This ventilatory mechanism would maximise the volume of O$_2$ in contact with the buccal and exposed gill surfaces while restricting the desiccatory water loss of rapid air movements. The opercular muscle twitches indicate that an aquatic respiratory drive may still function for some time after emersion. This may be while significant amounts of water are still held around the gills. The drive may decrease when this water layer is dispersed or other cues such as changes in acid-base status act on respiratory drive.

Mucus secretions exuded from the opercular chambers indicate enhanced mucus secretion, which may aid air-breathing mechanisms in several ways. It may retain moisture around the gill surfaces, lower surface tension effects and help to seal the opercula to allow tidal rather than flow-through ventilation. This latter function is seen in the ballooning of mucoid bubbles from the opercular margins.

Liem (1967b) and Peters (1978) described monophasic and biphasic ventilatory patterns in air-breathing anabantid fishes. However, Liem
(1980) has since written that cineradiography and electromyography must be employed to satisfactorily isolate the aerial ventilatory mechanisms of fish. He showed that the previously described monophasic and diphasic patterns in anabantids are actually more complex triphasic and quadriphasic patterns that are analogous to the aquatic feeding and coughing reflexes (Hughes, 1975). On this basis Liem suggested that many groups of air-breathing fishes may use existing reflex patterns for aerial ventilation. He also showed that the more specialised fish with obviously modified buccal and branchial muscles, such as Symbranchus, have developed unique ventilatory mechanisms that bear no relation to existing aquatic patterns.

The two ventilatory patterns described for mudfish resemble the monophasic and diphasic patterns described by Peters (1978), but without cineradiography or electromyography these cannot be confirmed as basic feeding and coughing reflexes. Furthermore, the previously described reflexes have only been described in species air breathing in water where reversed water flows and hydrostatic pressure play important roles in the ventilatory movements. In air, where these factors are not present such air-breathing mechanisms may not function very effectively. Liem (1984) stated that fish employing the cough reflex for air ventilation remain totally dependant on water and are therefore poorly adapted to explore terrestrial habitats. Therefore, such mechanisms may be of limited effectiveness or require modification in air.

Since the mudfish is only a seasonal facultative air breather, only existing or modified existing reflexes might be expected. This may be due to there being less selective pressures favouring development of complex or novel air-breathing patterns for mudfish compared to tropical swamp dwelling species (Liem, 1980). Thus, the resemblance of air-breathing patterns of mudfish to coughing and feeding reflexes may indeed be real.
The lack of the hydrostatic pressure component would aid retention of air in the oral cavities but would reduce the efficiency of tidal flushing. Lack of the reversed water flow would reduce the efficiency of tidal flushing also, but with the high $O_2$ capacitance of air (Dejours, 1978) and the low metabolic cost of aerial ventilation relative to aquatic ventilation (Shelton, 1970), adequate $O_2$ levels should be easily maintained adjacent to the exchange surfaces. The cutaneous accessory exchange would also supplement and therefore decrease the demand on the buccal and gill surfaces and mechanisms.

During ventilation, air appears to be in contact with many buccal, branchial and opercular surfaces. These have all previously been suggested to possess respiratory functions (Davidson, 1949) and therefore gas exchange roles are envisaged for a complex of surfaces during air-breathing.

The decrease in aerial ventilation frequency over the initial period of emersion is a pattern exhibited by many other air-breathing fish (Johansen, 1970). This again is a reflection of the high oxygen capacity of air compared to water and a consequence of $O_2$ rather than $CO_2$ tensions influencing respiratory control centres in fish (Randall, 1970). The further decrease in ventilation frequency beyond 3 hours of emersion may be due to fish adapting to the stress of sudden emersion and adjustment to breathing air. The time course of the change from the 3 to 24 hour value may match the pattern of aerial oxygen consumption. The lower rate may therefore be achieved by 4-6 hours. The maintenance of a uniform ventilation frequency beyond 24 hours after emersion shows that fish have achieved a stable state by this time.

The pattern of decreasing oxygen consumption during emersion, as shown by the Gilson respirometry data, is similar to the pattern of decreasing ventilation rate (Fig. 3.2). The initial increase in $V_O_2$
above aquatic rates during the first 4-6 hours of emersion probably reflects the combined effects of the stresses of handling, machine noise, shock of air exposure and adjustment to breathing air. This might be partially abolished if fish were emersed slowly over several hours without direct handling or disturbance. However, for experimental measurements this was not possible. Beyond 6 hours the $\dot{V}O_2$ fell to a level similar to the standard aquatic $\dot{V}O_2$ rate and there was no significant change in $\dot{V}O_2$ over the next 28 days. Therefore, mudfish settle down to a steady state over only a few hours. The actual settling period may however vary greatly, under differing experimental conditions, as illustrated by the other respirometry and partitioning results.

The uptake of oxygen in air does not appear to be a problem to mudfish as they are able to increase their $\dot{V}O_2$ above the aquatic rate and maintain the routine aquatic rate in air. This is not surprising as the same organs are used for both aquatic and aerial $O_2$ uptake (Meredith et al., 1982) and therefore little change may be necessary. This is also shown in the lack of any modulation of the blood oxygen transport properties of emersed and aquatic mudfish (Wells et al., 1984).

Changes in position by the fish so they lie on their dorsal surface, may be a behaviour to facilitate rehydration of the dorsal surface (van Dijk, 1960) and to enhance gas exchange efficiency either by exposing the ventral skin, or the ventral aspect allowing greater inflation of the opercular and buccal cavities. The physical act of rolling may also facilitate distribution and mixing of water within the opercular cavities in contact with the gills (Stebbins and Kalk, 1961), or allow some moisture to be taken up into the opercular cavities if the fish are resting on a moist surface.

The pattern of ventilation frequency changes during emersion suggests that frequency rather than changes in chamber volume
predominantly determine the $\dot{V}O_2$. Although only viewed subjectively, the amplitude of aerial ventilation did not vary and so may not influence routine respiration rates.

The maintenance of an oxygen consumption equivalent to the routine aquatic rate, and the stable $\dot{V}O_2$ and ventilation rates beyond 24 hours, shows that mudfish do not enter a torpid state during emersion but maintain a standard metabolic rate in air. The observations of fish making random movements throughout the chamber and their detection of stimuli also support this.

Dormant or torpid states have only been described in non-Teleost groups of fish including *Protopterus* (Smith, 1930), *Lepidosiren* (Thompson, 1969) and *Amia calva* (Neill, 1950), and in amphibians (Lee and Mercer, 1967; Reno et al, 1972). However, this is usually accompanied by the development of specialized structures or extensive preparations for drought. Mudfish obviously do not fall into this category.

Many amphibious fish are only voluntarily exposed to air for short periods (Graham, 1976). These fish are often relatively unspecialised oral and cutaneous air breathers. The respiratory responses of these amphibious species to emersion varies greatly, with some species maintaining aerial $\dot{V}O_2$ higher than the aquatic rate (Bicudo et al, 1979; Liem, 1967a; Sacca and Burggren, 1982; Teal and Carey, 1967) and some being unchanged, as in this study. Other fish reduce aerial $\dot{V}O_2$ below the aquatic rate (Berg and Steen, 1965; Jeukken, 1957; Tamura et al, 1976). However, factors such as duration of emersion and body size may affect the generalizations of aquatic/aerial $\dot{V}O_2$ ratios both between and within species. Clearly, Canterbury mudfish would be initially described as maintaining $\dot{V}O_2$ higher in air than water (Meredith et al, 1982) but over longer periods the mudfish shows an
equal ratio. Similarly, many species only survive for limited periods, or have only been studied over the first few hours of emersion and generalisations made from this. Also, in fishes with a greater adult size range than mudfish, the ratio can alter drastically as respiratory rate and efficiency varies with size (Ebeling et al, 1970). Graham (1976) concluded that marine amphibious fish generally have equal or higher metabolic rates in air than in water while freshwater or larger fish maintain lower metabolic rates in air. He suggested that the responses to air breathing in the two groups are essentially different. This would indicate that mudfish have affinities with the former group.

The terrestrial environment is prone to much greater and more rapid changes in temperature than the aquatic environment. One of the greatest problems for amphibious fish in air is the potentially greater respiratory demand at higher temperatures. The increased ventilation rate of mudfish at higher temperatures shows that the oral/branchial exchange becomes increasingly more important. The Q10_{vent} values show that ventilation increases exponentially, suggesting not only an increasing reliance on buccal ventilation at high temperature but also that ventilation efficiency may ultimately limit maximum respiration rates. Beyond a certain frequency, ventilation rates may not increase O$_2$ uptake efficiency but may even be associated with decreased efficiency due to the increased O$_2$ consumption of respiratory pump muscles. This has been shown for aquatically respiring fish (Kiceniuk and Jones, 1977). However, cost of ventilation in air would be considerably less than in water (Shelton, 1970).

Survival at higher temperatures and the scope for activity may be limited by the efficiency of the respiratory mechanisms. Meredith et al, (1982) showed the ability of the head of the mudfish to double the uptake of oxygen at 15°C when the cutaneous oxygen uptake had been restricted. Therefore, increased ventilation of the oral surfaces could
deliver more oxygen to the fish than the passively exchanging skin at higher oxygen demands. Conversely, at cool temperatures the cutaneous route may be able to supply almost all of the oxygen demand when fish are at rest, since the fish cease to ventilate.

The similarity of respiratory Q10 values with increased temperature range shows that mudfish have a uniform thermal sensitivity over the 5-25°C temperature range. They thus show no adaptation at these elevated temperatures to limit metabolic rates. In contrast Gordon et al (1977) found mudskipper fishes to be moderately sensitive to temperature in the 10-20°C range (Q10=1.5) but highly sensitive in the 20-30°C range (Q10=2.6).

Since ventilation rate increases more rapidly than respiration rate with temperature increases, it is possible that the cutaneous oxygen uptake rate remains fairly constant and that changes in total \( \dot{V}O_2 \) are brought about by changes in ventilation pattern alone and therefore via the oral and branchial exchange surfaces. This would mean the mudfishes ability to cope with high temperatures, disturbance and any necessary activity would be limited by the rate of diffusion across buccal and branchial oxygen uptake surfaces, and the ventilation of them. The buccal and branchial surfaces are therefore regarded as the primary gas exchange surfaces.

The \( \dot{V}O_2 \) values, measured by closed box respirometry, during increasing emersion periods did not decrease over the 12 hour period. The difference of this pattern from the decrease with time to basal levels of \( \dot{V}O_2 \), measured by Gilson respirometry, must reflect the different holding conditions. It highlights the importance of comparing the responses of fish in similar situations and conditions.

The \( \dot{V}CO_2 \) values increased with increasing emersion period to approach a value of 80% of the \( \dot{V}O_2 \). The data could describe a linear
increase in CO₂ excretion throughout the 12 hour period. The pattern generated by the individual points, however, showed a sinusoidal pattern like the VO₂ data but of greater magnitude. The deviations from linearity at 0-4 hours and 8-12 hours would indicate greater increases in CO₂ excretion at these periods. An early rapid rise in CO₂ excretion is expected upon emersion as the internal CO₂ levels rose to generate greater diffusion gradients, but the later rise is unexplained. Therefore, apart from a steep initial rise in V.CO₂ the linear increase is probably a better description of CO₂ excretion patterns in emersed mudfish.

The RQ versus time relationship, constructed from fitted values from the regressions of VO₂ and V.CO₂, showed a rise in RQ over the emersion period. If a mean RQ value of 0.8 is used to indicate respiratory equilibrium (Dejours, 1978), then mudfish did not achieve equilibrium until at least 11.4 hours after being first emersed. Respiratory quotients of 0.71-1.0 can indicate respiratory equilibrium, depending upon the main metabolic substrate (Schmidt-Neilson, 1979). Since most fish predominantly metabolise protein (van Waarde, 1983), a figure of 0.8 is to be expected. Mudfish, therefore, spend several hours in air accumulating CO₂ before reaching a stable state.

An estimate of the CO₂ load can be calculated from Fig. 3.8, assuming the necessary RQ value of 0.8 is accurate. Over the 11.42 hours before this is achieved, 5.51μM of CO₂ is accumulated per fish. This could be distributed throughout the fish in the blood, extracellular fluid or in all of the body fluid compartments. It will also be present in both the ionised form as bicarbonate and as gaseous CO₂, in a ratio determined by the Henderson-Hasselbach equation (Dejours, 1978). If all of the CO₂ was distributed in a 3% blood volume this would elevate blood concentrations by 5.51μM/l. It is more likely that CO₂ is distributed throughout much of the extracellular fluids. With this
estimated at 30% of body weight (Chapter 5) then the concentration would be elevated by 3.88mM/l, assuming uniform distribution. Heisler (1982) however, found that much of the bicarbonate accumulated by Symbranchus during emersion was retained intracellularly, for buffering of intracellular fluids. If this is also the case with mudfish then an 80% fluid space must be considered (Chapter 6) and with uniform distribution only a 1.45mM/l increase in CO₂ would be expected. The distribution of the accumulated CO₂ would therefore have important consequences to acid-base balance in emersed mudfish. Unfortunately neither in vivo blood values nor measurements of CO₂ excretion upon reimmersion could be obtained to verify the level of CO₂ retained by mudfish or its distribution.

The magnitude of CO₂ accumulation seems reasonable in light of current theories of CO₂ excretion and diffusibility. Piiper (1982) used models to predict, and then experimentally verified, the probable CO₂ levels across several gas exchange organs. He noted the similar diffusibility of CO₂ and O₂ in air but the much higher capacitance of CO₂ in water and hydrated tissues. In skin breathers he found that skin thickness gave a diffusion limitation but with the capacitance of tissues being 20 times higher for CO₂ than O₂, CO₂ movement should be much more rapid than O₂ or require much lower partial pressure gradients. However, factors such as rates of dissociation of bicarbonate complicate the calculation. In the skin breathing salamander Desmognathus (Gatz et al, 1974) blood CO₂ levels were predicted and verified as approximately 5 torr. Thus, relatively low accumulated levels of CO₂ would be expected in a skin breathing species.

Randall (1981) listed high CO₂ tensions in the blood of air-breathing species of fish compared to trout, and Heisler (1982) measured a four fold increase in the CO₂ tension of the blood of the emersed gill and skin breathing fish Symbranchus. DeLany et al
(1977), however, found a decrease in the CO₂ tension of the blood of lungfish when they increased skin perfusion during aestivation.

Overall, it appears that levels of CO₂ would be expected to rise in the blood of emersed mudfish but these levels would not be very great while the mudfish are at rest. This will disrupt acid-base balance, but since Wells et al (1984) measured high CO₂ buffering capacity of mudfish blood (19mM 1⁻¹ per pH unit), this may not greatly change blood pH.

By 12 hours after emersion, the rate of CO₂ excretion by mudfish probably matches that produced, and therefore CO₂ would no longer be accumulating. The increased rate of CO₂ excretion could be due to both the increased body PCO₂ levels generating a greater gradient for CO₂ excretion and increased endogenous carbonic anhydrase enzyme being produced.

Elevated RQ values, greater than 0.8, were not seen over any time period during emersion, therefore the CO₂ accumulated over the first few hours would be retained over the entire emersion period. This might lead to a slightly different blood ion and acid/base status in air but with the high buffering capacity of blood this may not be very great (Wells et al, 1984).

Low RQ values are reported for most emersed amphibious fish and accumulated CO₂ levels are seldom excreted until reimmersion (Graham, 1976). The problems of CO₂ excretion, therefore, appear the greatest potential problem for fishes. It is a potential problem for mudfish with increased CO₂ load and some disruption of acid-base and ion levels. However, the mudfish utilises an ability to withstand varying levels of CO₂ accumulations and possesses a permeable skin that permits adequate CO₂ excretion rates once an internal concentration gradient has been developed.
The large range of respiratory parameter values measured during different experimental manipulations (Table 3.1) shows the importance of considering stressors in physiological experiments. With the high degree of variation between fish within treatments, large changes in respiratory values between treatments must be observed for them to be significantly different. Therefore, the different effects of the stress on the individual fish restricted the sensitivity of the results. However, it must be noted that even the control fish were subjected to a substantial level of experimental manipulation. Further manipulation beyond this level may therefore not greatly disturb the fish.

Over the first 4 hours of air exposure the partitioned fish were not in respiratory equilibrium. Neither compartment was excreting sufficient quantities of CO₂ and therefore CO₂ must be accumulating in the fish, as seen in the previous experiment. Oxygen consumption was higher from the head (68%) but CO₂ was lost equally from both halves of the body. Thus, similar results were obtained to those of Meredith et al (1982).

Many factors affect CO₂ loss from fish and several must be considered before its loss from the mudfish can be explained. The dehydration of HCO₃⁻ to CO₂ in blood proceeds slowly (Forster and Crandall, 1975) and is a rate limiting step if the enzyme carbonic anhydrase is not present. Until recently, CA in fish was thought to be functional only in the gill epithelium or tissues derived from gills (Randall et al, 1981). Carbonic anhydrase in the red blood cell was thought to be inhibited by an unidentified factor that decreased erythrocyte permeability to HCO₃⁻ (Haswell and Randall, 1976). Therefore, HCO₃⁻ dehydration in tissues such as specialised air breathing organs would proceed at a slow rate that would preclude appreciable excretion from all tissues other than the skin. The in-transit time of blood in specialised air-breathing organs is too low for
appreciable bicarbonate dehydration, but with the longer in-transit time in skin, dehydration can proceed appreciably to allow CO₂ excretion. This premise of CA action and inhibition is no longer considered totally valid (Heming and Randall, 1982; Perry et al., 1982). Carbonic anhydrase, therefore, may be functional in the dehydration of HCO₃⁻ in the blood of many fishes and function in non-gill gas exchange organs. A role is still envisaged for membrane bound CA in gill epithelium and therefore enhanced CO₂ transport from the gill or gill derived tissues.

Manipulation of the systems governing CO₂ excretion has been carried out in several fish species (Burggren and Haswell, 1979; Daxboeck and Heming, 1982; Randall et al., 1978), to determine the predominant mechanisms governing CO₂ movements. These have yielded variable roles for CA and the related mechanisms for acid-base balance.

The effects of injections of CA and AZ on mudfish have indicated some of the possible roles of CA in the aerial CO₂ excretion of the mudfish. An injection of CA had no effect on CO₂ excretion over the first 4 hours of emersion showing there may not be a great enough pool of HCO₃⁻ for the injected CA to affect the CO₂ excretion rate. The enhancement of VO₂ by the AZ injection indicates a stressing effect which may be due to disruption of acid-base balance mechanisms within the fish. Randall (1981) suggested that CA action is central to acid/base regulation within fish. Injections of saline or CA did not cause an elevation of VO₂ so this must be an effect of the AZ itself. The VO₂ enhancement by AZ however confuses the interpretation of the VCO₂ data.

The VCO₂ of the head compartment shows no significant difference to control values, but in relation to the elevated VO₂, the RQ was significantly reduced. Thus, the amount of CO₂ anticipated to be excreted from the head, was not excreted. In the body compartment the VO₂ was not significantly increased but the VCO₂ was significantly reduced. Therefore, CA inhibition caused reduced cutaneous CO₂
excretion. This suggests that CA has a role in cutaneous, and therefore total CO\(_2\) excretion, via the blood. The decrease in total RQ by 40\% for the AZ treatment, shows that up to 40\% of the carbon dioxide excretion may be due to endogenous CA enzyme action. However, as the \(\text{VO}_2\) is enhanced by the AZ injection, the contribution of CA might be lower than this at lower rates of oxygen consumption.

Over the 4–8 hour period, control RQ values were not significantly increased compared to 0–4 hours, so a greater CO\(_2\) load within the fish was predicted. An injection of CA at 4 hours after emersion showed a near doubling of CO\(_2\) excretion from both compartments. Thus, there is a great enhancement of CO\(_2\) excretion due to enhanced levels of circulating CA. This indicates that a significant HCO\(_3^-\) pool has accumulated by 4–8 hours, that has not been cleared by endogenous CA. This pool may be present because of: insufficient endogenous CA present at the time of emersion, control of dehydration of HCO\(_3^-\) through CA inhibitors to allow buffering of the blood, or a diffusion limitation due to using the skin as the predominant exchange surface.

The control values obtained for fish emersed for 8–12 hours still show a low overall RQ. This departure from the results of the previous experiments for unrestrained fish shows one of the effects of partitioning upon fish. Thus, some of the degree of enhanced \(\text{VO}_2\), and CO\(_2\) loading, may be due to the level of stress upon the fish. An injection of CA over this time again showed an enhanced CO\(_2\) excretion from both compartments but no dramatic increase over the previous 4 hours. Therefore, either the pool of HCO\(_3^-\) is not substantially greater or the quantity of CA injected is saturated with plasma HCO\(_3^-\) and is therefore achieving a maximal effect. The former appears more likely as the quantity of CA injected was large and so unlikely to be saturated.

Although not giving a definitive account of CA action in the
mudfish respiratory system these experiments suggest several changes do occur. These are: that endogenous CA is acting in the CO$_2$ excretion of mudfish; that with air exposure a significant HCO$_3^-$ pool appears to develop in the fish; and that CA is active and possibly not inhibited in the blood. Thus, upon air exposure the CO$_2$ excretion system must change to enhance skin loss of CO$_2$. This would most likely require increased synthesis of CA and a change in buffering of the blood to account for the enhanced CO$_2$ levels. As indicated in these experiments, these would take several hours to achieve and would leave fish in a different acid base balanced state.

Carbonic anhydrase activity in the skin tissue itself has not been investigated in mudfish but has not been found in any other skin breathing fish species (Randall et al, 1981). CA is therefore likely to be active in the blood rather than the skin tissue of mudfish.

The RQ values calculated for the whole fish are generally lower in the partitioned fish compared to the unpartitioned fish in the previous section. As well as not achieving respiratory equilibrium within 12 hours, the results indicate that the concentration of CO$_2$ accumulated by the partitioned fish would be greater than that calculated in the previous section. This enhanced storage may also have magnified some of the mobilisation of CO$_2$ seen with CA but would not have affected the overall trends.

3.4.2 METABOLISM

The patterns of weight loss of fasted and emersed fish showed an increased rate of weight loss during the first 3 days of fasting, and a more linear rate beyond 3 days. These weight losses are predominantly metabolic rather than desiccatory since constant humidity and substrate substration were maintained. The initial high weight loss can be accounted for by a number of factors. For fasted fish these would have
been: the increased activity due to disturbance and handling; activity associated with light avoidance; exploratory activity, and food searches; faecal losses from previous meals; and interfish interactions for limited cover. In emersed fish they would include: enhanced activity as fish attempted to regain water or cover; faecal losses; non replaced urinary losses; and initial drainage of water from body surfaces and of water retained in the mouth around the gills. These would lead only to enhanced weight loss in the fasted fish, but both weight loss and a change to a new state of water balance in emersed fish. The former group had the greatest initial weight loss, probably due to the greater potential for extra activity outweighing the emersion hydration effects.

Since the initial rates were so much greater than the linear loss rates, they were noted but excluded from the calculation of mean linear loss rates. The linear loss rates may therefore have greater predictive power over longer periods.

The fasted fish showed greater linear rates of weight loss than the emersed fish for the same reasons that they had greater initial weight losses and increased activity, as they were not restricted in their movements.

Over longer periods of up to 85 days, both groups showed lower weight loss rates, indicating a progressive decrease in metabolic weight loss with starvation period. This would be expected as significant amounts of body tissue were lost. Mortality was a problem in long term emersed fish, but not fasted fish, with fish dying at irregular intervals throughout the 28–85 day period. This reduced the sample size of emersed fish and made comparison of treatments more difficult. Significant numbers of emersed fish, however, did survive over 80 days indicating good long term survival potential of mudfish in air. There appeared to be no pattern to the mortalities, but since fasted fish showed no mortality, it must have been due to factors of emersion
physiology, the artificial environment, or possibly the prior experimental history of the individual fish. Although the fasting did not correlate with the mortality it may be a contributing factor in combination with emersion effects, making fish more susceptible to disturbances.

The rates of weight loss of fasted and emersed fish, 0.42 and 0.30% per day over 28 days and 0.32 and 0.26% per day over 85 days respectively, are higher than those of the Brown mudfish studied by Eldon (1978a) or of eels (Dave et al., 1975; Larsson and Lewander, 1973). Eldon (1978a, 1979c) suggested that the mudfish could survive up to six months of aestivation. This period would lead to 55 and 47% weight loss of mudfish, using rates calculated from the two time periods respectively, and 20% and 47% weight loss for the two groups of brown mudfish studied by Eldon. This assumes the weight loss rates are constant throughout the six month period. Clearly, over six months the temperature, and microhabitat factors would change leading to variations in weight loss. The rates calculated in this study may also be an overestimate due to regular handling and disturbance of fish.

Interspecific comparisons are complicated by a number of factors, for example: eels hibernate and fast at low winter temperatures while mudfish aestivate at high summer temperatures. Fasting strategies and mechanisms may therefore be different in the two seasonal conditions with different demands placed upon the fish.

An important consideration in comparing fasting groups is the effect of body size. Both of Eldon's groups of brown mudfish differed in mean body size. The smaller sized group had a loss rate 2.25 times greater than the larger fish. The Canterbury mudfish used in this study had a smaller body size again, with even higher loss rates. Thus, with smaller fish having increasingly greater weight specific metabolic
rates but less mass of metabolisable body tissue, fish size would be expected to play a major role in determining maximum emersion survival times. Over longer drought periods only the larger fish may survive with the smallest fish being the first to perish.

The 85 day emersed mudfish lost considerable mass from the body trunk, becoming very slender. They are therefore able to metabolise much of their own body tissues. This ability has been documented for many fish species (Love, 1970). In one study an eel was noted to lose 76% of its body weight over a 4 year period (Love, 1970). Amongst amphibians, aestivating salamanders may lose 40-60% of their pre-aestivation body weight (Reno et al, 1972). The degree of weight loss able to be tolerated by mudfish was not determined in this study but may exceed 50% of initial body weight.

With a knowledge of drought periods, size structure and condition of a population, an idea of the percentage survival of a population may be estimated, assuming large numbers of fish were successful in finding favourable microhabitats. On a more gross level, durations of droughts that may lead to extinction of the population may be estimated.

Thus, like other fish species, Canterbury mudfish appear to be similarly able to withstand long periods of starvation and weight loss but total periods may be limited within the constraints of their small body size. Such periods, however, may indeed be up to six months as suggested by Eldon (1979c).

The metabolism of different compounds are important in the different stages and periods in the life of fishes. Fish predominantly metabolise dietary protein (van Waarde, 1983) although some species, such as herbivorous species, have specialised in carbohydrate metabolism (Love, 1970). Since the Canterbury mudfish is omnivorous, feeding on a wide variety of aquatic organisms (Eldon, 1979b), the primary metabolism would be protein based.
Both lipid and glycogen are stored in the body tissues of fish species but are thought to be only used in rapid activity and fasting, and anaerobic or anoxic conditions respectively (Van Waarde, 1983). The predominant metabolic substrates and decreases in different body constituents have been studied in several fish species during fasting (Dave et al, 1975; Moon, 1983b; Smith, 1937). Several studies on eels, *Anguilla anguilla*, indicated that lipids, mainly in the form of triglycerides are stored in the liver, muscle and gonads prior to fasting or migration phases (Lewander et al, 1974). It is generally agreed that the triglycerides are the lipids that can be mobilised for energy requirements while the phospholipids and cholesterol are mainly membrane constituents. Over experimental three month fasting periods of eels, neither Larsson and Lewander (1973) nor Moon (1983b) were able to measure decreases in triglyceride content, but Dave et al (1975) measured large decreases in body triglycerides and stated that metabolic energy was accounted for almost entirely by lipid metabolism. Smith (1937) similarly reported that only 25% of metabolism was protein catabolism in the early stages of fasting in the lungfish (*Protopterus*). Gehlbach et al (1972) also noted the predominance of lipid metabolism during aestivation in the amphibian *Siren intermedia*. However, over longer periods, often termed starvation rather than fasting, body protein is catabolised and can be observed as decreases in both red and white muscle fibre size (Moon, 1983a).

Eldon (1979c) found varying 'fat' levels in mudfish at different seasons with a peak at pre-aestivation periods. He related this to the possible use of 'fat' as a stored metabolisable energy source during aestivation. His method of lipid measurement was not described but probably measured total lipids, as in this study, and so non-metabolisable lipids such as the phospholipids and cholesterol would also be measured. His use of gonad free carcases also removed the
variability of gonad size and development, but it has often been noted
that gonads and viscera are often the sites of lipid deposition and are
also the first sites to be utilised (Moon, 1983b). El-Sayed et al
(1984) found the gonads of a female cichlid fish to be the highest lipid
containing tissue in the body. Roe lipids are also of note in many food
fishes (Kaitaranta and Linko, 1984). In this study, gonad and visceral
tissue had high lipid contents consistent with this trend.

The total lipid contents measured in this study (17%), would allow
for significant periods of fat metabolism before fish had to resort to
losses of structural body tissues. This level is not especially high in
comparison to other fish species. The most 'fatty' fish cited is
Salvelinus namaycush which averaged a 67% lipid content of muscle
tissue (Love, 1970). The total fat contents measured by Eldon (1978b)
appear low compared to those in this study. If these were
presented as percent wet weight then corrections to dry weight make
levels comparable with those of this study.

The lack of any significant decrease in total body lipid content
with fasting or emersion is unexpected. During the 4 week period 8-12%
of body weight was lost and much of this was assumed to be lipid loss.
Either, fish do not initially utilise appreciable amounts of lipid, or
any decreases are undetected in the large degree of variability between
fish. Due to the limited numbers that could be sacrificed the different
sexes could not be separated and only small numbers of fish were sampled.
Also, without peaks in food availability or seasonal cues, fish may not
be in similar reproductive states and therefore, not all actively or
selectively storing lipids. Thus, field experiments or experiments on
freshly caught fish may be more conclusive. However, freshly caught fish
are often highly stressed and show higher incidence of disease and
mortality if handled straight after capture (see Chapter 2.3).
Therefore, it is unfortunate that the importance and role of lipid
metabolism in aestivation is inconclusive and cannot be commented upon further.

In summary, the respiration and metabolism of air exposed mudfish _Neochanna burrowsi_ show several general trends. Oxygen uptake is not restricting and a standard rate of metabolism and weight loss is maintained. Thus, fish are in an active and responsive state rather than in a dormant or torpid state. This allows fish to use behavioural actions, such as rolling over, to avoid or to cope with environmental stressors. Fish could also actively seek water the moment it returns.

The problems of retaining a high metabolic rate are the high oxygen and energy demand, high weight loss and enhanced excretory loads. These would limit the maximum period of emersion. Drought periods would not be expected to last as long in temperate as in tropical regions and therefore the metabolic costs may not be as important. In tropical regions where droughts could be of indeterminate length and temperatures remain high, adaptations for survival for the longest possible period may be more important. Lungfish and other tropical fish make preparations for drought and enter torpid states, while it is more important for mudfish and other temperate fish to remain active and respond to water reappearance. With man's disruption of the environment (McDowall, 1976) possible drought periods are becoming longer in the remaining mudfish habitats with concomittant extinction of some populations (Eldon, 1978b).
CHAPTER IV

NITROGEN EXCRETION.

INTRODUCTION

The major nitrogenous excretion product in teleost fishes is ammonia which is largely a product of protein catabolism in the liver. Small amounts of urea are produced by teleost fish but this is thought to be produced by nucleotide degradation via uricolyis rather than by any active ornithine-urea cycle pathway (Forster and Goldstein, 1969). In the aquatic environment, ammonia and urea are rapidly excreted from the gills and kidney in large volumes of water.

Although fish are ammoniotelic in relation to their aquatic lives, the amphibious nature of some species raises questions about what effect changes in the environment may have on the patterns of waste nitrogen synthesis and excretion.

As mudfish are capable of living without access to free water for several weeks, they may be vulnerable to accumulation of the toxic end-products of protein catabolism. There is evidence that metabolic rate is not suppressed in emersed fish (Chapter 3), although a switch from protein to lipid metabolism is possible. Therefore, the rate of production of nitrogenous products may be somewhat suppressed. However, a rise in ammonia levels might threaten the acid-base status of the blood, and hence oxygen delivery, since at physiological pH levels it will be mainly in the form of the ammonium ion. Solutions to the problems of nitrogenous waste processing are therefore necessary.

The adaptations to waste nitrogen metabolism shown in previously studied amphibious fish are predominantly mechanisms allowing for
accumulation, rather than specialized routes of excretion during emersion (Goldstein and Forster, 1970; Morii et al, 1978, 1979). The long periods spent out of water by mudfish, may, however, limit the extent of nitrogenous accumulation as an effective strategy in such a small fish.

The permeable skin of the mudfish may also play a role in nitrogen excretion as well as in the respiration of the fish. If so, then it is possible that cutaneous vasoconstriction in hypoxia (Chapter 3) might alter the pattern of ammonia accumulation and excretion.

MATERIALS AND METHODS

4.2.1 Aquatic Nitrogen Excretion

Nitrogen excretion in mudfish was investigated using individual fish placed in 100ml of tapwater in 250ml glass conical flasks. Flasks were shielded from visual disturbances and kept under low light intensity by covering them with a dark cloth. Three 1 ml water samples were removed after 3 hours and analysed for ammonia (A) and urea (U) levels using the Nitroprusside/hypochlorite colorimetric technique (Trietz, 1970; Appendix 4), and total nitrogenous compounds using the colorimetric technique for Ninhydrin Positive Substances (NPS) (Clarke, 1973). In the latter technique, no correction was made for taurine or proline levels and glycine was used as a standard. From ammonia and urea standards and the measured values for ammonia and urea excreted, values of NPS-(A+U) were calculated, and thus the excretion of nitrogenous compounds other than A and U were estimated. Nitrogen excretion was expressed as weight and time specific excretion rates.

The nitrogen excretion rates of fasting fish were analysed at 3 day intervals over 28 days. Groups of fish were maintained for the 28 day period in a clean 80 L aquarium and not fed. Individuals were removed from the aquarium and placed in glass flasks for measurement of
excretion rates. Flasks were aerated with compressed air. Previous trials had shown that aeration did not affect different ammonia levels in tapwater. Levels of excreted A, U, and NPS-(A+U) were measured in water samples taken 3 hours after fish were transferred to the flasks. These rates were compared to control aquatic excretion rates as calculated above.

To assess the routes of nitrogen excretion, fish were partitioned in water (as described in section 2.6). 100ml of tapwater was placed in each chamber and the water was aerated with ammonia-free air. Water samples were removed at 2 hour intervals and nitrogenous excretion rates (A and U) measured. As cutaneous nitrogen excretion could not be isolated from renal nitrogen excretion in the body compartment (see section 2.6), excretion rates were calculated for both compartments and for the whole fish.

Nitrogen excretion was also measured for fish partitioned with their heads in normoxic water (PO$_2$>100 torr) and bodies in hypoxic water (PO$_2$<20 torr). The water in the body chamber was gassed with dry nitrogen and the chambers partially sealed to maintain a nitrogen atmosphere above the water. Oxygen levels were measured with a Beckman model 39553 oxygen electrode and Fieldlab analyser and O$_2$ tensions of less than 20 torr were maintained by adjusting the gas flow. The excretion rates were compared to those of fish partitioned in normoxic water.

4.2.2 Emersion nitrogen excretion

Nitrogen excretion was measured for groups of fish maintained in air for multiples of 4 hours up to a total of 28 hours. Fish were placed in perspex lunchboxes on tissue paper soaked with 10 mls of tapwater. After the end of each experimental time period, the fish were placed into flasks containing 100ml of tapwater and levels of A, U and NPS-(A+U)
excreted, were measured hourly for 6 hours. From the level of enhancement of nitrogen excretion rates above control rates after reimmersion, a measurement of the amount of ammonia and urea accumulated in the fish and therefore an estimation of blood ammonia and urea levels could be made. The perspex lunchboxes were rinsed with 10ml of distilled water and the A, U and NPS levels in 1 ml water samples measured to calculate the amount of A, U and NPS-(A+U) remaining in the lunchbox. This gave a measure of the nitrogen levels excreted by fish whilst in air.

Acid traps were designed, to collect any gaseous ammonia excreted whilst the fish were in air. Fish were blotted dry and placed in 35 or 60ml syringe barrels (monoject) with inlet and outlet ports. Chambers were flushed with a constant flow of air that had first been passed through dilute acid (0.01N H₂SO₄) to remove any residual ammonia, and then humidified by passage through distilled water. The outflowing air was bubbled through 30ml vessels containing 10ml of 0.01N H₂SO₄ to collect excreted ammonia. 1 ml volumes of the acid were analysed at intervals of 4 hours for 28 hours for ammonia content and gaseous ammonia excretion rates were calculated. Both blanks and standards were prepared from the same stock acid solution and the efficiency of colour development was not affected by the acid conditions. The efficiency of the acid traps was assessed by placing four acid traps in series. Efficiencies of greater than 95% were found for the first trap in three trials and thus only 1 acid trap was used per chamber.

Fish that had been emersed for 24 hours in perspex lunchboxes were partitioned in air to isolate the gaseous ammonia excretion surfaces. Fish were partitioned in respirometry type chambers (Section 2.6) and each chamber was flushed with a constant flow of ammonia-free air. Gaseous ammonia was collected in the outflowing air in acid traps over 4
hours and individual chamber and total excretion rates were calculated.

4.2.3 Blood Nitrogen

Blood concentrations of nitrogen excretion products were measured in pooled plasma samples from aquatic, starved and emersed fish. Plasma samples were obtained as in Section 2.5 and added to 1ml volumes of distilled water. Samples were analysed for A, U and NPS. Measured blood nitrogen levels were compared to the values calculated in the previous section and published values for other fish species. Due to the use of pooled samples, the significance of differences between values could not be readily tested.

RESULTS

4.3.1 Aquatic nitrogen excretion

Mudfish ammonia, urea and NPS excretion rates are shown in Table 4.1. Over 80% of the nitrogenous excretion was ammonia (8.28 µgN g\(^{-1}\) hr\(^{-1}\)) with the remainder being predominantly urea. Excretion of other nitrogenous compounds, detectable as NPS, did not exceed 3% of the total budget and was thereafter assumed to be insignificant.

Table 4.1. Aquatic nitrogen excretion rates of Canterbury mudfish.

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>5.66 ± 0.60 (n = 8)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Excretion rate µgN g(^{-1}) hr(^{-1})</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>8.28 ± 0.90</td>
<td>83.3</td>
</tr>
<tr>
<td>Urea</td>
<td>1.45 ± 0.21</td>
<td>14.6</td>
</tr>
<tr>
<td>NPS-(A+U)</td>
<td>0.21 ± 0.06</td>
<td>2.1</td>
</tr>
<tr>
<td>Total</td>
<td>9.94 ± 0.98</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 4.1 shows the ammonia and urea excretion rates of fish during a 28 day fasting period. Urea excretion rates were constant.
throughout the fasting period. Ammonia excretion rates were more variable but were not significantly different from the control excretion rate at any stage. There was no significant excretion of other NPS compounds above that of the controls at any stage. Therefore, fasting did not stimulate any significant change in nitrogen metabolism.

![Graph of ammonia and urea excretion rates of mudfish during a 28 day fasting period in water at 15°C.](image)

**Figure 4.1** Graph of ammonia and urea excretion rates of mudfish during a 28 day fasting period in water at 15°C.

Fish partitioned in water excreted 27% of total ammonia and 37% of total urea excretion via the body compartment (Table. 4.2). In fish partitioned with their bodies in hypoxic water, excretion rates of ammonia from the body compartment were significantly decreased (p<0.01) and excretion rates from the head significantly increased (p<0.05), but urea excretion was unchanged from normoxic values (Table.4.2). Total nitrogen excretion was slightly but not significantly increased.
Table 4.2  Partitioned nitrogen excretion of mudfish in either normoxic water, or with the head in normoxic water and body in hypoxic water (P0₂<20 torr). Units - μgN g⁻¹ hr⁻¹.

<table>
<thead>
<tr>
<th>Fish Weight (g)</th>
<th>n</th>
<th>Head</th>
<th>Body</th>
<th>Total</th>
<th>%Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish in Normoxia</td>
<td>A</td>
<td>5.52</td>
<td>2.07</td>
<td>7.59</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>±.63</td>
<td>±.45</td>
<td>±.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight 8.00 ±1.78</td>
<td>U</td>
<td>1.13</td>
<td>0.65</td>
<td>1.78</td>
<td>37.0</td>
</tr>
<tr>
<td></td>
<td>±.15</td>
<td>±.12</td>
<td>±.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxic Head,</td>
<td>A</td>
<td>7.98*</td>
<td>1.11**</td>
<td>9.09</td>
<td>12.1</td>
</tr>
<tr>
<td>Hypoxic Body,</td>
<td></td>
<td>±1.09</td>
<td>±.17</td>
<td>±1.14</td>
<td></td>
</tr>
<tr>
<td>Weight 8.00 ±1.08</td>
<td>U</td>
<td>1.21</td>
<td>0.70</td>
<td>1.91</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>±.18</td>
<td>±.12</td>
<td>±.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance. *= p<0.05; **= p<0.01.

4.3.2 Excretion of Emerged Fish

Nitrogenous compounds accumulating in the damp tissue paper below emersed fish indicated that significant levels of ammonia were being excreted over the whole emersion period, but urea levels did not significantly increase in the substrate beyond 8 hours after emersion (Fig. 4.2). No significant quantity of any other nitrogenous compound was observed.

Ammonia and urea were accumulated during emersion and were rapidly excreted upon reimmersion in water (Fig. 4.3). The accumulated ammonia was excreted more rapidly than the urea but excretion rates of both were back to control levels by 3 hours after reimmersion. The calculated levels of ammonia and urea accumulated in the fish are shown in Fig. 4.4. The accumulation of ammonia ceased by 24 hours after emersion; urea retention was still occurring at this time, but had ceased by 48 hours.

The rate of excretion of gaseous ammonia from fish in dry chambers is shown in Fig. 4.5. The ammonia excretion rate increased during the
Figure 4.3 Graph showing the amount of nitrogenous compounds excreted by mudfish whilst emersed on damp tissue paper for 48 hours.

Figure 4.4 Graph showing the rates of ammonia and urea excretion of mudfish reimmersed after 24 hours on damp tissue paper.
Nitrogen Accumulation
\( \mu gN \ g^{-1} \) in substrate

Nitrogen Excretion Rate
\( \mu gN \ g^{-1} \ h^{-1} \)

Time after Emergence (Hours)

Time (hours) After reimmersion
Figure 4.5 Graph showing the levels of ammonia and urea accumulated by mudfish whilst in air, calculated from reimmersion excretion rates.

Figure 4.6 Graph showing rates of gaseous ammonia excretion by mudfish at up to 28 hours emersion in dry chambers.
Ammonia Accumulation

Time (hours) in air.

Ammonia Accumulation

\( \mu g N/g \)

Time (hours) in air.

Aerial Ammonia Excretion

\( \mu g N g^{-1} hr^{-1} \)

Time (hours) in air.
period of emersion to a stable rate by 12 hours after emersion, but never achieved a level higher than 15% of the aquatic ammonia excretion rate. Fish partitioned within dry chambers showed equal rates of aerial ammonia excretion from both chambers (Table 4.3).

Table 4.3 Partitioned ammonia excretion of mudfish in dry chambers after 24 hours emersion compared to ammonia excretion in water. Units μgN g⁻¹ hr⁻¹.

<table>
<thead>
<tr>
<th>Treatment &amp; Weight</th>
<th>n</th>
<th>Head</th>
<th>Body</th>
<th>Total</th>
<th>%Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic water</td>
<td>8</td>
<td>5.52</td>
<td>2.07</td>
<td>7.59</td>
<td>27.3%</td>
</tr>
<tr>
<td>8.00 ± 1.78</td>
<td></td>
<td>±.63</td>
<td>±.45</td>
<td>±.72</td>
<td></td>
</tr>
<tr>
<td>In Dry Air</td>
<td>8</td>
<td>0.44***</td>
<td>0.54***</td>
<td>0.98***</td>
<td>55.7%</td>
</tr>
<tr>
<td>7.45 ± 0.95</td>
<td></td>
<td>±.08</td>
<td>±.04</td>
<td>±.07</td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance. *-p<0.05; **-p<0.01; ***-p<0.001.

4.3.3 Blood Nitrogen

Levels of ammonia, urea, and NPS-(A+U) in the blood of aquatic, starved and 1 and 28 day emersed mudfish are shown in Table 4.4. Low levels of ammonia and no measurable urea levels were seen in the blood of both control and starved fish. Both ammonia and urea levels were increased in the blood of 1 day and 28 day emersed fish. NPS-(A+U) levels, however, were not different from control levels. Since the results were from pooled samples, sample sizes can be quoted but levels of significance could not be tested.

The estimates of blood nitrogen levels at 1 and 28 days are higher than those measured in the blood except for the urea level at 24 hours.
Table 4.4 Blood nitrogen levels in the plasma of mudfish that were starved or emersed for different periods. Measurements on pooled samples. Units - mM/l.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Ammonia</th>
<th>Urea</th>
<th>NPS-(A+U)*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (aquatic)</td>
<td>20</td>
<td>0.58</td>
<td>N.D.</td>
<td>2.10</td>
<td>2.68</td>
</tr>
<tr>
<td>Fasted (28 days)</td>
<td>10</td>
<td>0.40</td>
<td>N.D.</td>
<td>2.40</td>
<td>2.80</td>
</tr>
<tr>
<td>Emersed (24 hours)</td>
<td>10</td>
<td>2.71</td>
<td>2.47</td>
<td>1.80</td>
<td>6.98</td>
</tr>
<tr>
<td>Emersed (28 days)</td>
<td>10</td>
<td>2.13</td>
<td>2.80</td>
<td>2.21</td>
<td>7.14</td>
</tr>
</tbody>
</table>

**Estimations**

| Emersed (24 hours) | 5.20 | 1.80 | ---  |
| Emersed (28 days)  | 145.60 | 50.40 | ---  |

* Calculated as glycine equivalents.
** Calculated from estimated rate of accumulation. See text.
N.D. - non detectable.

DISCUSSION

4.4.1 Aquatic Nitrogen Excretion

Mudfish are primarily ammoniotelic during their aquatic mode of life (Baldwin, 1964). In this respect they are similar to most other teleost fish species. The rates of excretion and relative proportions of ammonia to urea excreted are also similar to the majority of other small salmoniform fish (Love, 1980). Urea nitrogen ranged from 17-25% of the total nitrogen excretion in 5 freshwater fish species studied by Vellas-Clos (1973; cited in Love, 1980). The low level of urea excretion in mudfish indicates that, as in most other fish species, urea excretion is probably derived from nucleotide degradation rather than routine metabolic origins (van Waarde, 1983). Excretion of free amino acids or similar amino compounds does not appear to play any significant role in aquatic nitrogen excretion of mudfish but have been implicated in the nitrogen balance of other amphibious teleosts (Iwata et al, 1980;
Morii et al. (1978) found several other compounds including uric acid, creatine, creatinine, TMA, TMAO and amino acids excreted by two amphibious gobiid fishes but they totalled less than 3.5% of the total nitrogen excretion. The 2.1% non-(A+U) nitrogen excreted by mudfish is probably a similar assemblage of these compounds that are filtered by the kidney or diffuse across the gill surfaces. As such they are probably not important excretory products for removing waste nitrogen.

The patterns of nitrogen excretion of fasted fish showed that nitrogen excretion rates of mudfish are independent of starvation status. Thus, starvation per se does not seem to stimulate any significant readjustment in nitrogen metabolism, that may be reflected in physiological changes during emersion. Gordon et al. (1977) similarly found that starvation did not affect the excretion rates of ammonia in Periophthalmus species but did significantly decrease urea excretion. This was an unexpected result, as urea excretion is normally considered to be independent of routine metabolism (van Waarde, 1983).

Nitrogen excretion rates from the two body regions of fish partitioned in water show that the body contributes significantly to the excretion of both ammonia (27%) and urea (37%). The body compartment contains both the renal excretion and a possible cutaneous excretory route but these could not be directly isolated from one another (section 2.6).

Forster and Goldstein (1969) and Smith (1929) suggested that freshwater teleost fish rarely excrete more than 15% of their total nitrogen excretion via the renal system. Vellas-Clos (1973; cited in Love, 1980) found even smaller contributions from renal excretion (6.1–7.8%) in three freshwater teleosts, and Fromm (1965) found only 3% of total nitrogen excreted by the kidney in rainbow trout (Salmo gairdneri). If the magnitude of the mudfish renal excretion is similar
to the 15% value most often cited for freshwater fish, then possibly 10-15% of the total, and 50% of the body compartment excretion may be via a cutaneous excretion route. This would further implicate the skin as an important exchange surface.

Urea was lost from both the head and body of mudfish with the greatest urea excretion from the head (63%). Vellas-Clos (1973) found only 5-27% of urea excretion to be via the kidney in three freshwater fish species. However, Morii et al (1978) found 40-50% of urea excretion to be lost from the body of two gobiid species and Eddy et al (1980) found 60% of urea to be lost from the body in the African catfish. Therefore, although the kidney may be either the major or the secondary nitrogenous excretory route for urea in fish, in mudfish, it is only the secondary route, with the gill being the predominant excretion site for both ammonia and urea. This is important when also considering excretion in air.

On exposure to hypoxic conditions, the decrease in ammonia excretion with no concommitent decrease in urea excretion from the body, indicated a disruption to body excretion patterns. A possible cutaneous vasoconstriction, as suggested by Meredith et al (1982), may reduce any cutaneous excretion but not effect the renal excretion. Ammonia may diffuse readily across the skin in its unionised form but skin may be relatively impermeable to urea. Urea is a large inert dipolar molecule and although having a similar diffusion coefficient to ammonia (Forster and Goldstein, 1969) it must pass through aqueous pores rather than the lipid-protein areas that unionised ammonia moves through. Therefore, cutaneous loss of ammonia is likely, but cutaneous loss of urea is unlikely to occur. Urea excretion from fish is therefore probably restricted to the filtration mechanisms of the kidney or across the thin gill epithelium.
The changing patterns of excretion due to the hypoxic stimulus may indicate a decreased cutaneous excretion of ammonia but no change in renal excretion patterns. The decreased body ammonia excretion is compensated for by a significantly increased excretion of ammonia from the head. However, total ammonia excretion is not significantly changed. The proportion of total nitrogen excretion being excreted from the body during hypoxia is similar to the renal excretion rates of most fish species. Therefore, we might speculate that the pattern under hypoxia represents solely renal excretion when cutaneous excretion is prevented. This would give an excretion rate for the 80% of skin in the body compartment of 0.96 μgN g⁻¹ hr⁻¹ or 15.2% of total. Since the magnitude of the urea excretion from the body does not change, all of the urea excreted from the body compartment is probably excreted via the urine and therefore urea may not be involved in cutaneous exchange as initially hypothesised.

Upon emersion, nitrogen excretion is greatly disrupted with changes in the patterns of excretion and accumulation of nitrogenous products. The lack of any significant excretion of urea beyond 8 hours of emersion indicates that mechanisms for its excretion are lost by 8 hours. This route of initial urea loss could be either from the gill or via the urine, but since the gill is no longer irrigated the urea loss must be of urinary origin. The cessation by 8 hours, therefore, indicates that there may be significant urinary losses up to 8 hours after emersion, but this ceases, probably as a water conservation mechanism. Cessation of urinary flow is also indicated by the change in rate of weight loss of fish over this period after emersion (Chapter 6.3.4). Ammonia is still being excreted beyond this time probably via alternative pathways such as the skin.
The accumulation of ammonia and urea within the fish would enhance gradients across the body surfaces for excretion to both air and moist surfaces. The time taken for the calculated ammonia accumulation to stabilise indicates appreciable levels of ammonia must be accumulated before gradients for excretion are large enough for appreciable excretion or for other mechanisms to limit further production of ammonia. The mechanism controlling body ammonia levels has therefore stabilised by 24 hours but urea levels continue to increase for several hours beyond 24 hours. The mechanisms controlling body levels of ammonia and urea may therefore be different.

The postulation that internal ammonia levels must increase to generate a diffusion gradient is similar to that suggested for CO$_2$ excretion and a similar time course of excretory patterns is found. Whereas CO$_2$ accumulation would lead to an acidosis, ammonia accumulation would lead to an alkalosis so dual accumulations might cancel some of the acid-base disruption and lead to an increase in blood osmolarity. Equally, some of the accumulations and buffering mechanisms may be at the intracellular rather than the extracellular level, as suggested by Heisler (1982) and therefore may not all be directly seen in the blood. Ultimately though, all the ions must pass through the blood to be excreted.

Unlike CO$_2$ excretion, ammonia and possibly urea production may be reduced by allosteric feedback of plasma levels, or a switch in metabolic substrates to limit the amount of waste nitrogen produced (van Waarde, 1983).

The time course of the stabilisation of the gaseous ammonia excretion pattern is similar to that of ammonia accumulation. Therefore, it is likely that the two are linked and plasma ammonia is increased to develop a gradient for gaseous ammonia excretion. Thus, it takes 24 hours for ammonia accumulation to generate a sufficient gradient for
appreciable gaseous ammonia excretion and the development of a stable excretion pattern.

The existence of gaseous excretion of ammonia from mudfish poses an interesting problem. Ammonia is a toxic substance and must pass across the respiratory surfaces. At a blood pH of 7.8-7.2, 99% of ammonia in the blood will be in the form of ammonium ions and increasing levels would lead to an alkalosis of the blood. The ammonium ions may diffuse directly across the epidermis but must be dissociated to ammonia at the skin surface to be lost. Ammonia in the non-ionized lipid soluble form may also diffuse across the epidermis in the lipid layers and since the conversion of ammonium to ammonia is relatively instantaneous this would not be a rate limiting step (Forster and Goldstein, 1969). Ammonia arriving at the moist skin surface would immediately reionise under neutral conditions. When the fish lie on a moist surface ammonium ions may be excreted directly into the surface moisture. This may be further enhanced by the previously described rolling behaviour that would allow diffusion from the different body surfaces whilst in contact with the moist surface. When the fish are on a dry surface, the gaseous excretion may be accomplished either by a large accumulation of ammonium at the skin surface allowing an appreciable rate of dissociation of ammonium ions or the development of alkaline conditions at the surface to aid dissociation. Large accumulations of ammonium would be toxic and harmful to skin surfaces unless protected by special mucoid compounds. Strongly alkaline conditions were not present on several fish whose body surfaces were tested with narrow range pH papers (BDH Ltd, U.K.). The skin surface was always close to neutrality (6.7-7.2). There could be a role for basic mucus substances in the mucus and cuticular coating on the fish skin in aiding dissociation of ammonium although only neutral and acidic muco-substances are normally described from fish skin (Mittal and Banerjee, 1980).
Gaseous ammonia is lost from both the body (56%) and the head (44%). The head region may contain both cutaneous and branchial ammonia excretion routes. Gill epithelia contain Na\(^+\)/NH\(_4\)^+ exchange pumps that are used for aquatic ion balance and acid-base balance (Maetz and Garcia Romeu, 1964) but these could not function in air where the external cation is not present. Therefore, in air, branchial surfaces may only move ammonia/ammonium by passive diffusion processes, and as with cutaneous CO\(_2\) excretion, significant diffusion gradients have to be developed.

At no stage was urea seen to be excreted beyond 8 hours of emersion. Since it does not appear to be accumulated beyond 48 hours, some further method of conversion of urea for storage, or suppression of ureogenesis must take place. Conversion of urea for further storage has not been described in fish (Goldstein and Forster, 1970). Mechanisms of suppression are similarly undescribed. In many species, levels of urea increase in the body throughout the aestivation or emersion period. Studies have shown elevated blood urea levels in several fish species during these periods (Smith, 1930; Janssens, 1964; Morii et al., 1978, 1979). Forster and Goldstein (1970) described a 140mM/l urea level in long term aestivating lungfish.

Most studies of fish showing significant urea accumulations upon emersion are of fish that either enter torpid states to decrease energy usage and excretion production of nitrogenous wastes, or only remain emersed for short periods. With mudfish being small fish and maintaining a routine metabolic rate in air, accumulating urea load may become very large unless the urea production is limited. The rate of accumulation of urea over 24 hours (Fig. 4.4) was 0.625 µgN g\(^{-1}\) hr\(^{-1}\) which is 43% of the aquatic excretion rate. A further 6% is accounted for by that excreted during the first 8 hours of emersion. Therefore upon emersion urea excretion is at least halved. If the urea were to accumulate at
this rate, blood urea levels of 1.8, 12.5 and 50 mM/l would be expected after 1 day, 1 week and 1 month of emersion respectively. This is assuming the urea contained within the fish was distributed throughout the extracellular fluid space (30% of body weight; Chapter 5).

The blood urea level measured at 24 hours, 2.5mM/l, was 39% higher than that predicted from accumulation data (Fig. 4.5). Therefore, urea is either not all excreted upon reimmersion leading to higher maintained blood levels, or the urea is not so widely distributed in the body. If the extracellular fluid space of urea were less than 30% of body weight then this would effectively concentrate the urea. It is well known that different compounds have different diffusibilities in extracellular fluid and larger molecules fail to penetrate some compartments. They thus yield different estimates of fluid space (Lutz, 1972). It would, therefore, not be surprising if ammonia and urea had different fluid space distributions and if both differed from that of the sodium space measured in chapter 5. The longer term accumulations of urea suggested may therefore be underestimates.

The high levels of urea calculated to accumulate, if ureogenesis was not supressed, would be tolerable for up to 1-2 months as these levels are tolerated by other species (Goldstein and Forster, 1970). However, urea does not appear to accumulate much beyond 24 hours, so tolerance to these levels does not appear necessary. The 28 day plasma urea concentration is little greater than the 24 hour concentration. Therefore, suppression of ureogenesis must occur. Mechanisms of this are unknown but could be brought about by allosteric feedback of enhanced plasma levels on the ureogenic processes.

Ammonia accumulation, calculated from Fig. 4.4, gives a linear accumulation rate of 5.5 μgN g⁻¹ hr⁻¹ which is 66% of the control aquatic rate of production. However, this rate of accumulation appears to be rapidly decreasing after 6-8 hours emersion. Such a rapid
accumulation would kill the fish if not supressed or detoxified. A calculated level of 5.2mM/l would accumulate by 24 hours with toxic levels of 35 and 150mM/l by 1 week and 1 month respectively. However, like urea, a stable accumulated level appears to be achieved by 24 hours. Measured blood levels were only 50% of those calculated to be present at 24 hours and the level at 28 days was slightly lower.

The lack of the expected level of enhancement of ammonia levels in the blood may be an artifact of blood extraction and handling techniques rather than being indicative of ammonia storage elsewhere in the fish. Since blood is not taken anaerobically and is handled several times before the assay, there is scope for diffusive loss of ammonia to the air, especially if the blood became warmed during centrifugation. If accumulated ammonia was involved in buffering and in association with CO₂ levels, as previously suggested, then exposure of blood to air or the return of fish to water would result in rapid CO₂ loss and accompanying ammonia loss. However, the plasma ammonia data does show an increase in ammonia levels on emersion, indicating that ammonia loading in the blood does take place, but the magnitude of the increase may be an underestimate of the actual level of loading.

Pandian (1975) suggested that buffering or protective mechanisms are necessary against ammonia poisoning in situations of enhanced ammonia accumulation and proposed a role for glutamic acid to detoxify (absorb) ammonia for later liberation. A stronger case has also been made for a role for glutamine (Vellas and Sefarty, 1974) since this is not excreted at the gill as are other amino acids. None of these mechanisms appear to act in mudfish at the blood level as concentrations of NPS did not significantly change at any stage. However, levels in the liver or other tissues could not be measured and storage or detoxification in these tissues cannot be isolated. Any change in these tissues would however be expected to be reflected somewhat in plasma levels and therefore may
either not occur or not be appreciable. However, blood urea would not be prone to loss by gaseous diffusion and therefore probably does present a reliable estimate of blood urea accumulation.

The levels of ammonia and urea in plasma of aquatic mudfish are lower than those reported in most studies. This may be due to the small size of mudfish and the high proportional exchange areas, making excretion more efficient and therefore leaving lower residual body levels. Most fish previously studied have been large (>100g), and have had blood ammonia and urea levels ranging from 0.2-1.8 and 1.32-20.9 mM/l respectively (Holmes and Donaldson, 1969; Pandian, 1975). Most essential and non-essential amino acids and traces of other amino compounds are found in fish plasma (Goldstein and Forster, 1970; Hille, 1982). The total levels seen in mudfish plasma are lower than those described from other fish. Since the levels do not change they may not play any significant role in storage or detoxification of nitrogen.

Although results show significant gaseous ammonia release from the skin and gills, the magnitude of release in emersed fish is still less than 15% of the standard aquatic excretion rate. Thus, 85% of the expected aquatic production rate is being excreted (but undetected), stored, or not being produced. An explanation for the latter may be that upon emersion a switch in primary metabolic substrates occurs.

Many studies of fish metabolism have shown that proteolytic pathways may be the primary source of energy metabolism rather than carbohydrate or lipid metabolism, as in mammals (van Waarde, 1983). It has been shown in several fish species that dietary protein can completely cover the requirements for routine metabolism (Brett and Zala, 1975), while increases in metabolic rate are compensated for by lipid metabolism. Carbohydrate metabolism plays little role in fish metabolism, being reserved for conditions of environmental hypoxia and
tissue anoxia (van Waarde, 1983).

An ammonia quotient relating oxygen usage to ammonia production can be used to estimate the relative contribution of protein catabolism to total energy production, as almost all excreted ammonia originates from proteolysis (van Waarde, 1983). Ammonia quotients calculated for a group of mudfish in water 24 hours after feeding, show 73% of the energy requirement is protein derived (Table 4.5). The stable ammonia production rate and and standard $\dot{V}O_2$ of fasted fish showed that under fasting protein metabolism still predominates. Proteolysis therefore continues throughout fasting and little reduction of lipid levels may be expected. However, over longer fasting periods mudfish may switch to metabolism of lipid reserves. Moon (1983b) found that eels delayed switching to lipid metabolism until 3 months after the beginning of starvation.

Table 4.5 Calculation of Ammonia Molar Quotient and estimation of proportion of energy covered by protein catabolism for aquatic, fasted and emersed mudfish.

PROTEIN CATABOLISM

$$\text{C}_{43}\text{H}_{70}\text{O}_{14}\text{N}_{12}\text{S}_{0.3} + 44.5\text{O}_2 \rightarrow 43\text{CO}_2 + 12\text{NH}_3 + 17\text{H}_2\text{O} + 0.3\text{H}_2\text{S}$$

After van Waarde (1983).

<table>
<thead>
<tr>
<th>Medium</th>
<th>$\dot{V}O_2$</th>
<th>NH$_3$</th>
<th>AMMONIA MOLAR QUOTIENT</th>
<th>% OF ENERGY COVERED BY PROTEIN CATABOLISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER</td>
<td>50.2 ± 2.8</td>
<td>7.61 ± 0.91</td>
<td>0.199</td>
<td>72.3%</td>
</tr>
<tr>
<td>FASTING</td>
<td>56.2 ± 2.9</td>
<td>8.21 ± 0.87</td>
<td>0.146</td>
<td>53.0%</td>
</tr>
<tr>
<td>AIR</td>
<td>71.2 ± 3.1</td>
<td>1.10 ± 0.20</td>
<td>0.024</td>
<td>9.1%</td>
</tr>
</tbody>
</table>
In emersed fish, the patterns are very different. If the aerial ammonia excretion values are accepted as the total ammonia production during emersion, then 9% of the energy requirement is calculated to be protein derived. To switch from 73% to 9% proteolysis upon emersion would require a switch to predominantly lipid metabolism. Such a switch to lipid metabolism may be controlled by either rising ammonia or urea levels or changes in acid-base balance acting to inhibit some key proteolytic reactions. This inhibition would enhance other pathways and thus severely restrict ammonia production. This state would continue while the fish had appreciable lipid reserves and levels of the controlling factor remained high in the blood. Such a mechanism could cause very rapid switches in metabolism under these conditions.

In the previous chapter, mudfish were shown to store appreciable levels of lipids in the body and gonads. Eldon (1979c) also showed pre-aestivation peaks in mudfish lipid levels. Therefore, levels of lipid are available for such a switch in metabolism. It is unfortunate that I was unable to demonstrate significant depletion of lipid reserves. However, as discussed in the previous chapter, the negative finding does not preclude a role for lipid metabolism.

Lipid metabolism is a viable solution for limiting production and accumulation of toxic levels of ammonia which could be produced during emersion, but may not be adequate to explain metabolism during some of the long aestivation periods experienced by mudfish in their natural habitat (Eldon, 1978b, 1979c). Over long periods, mudfish exhibit weight losses that far exceed the possible lipid contents of their bodies. Some fish are known to have marked abilities to consume their own structural proteins (Creac'h and Sefarty, 1974) and when this occurs the accompanying nitrogen load would again become a problem. The mechanisms of nitrogen excretion during these periods is unknown.
Many details of the nitrogenous excretion of the gobiid fishes of the *Periophthalmus* genus show similarities to the patterns of nitrogen excretion in this study. Like the mudfish, several species have been shown to possess a well vascularised skin (Schottle, 1932) and use cutaneous respiration (Teal and Cary, 1967; Tamura et al, 1976). Morii et al (1978) implicated the skin in the cutaneous excretion of both ammonia and urea when in water, and found respectively, 15 and 21% of ammonia and 43 and 53% of urea to be excreted by the body region of 2 species of mudskipper when partitioned.

None of the authors described aerial ammonia excretion but since ammonia appears to be cutaneously excreted in water then it is conceivable that mudskipper fishes may also excrete some ammonia by gaseous diffusion. All of the mudskipper studies described a rapid excretion of accumulated nitrogen upon reimmersion and also that the amount released was less than that predicted from a constant aquatic excretion rate. The explanations of depressed ammonia production rates in air, even though respiration rates were constant, may also be explained by an unmeasured gaseous excretion of ammonia as seen in mudfish in this study.

The descriptions of urea excretion rates and accumulations in mudskippers are varied and range from suggestions of conversion of ammonia to urea (Gordon et al, 1969), to a constant rate of production and accumulation (Morii et al, 1978), and to roles for free amino acids in detoxification of ammonia in the body (Iwata et al, 1980). Gregory (1977) showed the lack of a functioning ornithine—urea cycle in mudskippers, and although the enzymes have not been looked for in mudfish, it is unlikely that a full compliment of the enzymes would be found here either. Therefore, Gordon's theory of an evolutionary move to ureotelism in relatively unspecialised amphibious fish appears unlikely in both the mudskippers and the mudfish.
Overall there are many similarities between the mudskippers and mudfish and many of the responses and initial mechanisms for nitrogenous excretion are the same. The main difference between the two groups is that the mudfish survives long periods out of water while emersion periods for mudskippers are frequently limited to less than 48 hours. While mechanisms of nitrogen excretion described are adequate to explain excretion in the short and medium terms, mechanisms for limiting ureogenesis and allowing the use of proteolytic metabolism at longer aestivation periods remain unexplained at present.
CHAPTER V

SODIUM FLUXES AND ION BALANCE

INTRODUCTION

Ion balance in a freshwater fish such as the Canterbury mudfish may be compromised by increased permeability of the body surfaces which allows cutaneous respiration. The cutaneous excretion of ammonia, shown in the previous chapter, may indicate the ease with which molecules other than gases may cross the epidermis of mudfish. This may either place increased load on osmoregulatory functions or limit the homeostatic regulation of body ion levels.

Freshwater fish are hyperosmotic regulators, maintaining the tissues and fluid ions at much higher concentrations than the external medium. Any disruption to patterns of ion loss or uptake could therefore have dire consequences on ion levels and physiological processes within the fish. The gills of fish are generally regarded as the primary site of active ion uptake (Payan et al., 1984), while the kidney serves for osmotic regulation and an accompanying loss of ions. However, the external skin may function in ion regulation at least in some larval fishes (Roberts et al., 1973). Marshall (1977) has since shown a marine gobiid fish to be capable of active ion uptake across regions of opercular skin, but skin active transport is not normally assumed to occur in fish.

It is interesting to speculate on the permeability of mudfish skin to ions and whether they support significant active or passive fluxes of ions across the epidermis. Ability to change the permeability through perfusion control, as suggested under hypoxia (Chapter 3, 4), may influence such fluxes.
Upon emersion of mudfish and with the loss of gill irrigation for ion uptake, the extent of disruption of ionic homeostasis may be considerable, if urinary and cutaneous losses are maintained. With loss of the uptake surfaces, the compensation for changing body levels and the roles of ions in acid base balance may similarly be lost. Net ion fluxes and extent of possible ion regulation while emersed may therefore be important.

Correlations of cutaneous permeability and cutaneous respiration with marine and euryhaline species suggests that the Canterbury mudfish may have marine affinities from which the potential for cutaneous exchange is derived. Euryhaline ionic regulatory abilities and wide salinity tolerance may confirm this nature of the mudfish.

MATERIALS AND METHODS.

5.2.1 Blood ion levels

Mudfish which had been acclimated to the aquarium system and were feeding regularly were sampled for measurement of blood ions. Blood samples were obtained by heart puncture and prepared as in section 2.5. Blood ion concentrations were measured using the following methods: plasma sodium was measured in 1:1000 dilutions of plasma in distilled water in an EEL 100 flame photometer against NaCl standards, plasma chloride was measured from 10μl volumes in a Corning EEL 920 Chloride meter, and osmolality in 5μl samples using a Wescor Inc. 5100C vapour pressure osmometer. Blood samples were taken from feeding fish (controls), from fish fasted for 28 days, and from fish maintained in air for 1 and 28 days after ether anaesthesia.

5.2.2 Aquatic Sodium Regulation

Sodium effluxes from mudfish were measured by sodium loss into
distilled water, with the sodium concentration of water samples being measured using an EEL 100 flame photometer. This method of determining sodium loss rates gave variable results and so sodium efflux rates were also obtained by use of the $^{22}$Na radioisotope. For measurement of radioisotope efflux, fish were anaesthetised in an ether atmosphere and then labelled by an intraperitoneal injection of $0.2\mu$Ci $^{22}$Na in $10\mu$l of physiological saline (0.9% NaCl) using a $10\mu$l Hamilton syringe (Hamilton Co, Reno, Nev.). The isotope was obtained from New England Nuclear as a 200mCi/ml solution of NaCl. Fish were held in air for 10 minutes to allow distribution of the isotope within the fish before the fish were placed in $150\text{ml}$ of tapwater (0.4mM Na$^+$) in a $250\text{ml}$ conical flask. The water was lightly aerated with compressed air during experiments. The medium was sampled at hourly intervals and the radioactivity of $1\text{ml}$ water samples in scintillation cocktail was measured using a Beckman LS2800 liquid scintillation counter. A toluene based cocktail was prepared and used for all scintillation counting. The efflux rate was calculated from the linear rate of appearance of activity in the medium compared to the total activity injected. Activity injected was calculated from the mean of triplicate measurements of known quantities of the injection tracer substock.

Linear effluxes of tracer were measured rather than using compartmental analysis (Motais, 1967), to facilitate comparison with partitioned ion fluxes.

Sodium influx was measured by immersing fish in $150\text{ml}$ of tapwater of known sodium concentration labeled with $0.1\mu$Ci/ml NaCl. Influx was calculated from the linear rate of decrease of activity and the initial sodium concentration of the water. The effects of anaesthesia and injection, on sodium influx, was assessed for fish anaesthetised and injected with $10\mu$l of unlabeled physiological saline prior to measuring.

The extracellular sodium spaces (ECSS), (or volumes of
distribution of sodium), of mudfish were calculated from a single blood sample taken from the fish used for measurement of sodium influx and efflux. A blood sample was taken by cardiac puncture (Section 2.5), 12 hours after the injection of tracer, or immersion in tracer, and the activity of a known volume of plasma measured. The plasma sample was diluted with 1ml of distilled water and added to scintillation cocktail for measurement of activity in the scintillation counter. Sodium space was calculated as the activity injected, less the activity lost by the fish, divided by the activity of 1µl of plasma. It was assumed that the blood and extracellular fluid space were freely exchanging and in equilibrium. Only the single blood sample could be obtained from each fish for measurement of blood activity so no check on the equilibrium status of the tracer could be obtained. However, the influx and efflux data indicated, by their deviation from linearity, that the two compartments should be in equilibrium by this time.

The relative roles of branchial, cutaneous and renal sodium fluxes were measured with fish partitioned as in Section 2.6. The partitioning chambers were 200ml perspex pottles attached to short syringe barrels. Each chamber was filled with 100ml of tapwater and stirring action was provided by aeration with compressed air. To measure partitioned sodium efflux, fish were lightly anaesthetised in an ether atmosphere and injected with 0.2µCi $^{22}$Na in 10µl saline immediately prior to partitioning. Samples of medium were counted at intervals over six hours and efflux rates calculated for both compartments as previously described. Fish were also partitioned with bodies in hypoxic water ($P_{O_2} <$ 20 torr), and with heads in air and bodies in hypoxic water. Hypoxic conditions were generated in the chambers by gassing with dry nitrogen as previously described.

Sodium influx was measured in partitioned fish in a similar
manner to the unrestrained fish, although only one chamber was labelled in each experiment. The influx was calculated from the disappearance of activity from the chamber.

In all tracer experiments, the duration of the experiments from which linear losses were calculated were very short compared to the time for significant equilibration of the tracer between both compartments, so backflux of tracer was insignificant (<1%).

5.2.3 Emersion sodium balance

To measure sodium losses from mudfish during periods out of water, individual fish were labelled with 0.2µCi of $^{22}$Na by injection as previously described and placed in individual plastic containers on tissue paper soaked with 10ml of tapwater. The water volume allowed moistening of the fish body surface but did not allow free water to be taken into the buccal and branchial cavities. 0.2ml samples of the water under the fish were removed at intervals with replacement of equivalent volumes of tapwater and the activity was measured in scintillation cocktail in the scintillation counter as previously described. Cumulative sodium loss was calculated from the ratio of the radioactivity injected into the fish to that lost from the fish and the total sodium content of the fish. The exchangeable sodium content of the fish was estimated using values of 30% body weight ECSS and a plasma sodium concentration of 160mM/l (see results).

5.2.4 Salinity Tolerance

The ability of mudfish to tolerate a salinity range from tapwater (0.4 mM/l Na) to 100% seawater (470 mM/l Na) was tested by observations of fish behaviour, and measurements of plasma sodium and chloride concentrations after 1 week in different salinities. Groups of eight mudfish of comparable size were placed in 10L aquaria containing dilute seawater prepared from tapwater and Lyttelton seawater. Seawater
strengths of 5, 10, 20, 30, 45, 60, 75, and 100% seawater (SW) were used. At salinities greater than 20% seawater, mudfish were first exposed to 20% seawater for 1 hour and then appropriate volumes of seawater added to bring it slowly up to the required strength. This prevented fish from being exposed to sudden hyperosmotic shock.

Buoyancy control, behaviour and mortality of fish were observed during the first day of exposure to the salinity, and then monitored daily. After 7 days, fish were removed, anaesthetised in an ether atmosphere and blood taken by ventral cardiac puncture (section 2.5). Plasma was prepared and sodium and chloride levels measured as previously described. Blood osmolality was unable to be measured at this time.

Bled fish were placed in 10% seawater for 1 day before being returned to freshwater aquaria. Neither the blood sampling nor the reverse transfer caused significant mortality.

RESULTS

5.3.1 Blood ion levels

The osmolality, sodium and chloride concentrations in mudfish plasma were higher than those found in most freshwater fish species and similar to euryhaline marine species (Table 5.1, Table 5.5; Evans, 1979).

Mudfish starved for 28 days showed a significant decrease in sodium concentration ($p<0.01$) and plasma osmolality ($p<0.05$) but no significant change in plasma chloride levels (Table 5.1). Thus, there may be some adjustment in cationic levels and a decrease in the concentration of other plasma contents during starvation. Mudfish kept in moist air for 28 days showed a similar decrease in plasma sodium concentration but no change in chloride or total osmolality (Table 5.1). Thus, during emersion there is a cationic change similar to starvation but no significant change in overall osmotic strength.
Table 5.1 Ion levels in the plasma of starved and emersed groups of mudfish.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Sodium mM/l</th>
<th>Chloride mM/l</th>
<th>Osmolality mOsM/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquatic (control)</td>
<td>9</td>
<td>176.8 ± 2.2</td>
<td>123.1 ± 2.7</td>
<td>308.8 ± 5.2</td>
</tr>
<tr>
<td>Fasted</td>
<td>8</td>
<td>163.3 * ± 5.3</td>
<td>119.0 ± 3.4</td>
<td>283.0 * ± 7.2</td>
</tr>
<tr>
<td>Emersed (28 days)</td>
<td>8</td>
<td>159.2 ** ± 4.1</td>
<td>123.9 ± 2.3</td>
<td>295.0 ± 9.8</td>
</tr>
</tbody>
</table>

Significance (T-Test) - *-p<0.05; **-p<0.01; ***-p<0.001.

5.3.2 Aquatic Sodium exchanges

Sodium efflux measured by sodium loss to distilled water gave a mean efflux rate 2.5 times higher and more variable than those measured by tracer fluxes (Table 5.2).

Measurements of sodium influx were significantly lower than for the measured sodium effluxes (p<0.01; Table 5.2), indicating a net sodium loss under the experimental conditions. The added stresses of anaesthesia and intraperitoneal injection did not significantly affect sodium influx rates (Table 5.2).

Table 5.2 Measurements of total sodium efflux and influx of Canterbury mudfish measured by washout of radiotracer.

Units - μgNa g⁻¹ hr⁻¹ (sample size in brackets).

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>EFFLUX</th>
<th>INFLUX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washout to distilled water</td>
<td>0.60</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>±0.05  (10)</td>
<td></td>
</tr>
<tr>
<td>tracer, with injection and prior anaesthesia</td>
<td>0.247</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>±0.032 (16)</td>
<td>±0.033 (8)</td>
</tr>
<tr>
<td>tracer, uninjected and undisturbed fish</td>
<td>-</td>
<td>0.154</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.071 (9)</td>
</tr>
</tbody>
</table>
Measurements of ECSS from influx and efflux experiments were significantly different (p<0.05), (Table 5.3). The similarity of the two values, however, indicates radioactive sodium was close to equilibrium in both experiments and therefore both ECSS and flux data may be good estimates of true sodium distribution and movements.

Table 5.3 Measurements of extracellular sodium space of mudfish from sodium influx and sodium efflux methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Sodium Space (% body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Efflux</td>
<td>16</td>
<td>33.2 ± 1.4</td>
</tr>
<tr>
<td>Sodium Influx</td>
<td>11</td>
<td>28.5 ± 1.4 *</td>
</tr>
<tr>
<td>Mean</td>
<td>27</td>
<td>30.9 ± 1.2</td>
</tr>
</tbody>
</table>

Significance * p<0.05

Fish partitioned in normoxic water showed a 4.5 fold increase in total sodium efflux compared to the previously measured unrestrained fish. Over 70% of the sodium efflux was from the body compartment indicating a greatly increased cutaneous or renal salt loss (Table 5.4).

Neither exposure to hypoxic water, nor a combination of emersion and hypoxic water caused any significant change in the sodium efflux rates from the body but total sodium loss was decreased in partially emersed fish (p<0.05) due to the loss of the head exchange surfaces.

Sodium influx by the head compartment of partitioned mudfish was slightly less than the total influx from unrestrained mudfish (Table 5.2), while there was no significant sodium influx from the body compartment. Therefore, the gills may be the only route of ion uptake.
Table 5.4  Sodium fluxes of mudfish partitioned in normoxic water, with heads in normoxic water and bodies in hypoxic water and with heads emersed and bodies in hypoxic water. (μM g⁻¹ hr⁻¹.)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>HEAD</th>
<th>BODY</th>
<th>TOTAL</th>
<th>Body % of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium efflux</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>6</td>
<td>0.228 ± 0.070</td>
<td>0.628 ± 0.141</td>
<td>0.865 ± 0.156</td>
<td>73.4</td>
</tr>
<tr>
<td>Normoxic head</td>
<td>6</td>
<td>0.201 ± 0.030</td>
<td>0.584 ± 0.122</td>
<td>0.785 ± 0.130</td>
<td>68.7</td>
</tr>
<tr>
<td>Hypoxic Body</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emersion and</td>
<td>6</td>
<td></td>
<td>0.595 ± 0.128</td>
<td>0.595 ± 0.128</td>
<td>100</td>
</tr>
<tr>
<td>Hypoxic Body</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Influx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>6</td>
<td>0.138 ± 0.028</td>
<td>0.138 ± 0.028</td>
<td>0.138 ± 0.028</td>
<td>0</td>
</tr>
</tbody>
</table>

Significance — * p<0.05.

5.3.3  Emersion sodium balance

Fish held in air on a damp substrate lost a significant amount of sodium with increasing periods spent out of water (Fig. 5.1). Fish had rapid sodium loss rates up to 6 hours after emersion by which time a lower more stable loss rate had developed. Thus, sodium loss may be modulated by 6 hours after emersion but is still appreciable while in air.

5.3.4  Salinity Tolerance

In all salinities greater than tapwater, mudfish exhibited initial positive buoyancy. All groups ventilated their gills at maximal amplitude. At increasing salinities they initially showed increased activity and agitation. Many fish attempted to leave the experimental salinities by jumping from the water.

Buoyancy compensation was achieved within 1 hour in 5%SW but took 24 hours in 45%SW. Although surviving for 1 week, 62% of fish in 60%SW
Figure 5.1 Graph showing cumulative sodium loss in mudfish emersed upon damp tissue paper.

Stable loss t=6–24 hours: 0.36 μM g⁻¹ hr⁻¹.
did not manage to achieve negative buoyancy. None of the fish in 75% and 100%SW managed to regulate buoyancy or survive for greater than 36 or 10 hours respectively.

Sodium levels in plasma were always higher than chloride levels in all treatments (Fig. 5.2). Plasma sodium levels dropped in 10%SW and then rose at salinities of 20% and 30%SW. At 45% and 60%SW, plasma sodium concentration remained at a level 35mM or 22% higher than freshwater plasma levels. Therefore, at hyperosmotic salinities, mudfish were able to regulate their plasma sodium levels with small ion increases.

Plasma chloride levels were more stable than sodium levels, only rising from control values as the isoionic line was reached. Beyond this, plasma chloride only rose slowly with increasing salinity. At 60%SW plasma chloride levels were 80mM (65%) higher than the freshwater plasma levels. Thus, chloride concentrations were well regulated in hypoionic conditions and moderately well regulated in hyperionic conditions.

Thus, mudfish are capable of surviving at up to 60%SW regulating hypo-osmotically and hyper-osmotically.

Figure 5.2 Graph of plasma ion levels versus dilute seawater ion strengths for mudfish in dilute seawaters for 7 days.
DISCUSSION

The blood osmolarity and ion levels vary greatly within freshwater and euryhaline fish species (Table 5.5). Marine and primarily marine euryhaline fish species generally maintain higher blood ionic concentrations both in seawater and in freshwater relative to freshwater fish (Evans, 1979). The blood sodium concentration of mudfish is higher than that normally found in freshwater fish and closer to that of marine fish (Table 5.5), while chloride concentrations and total osmolality, in contrast, are similar to the levels seen in many freshwater fish.

Since it has been shown that the mudfish has a skin permeable to respiratory gases and ammonia, increased ionic permeability was considered possible. If increased regulatory problems were associated with increased permeability, then lower blood ion levels might be expected. Such a decrease in ion levels would decrease ionic gradients and therefore ionic losses. The high ionic levels measured in mudfish plasma would in contrast, increase any ionic regulation problems.

The mudfish is generally considered to be an exclusively freshwater fish as it lives its full life cycle in freshwater, lacking the marine whitebait stage of many of its closer relatives (McDowall, 1978). The high concentrations of sodium and chloride ions may therefore reflect marine stages in the mudfish's ancestry.

Since both starved and emersed mudfish showed significant decreases in blood sodium levels, there must be some cationic adjustments taking place during these periods. Results from previous chapters suggest that blood $\text{CO}_2/\text{HCO}_3^-$, $\text{NH}_4^+$ and urea plasma concentrations increase during emersion and that these would effectively increase total osmolality. This hypothesised increase in plasma osmolality may however not be seen in the blood samples due to the previously discussed
Table 5.5  Blood Ion Levels of the Canterbury Mudfish and some other Freshwater and Euryhaline Teleost Fish.

<table>
<thead>
<tr>
<th>Species</th>
<th>Na (mM/l)</th>
<th>Cl (mM/l)</th>
<th>Osmol (mosM/l)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canterbury mudfish Neochanna burrowsius</td>
<td>177</td>
<td>123</td>
<td>308</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Freshwater Species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coregonus clupoides</td>
<td>141</td>
<td>117</td>
<td>---</td>
<td>Robertson (1954)</td>
</tr>
<tr>
<td>Micropterus dolomieu</td>
<td>128</td>
<td>111</td>
<td>---</td>
<td>Shell (1959)</td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>130</td>
<td>125</td>
<td>274</td>
<td>Houston and Madden (1960)</td>
</tr>
<tr>
<td><strong>Euryhaline species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fundulus heteroclitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.W.</td>
<td>172</td>
<td>126</td>
<td>---</td>
<td>Potts and Evans (1967)</td>
</tr>
<tr>
<td>S.W.</td>
<td>183</td>
<td>146</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Poecilia latipinna</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.W.</td>
<td>145</td>
<td>100</td>
<td>---</td>
<td>Ball and Ensor (1969)</td>
</tr>
<tr>
<td>S.W.</td>
<td>195</td>
<td>135</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Pleuronectes flesus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.W.</td>
<td>124</td>
<td>132</td>
<td>240</td>
<td>Lahlou (1967)</td>
</tr>
<tr>
<td>S.W.</td>
<td>142</td>
<td>168</td>
<td>297</td>
<td></td>
</tr>
<tr>
<td>Salmo trutta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.W.</td>
<td>155</td>
<td>124</td>
<td>351</td>
<td>Gordon (1959)</td>
</tr>
<tr>
<td>S.W.</td>
<td>166</td>
<td>138</td>
<td>356</td>
<td></td>
</tr>
<tr>
<td>Anguilla anguilla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.W.</td>
<td>150</td>
<td>105</td>
<td>328</td>
<td>Sharratt et al (1964)</td>
</tr>
<tr>
<td>S.W.</td>
<td>175</td>
<td>154</td>
<td>377</td>
<td></td>
</tr>
</tbody>
</table>
criticism of the blood sampling method (Chapter 4), where gaseous diffusible metabolites may be lost during blood handling.

The decrease in sodium concentration in emersed fish may be due to the different excretory routes of sodium and chloride. During the first few hours of emersion sodium may be lost in the diminishing urinary flows and cannot be replaced, while chloride, not usually lost to the same extent in urine, is conserved (Evans, 1979). Ammonium ions accumulating during this period may maintain the cationic balance.

The reason for lowered blood sodium concentrations in starved mudfish is unknown as starvation has previously been shown to have little effect on metabolism or excretion parameters and therefore may not be expected to disrupt ionic balance. Unless starved mudfish were in a weakened condition and more susceptible to incipient disease, ionic exchange mechanisms should be identical. Moon (1983a) found decreasing sodium levels in the blood of long term starved American eels and related this to increases in muscle and tissue sodium levels. These changes were however only observed in eels starved for greater than 6 months, when more than 40% of body weight had been lost. Mudfish were not subjected to anywhere near this degree of starvation so it is uncertain whether a similar redistribution of sodium takes place in mudfish during starvation.

Decreased osmolality may be due to a combination of the decreased sodium concentration and lower levels of circulating plasma proteins and organic compounds during starvation (Dave et al., 1975; Joshi, 1974; Siddiqui, 1975).

Measurements of unidirectional sodium efflux in distilled water can overestimate realistic sodium fluxes due to the absence of external calcium concentrations. In many fish species, external calcium concentrations have been shown to have a direct effect upon both flux rates and permeabilities (McDonald, 1982). In mudfish, the effluxes in
distilled water were 2.5 times higher than those measured by tracer movements in tapwater (water hardness = 30-40 g.m\(^{-3}\) as CaCO\(_3\)).

Other fish species have shown even greater increases in sodium efflux in calcium-free media (Table 5.6).

Sodium efflux rates of freshwater fish vary greatly but are much lower than the unidirectional fluxes of seawater fish (Evans, 1979). Both the distilled water and tracer measured sodium effluxes of mudfish fall in the range of published values for freshwater fish (Table 5.6). Despite the mudfish having a permeable skin used for respiration, total body sodium efflux was fairly low, possibly indicating that the skin may not be very permeable to ions and therefore not contribute significantly to ion loss.

The sodium influx of mudfish was lower than the measured sodium efflux, therefore mudfish suffered a net sodium loss during the experimental periods. The higher efflux rates are not readily explained by the added stresses of anaesthesia or the intraperitoneal injection as these had no significant effect on the measured sodium influxes (Table 5.2). However, anaesthetics are often implicated in the temporary alteration of the permeability of exchange surfaces (Maddren and Houston, 1976). It may be that handling stress has caused an increased ventilation or altered passive permeability that is not immediately compensated for by ion uptake mechanisms. Since influx and efflux mechanisms are only linked in as much as they maintain a uniform body ion level, immediate compensation for stresses may not be expected and some ion imbalance is conceivable for the duration of the experiment. The difference between the two values, however, is not so great as to undermine confidence in the flux data and the methods used to obtain them.

Extracellular fluid spaces have been measured in different fishes in a number of ways, which differ in the distributions and types of
Table 5.6 Sodium effluxes across the body surfaces of the Canterbury Mudfish and other freshwater Teleost fish.

<table>
<thead>
<tr>
<th>Species</th>
<th>Regulatory Ability</th>
<th>Sodium Efflux $\mu$M g$^{-1}$ hr$^{-1}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canterbury Mudfish</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>Neochanna burrowsius</td>
<td>FW?</td>
<td>0.60</td>
<td>Maetz et al (1964)</td>
</tr>
<tr>
<td>Distilled water:</td>
<td></td>
<td></td>
<td>Eddy (1975)</td>
</tr>
<tr>
<td>Tapwater:</td>
<td></td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Carassius auratus</td>
<td>FW</td>
<td>0.09</td>
<td>Eddy et al (1980)</td>
</tr>
<tr>
<td>Ca free water:</td>
<td></td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>Clarias mossambicus</td>
<td>FW</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Fundulus heteroclitus</td>
<td>E</td>
<td>0.60</td>
<td>Potts and Evans (1967)</td>
</tr>
<tr>
<td>Tilapia mossambica</td>
<td>E</td>
<td>2.24</td>
<td>Potts et al (1967)</td>
</tr>
<tr>
<td>Salmo salar</td>
<td>E</td>
<td>0.68</td>
<td>Potts et al (1970)</td>
</tr>
<tr>
<td>Salmo gairdneri</td>
<td>E</td>
<td>0.23</td>
<td>Greenwald et al (1974)</td>
</tr>
<tr>
<td>Cottus morio</td>
<td>E</td>
<td>0.55</td>
<td>Foster (1969)</td>
</tr>
<tr>
<td>Platichthys flesus</td>
<td>E</td>
<td>0.22</td>
<td>Motais (1967)</td>
</tr>
<tr>
<td>Anguilla anguilla</td>
<td>E</td>
<td>0.48</td>
<td>Motais (1967)</td>
</tr>
<tr>
<td>Opsanus tau</td>
<td>E</td>
<td>0.057</td>
<td>Lahlou and Sawyer (1969)</td>
</tr>
</tbody>
</table>

FW = Stenohaline freshwater species; E = Euryhaline species.
measured compounds. The usual methods assume that the injected compound does not penetrate into cells but distributes itself evenly throughout the extracellular body water and is not readily metabolised. Measurements of sodium and chloride space have been criticised due to fractions of the ions penetrating cells (Lutz, 1972). Sodium concentrations within cells, however, are usually low (<5mM). They can however be useful as an interspecies or intersstudy comparison.

The similarity of the ECSS calculated from influx and efflux experiments suggest that in both methods an even distribution of $^{22}$Na was achieved within the sodium pool. This similarity validates the methods of flux measurement and ECSS determinations. The actual space of distribution probably falls between the two values of ECSS. Therefore, the mean value of 30% body weight is probably of greatest use in estimating body concentrations of metabolites and ions in the ECSS.

Both of the calculated values of ECSS fall within the range of values found for most other fish species (Lutz, 1972). The mean value of 30% is also identical to that used as an estimate for ECSS in many studies where it has not been empirically determined (Eddy et al., 1980). Therefore, there is nothing unusual about the exchangable ion spaces within mudfish.

The patterns of sodium efflux in partitioned mudfish cannot be easily explained. Although the sodium efflux from the head was equivalent to 92% of the total efflux of the unrestrained fish, the massive loss of sodium from the body was not expected. A value of 90% of sodium loss being via the gills is conceivable for freshwater fish, since sodium and small monovalent ions are usually lost via the gills, and divalent ions and larger molecules are usually lost in the urine (Evans, 1979). However, up to 25% of sodium loss may be via the urine due to the large volumes of dilute urine voided by freshwater fish (Motais, 1967; Maetz, 1971).
The large sodium loss from the body is likely to be either a greatly enhanced cutaneous loss or an increase in urine flow rates. The patterns of partitioned urea excretion, described in the previous chapter, may indicate that a diuresis was occurring that would explain this ion loss.

The level of body urea excretion was double that seen in many fish. Such a doubling of urea excretion may be achieved by an increase in glomerular filtration rate (GFR). A doubling of GFR would therefore produce potentially greater urine volumes and therefore sodium loss. Increased urinary flow rates associated with increased GFR may also reduce the amount of sodium reabsorption in distal and proximal convoluted tubules and the urinary bladder through decreased residence time (Evans, 1979).

Partitioned body sodium loss in mudfish is 5.6 times greater than the weight specific loss found in African catfish, another air-breathing fish (Eddy et al, 1980). However, Oduleye (1976) found a ten fold increase in electrolyte loss in trout, due to a diuresis caused by handling stress. Therefore, such a high rate of ion loss could very easily be accounted for by a diuretic response to handling and partitioning in mudfish.

An increased cutaneous extrusion of sodium could also be caused by enhanced skin capillary perfusion or a change in the permeability of the skin epidermis. A hypoxic cutaneous vasoconstriction has been postulated and indirect evidence produced for one from data on respiration and nitrogen excretion. However, neither a hypoxic stimulus, nor emersion and hypoxia stimuli caused any significant decrease in body efflux rates. Therefore, either the suggested vasoconstriction does not occur or sodium loss is not linked to skin blood perfusion.

The sodium loss is not compensated for by sodium uptake mechanisms and therefore a considerable net sodium loss occurs during partitioning.
The efflux differs so greatly from unpartitioned fluxes. The sodium loss may, therefore, be an artifact of the experimental conditions rather than indicative of any special ionic regulation of mudfish. Also, skin ion permeability is not likely to change to this extent as the skin of freshwater fish is generally regarded to be ion impermeable unless the surface is damaged (Parry et al., 1967). Therefore, it is also possible that the body ion loss may be caused by tissue damage in the region of the partitioning dam, either from physical abrasion damage by the dam itself, or physical or chemical damage by the cement used. Partitioned fish were often noted to be scarred after partitioning and to take up to several weeks to regenerate complete skin pigmentation. Some mucus loss also occurred in this region and therefore the mucus would not be acting as a barrier to diffusion (Parry et al., 1967). From the orientation of the dam, and the cementing occurring on the body side of the dam, most of the damage would occur on the body side margin of the dam. Therefore, negligible effects of partitioning damage would be expected in the head compartment, as seen.

Either explanation could explain the enhanced body sodium efflux, although a diuresis seems more probable and has been previously noted in other fish. The ion loss could also be due to a combination of the two effects, both of which may initially generate uncompensated ion depletion.

Thus, the partitioning data provide little information on the relative roles of the gills, skin and kidney in the sodium movements in mudfish. The unrestrained fish efflux rates and head compartment rates do conform to normal teleost sodium efflux patterns and therefore mudfish skin may be an ion impermeable surface. Eddy et al. (1980) estimated that up to 22% of the sodium and chloride efflux from the freshwater African catfish may be via the skin. Thus, they suggested that the skin may be involved in ion balance in many amphibious
freshwater fish. This does not however appear to be the case with mudfish.

In the interpretation of all of the flux results, Maetz's (1974) warning should also be considered. Because the stress of handling can drastically upset the water and mineral balance, he suggests that mismatching of ion fluxes and mineral imbalance can be expected in most studies as a matter of course.

Emersed fish showed significant changes in the loss of sodium during the first 6 hours out of water. The route of this ion loss may be either cutaneous or urinary loss and therefore the change in loss rates must be related to changes in one of these routes. I have previously suggested from urea excretion, and weight loss data (Chapter 6), that urinary loss may cease at 4-8 hours after emersion. Therefore, the high sodium loss rate, decreasing by 6 hours may initially represent a urinary loss rate. The uniform loss rate beyond this time may be either a small continuing urinary loss, ventral cutaneous sodium loss, or both of these.

Since the mudfish were on a water saturated surface they may face a cutaneous osmotic influx of water that could lead to water loading, and therefore a small urinary excretion of excess water. Such a urinary flow would maintain water balance but would ultimately lead to a depletion of sodium ions. The stable sodium efflux in air represents only a 0.13% loss of total exchangeable body sodium per hour which, although not harmful in the short term, may lead to a 50% depletion of body sodium in 16 days. This magnitude of decrease in body sodium content is not seen in the blood concentration for 28 day emersed fish, although a small decrease was seen. The sodium loss must therefore decrease further over longer emersion periods.

This predicted urinary flow and ion loss may not occur in the natural environment where mudfish aestivate, as there would rarely be a
saturated substrate with free water to generate such a water and ion turnover. Clay and organic material would absorb water, and surface water may rapidly drain away leaving a humid microclimate but no free exchangable water. Eddy et al (1980) found significant efflux of sodium in emersed African catfish and loss rates exceeded possible urinary sodium excretion. They therefore suggested that over 50% of emersion sodium loss was via ventral cutaneous efflux. However, they also used fish on a water saturated surface, and this may have also allowed significant water turnover and therefore unrestricted ion loss through urinary flows.

Therefore, mudfish on very damp substrates do suffer a considerable sodium loss but it is not possible to discern cutaneous or urinary fluxes. In a drier microhabitat this loss of sodium would be expected to cease almost completely by 6 hours after emersion.

The lack of any sodium uptake from the body of the mudfish while in water further shows that the skin is a primitive passively exchanging barrier and has not evolved to the level of the actively transporting epithelia found in amphibians (Maetz et al, 1976). The mudfish is thus similar to most freshwater fish in most respects of its ionic regulation with a low passive ionic permeability that does not appear to be compromised by previously found cutaneous respiratory exchange mechanisms. They may however be susceptible to ion losses through stress responses.

Mudfish survived a wide range of salinities but could not adapt to rapid transfer to full strength seawater. As the specific density of the water was increased, the mudfish became more buoyant as the specific gravity of the water exceeded that of the fish body. To regain overall negative buoyancy mudfish needed to either alter the swimbladder volume or overall fluid and tissue density. At no stage were gas bubbles seen
to be voided by the mudfish indicating active emptying of the
physostomous swimbladder (Davidson, 1949). Specific density was
therefore increased, either by increased tissue/fluid osmolality (osmotic
dehydration), or removal of swimbladder gas at the gas gland. The
process of regaining buoyancy control was slow and the length of time
taken to adapt correlated with external salinity. 60%SW was possibly at
the limit of adaptation under these conditions as shown by the inability
of many fish to regulate buoyancy at this concentration.

Survival in 45% and 60%SW could be achieved by either
osmoconforming to the hyperosmotic seawater or by regulation of fluid
levels below the level of the external medium (Evans, 1979). From the
variation in blood ions with external salinity, mudfish can regulate both
hypo-osmotically and hyper-osmotically. Mortality beyond 60%SW may be
due to a failure of homeostasis beyond this level.

Kirsch (1972) found that eels retained more chloride than sodium
in association with increased bicarbonate excretion at higher salinities.
However, Evans (1979), in reviewing the plasma ion levels in many fish
species, found that no generalisation of proportional ion changes could
be made except that all blood ions show some degree of change in relation
to salinity changes. In mudfish, the chloride levels rose to a greater
degree than sodium but never exceeded sodium levels. Therefore ion
regulation mechanisms in mudfish may be similar to that of Kirschs' study
of eels, favouring chloride retention. Chloride levels were more stable
than sodium but showed a more pronounced rise at isoionic conditions,
suggesting chloride may be more closely regulated than sodium except for
disruption at the change from hyper to hypoionic regulation.

At 45% and 60%SW overall blood osmolality had probably risen
35-40% due to sodium and chloride rises. This does not indicate highly
efficient regulation at these salinities as the rise is higher than seen
in other euryhaline fish species (Table 5.5). Survival and adaptation
to salinities over longer periods, and to higher salinities, may be
greater if fish were exposed for longer and if salinity change was more
gradual. Thus, this experiment was not a definitive treatment of
salinity tolerance in mudfish but served to illustrate that the mudfish
do retain euryhaline regulatory abilities. These abilities are
presumably characters retained from their ancestral galaxiids marine
stages. Other studies have shown that species such as the lamprey
Petromyzon marinus, which have been landlocked for thousands of
generations, still retain effective undiminished euryhaline osmoregulatory
abilities (Mathers and Beamish, 1974). Such euryhaline ability may
therefore be retained and not readily lost in species initially
possessing such mechanisms. Thus it is not surprising, therefore, that
mudfish retain euryhaline abilities along with cutaneous respiratory
mechanisms.
CHAPTER 6

WATER BALANCE

INTRODUCTION

Maintenance of water balance in an amphibious fish such as the Canterbury mudfish may include mechanisms of maintaining both hydration status in water and preventing dehydration in air.

In freshwater, the body surfaces must remain a barrier to water movements to prevent rapid water loading and therefore dilution of the body tissues and fluids. The gills are generally regarded to be the major site of water exchange in fish (Evans, 1979), although in fish such as the mudfish with extensive cutaneous gas exchange, the skin may have significant water permeability. This might increase overall water permeability and therefore lead to greater rates of turnover of body water, due to greater water loading of the body.

Water movements across the integument due to epidermal permeability may also be influenced by the relative perfusion and ventilation of the respective surfaces. Control of skin perfusion may therefore allow variation in the apparent permeability of the mudfish under different conditions. Cutaneous vasoconstriction during hypoxia, as described previously, may decrease cutaneous water flux during these periods, and thus may supply further evidence for regulation of cutaneous exchange.

Maintenance of water balance during emersion may be predominantly directed towards restricting water loss through urinary excretion and desiccatory losses. As suggested in the previous chapters, urinary losses should cease soon after emersion, preventing a rapid net loss of water. Cutaneous permeability may, however, allow unrestricted loss of water to subsaturated atmospheres through desiccation with concomittant
concentration of body fluids. Such high cutaneous permeability may however, also allow rehydration from saturated substrates and appreciable exchange of water and metabolites with the substrate. Control of the extent of these exchanges may also be important.

The extent of cutaneous and total water permeability may, therefore, greatly influence the mechanisms and strategies allowing survival of mudfish in both air and water.

MATERIALS AND METHODS

6.2.1 Aquatic water balance

Body water content of healthy mudfish, acclimated to the aquarium system, was calculated from fish which were killed, weighed, and dried to a constant weight at 80°C. The difference between wet and dry weights accounted for total body water content. The mean water content was used in the calculation of water movements.

The water turnover rates (or apparent water permeability) of mudfish were measured by the efflux of tritiated water from fish labeled by injection of tritiated water in saline. Mudfish were lightly anaesthetised in an ether atmosphere and injected intraperitoneally with 10μCi of $^3$H$_2$O in 10μl of isotonic saline with a 10μl Hamilton syringe (Hamilton Co, Reno Nev.). Fish were maintained in air on a dry surface, but in a humid atmosphere, for 10 minutes to allow time for distribution of the tracer within the fish. In these conditions there was no significant loss of labelled water. Fish were then placed in 150ml of tapwater maintained at 15°C, and 0.5ml water samples taken at 10 minute intervals. Radioactivity in water samples was counted in a 1:10 mix with scintillation cocktail using a Beckman LS2800 Liquid Scintillation Counter. The scintillation cocktail used was as described in the previous chapter. A linear efflux of the radioisotope was calculated over the
first 40 minutes of sampling and water turnover rate as % body water per hour calculated from the initial activity injected. Water turnover rates were used as a measure of apparent water permeability.

Water turnover of partitioned fish was measured with fish partitioned as in section 2.6 using 200ml chambers. Fish were anaesthetised and injected with tritiated water, as in unrestrained fish, partitioned, and each chamber filled with 100ml of unlabelled tapwater. Water samples were taken from each chamber at 10 minute intervals and counted as previously described. Water turnover was calculated for each chamber. Further partitioned experiments were performed with fish with heads in normoxic water / bodies in hypoxic water ($P_O_2<20$ torr), and with heads in air / bodies in hypoxic water. Hypoxic conditions were generated by gassing the water with dry nitrogen as previously described.

Backflux of tracer was assumed to be insignificant (<1%) due to the short duration of experiments and the large volume of water relative to fish volume (30:1). Volumes removed were small relative to total volumes.

6.2.2 Emersion water balance

Water efflux of emersed fish was measured with fish labelled with 10μCi of tritiated water in 10μl of saline, as previously described, and placed in small plastic pottles containing tissue paper and 10ml of tapwater. This volume of water allowed the fish to remain moist but not to take water into the branchial chambers. Pottles were capped to maintain a humid atmosphere surrounding the fish and to restrict evaporative loss of the tracer. Tritiated water activity was measured in 100μl water samples taken every 15 minutes. Water samples were diluted to 1ml with distilled water and activity counted in scintillation cocktail as previously described. Water loss rate could therefore be calculated from initial activity injected and the rate of accumulation of
tritiated water in the substrate using the compartmental analysis of Motais (1967).

Water uptake of emersed fish was measured with fish resting on tissue paper soaked with 10ml of tapwater labelled with 1.0pCi/ml tritiated water. 100μl water samples were taken at 15 minute intervals and tritium activity measured as previously described. Water influx was therefore calculated from the disappearance of radioisotope from the water (Motais, 1967).

Evaporative water loss rates of mudfish were measured with individual mudfish in plastic netting baskets suspended 2cm above either a saturated NaCl solution (75% relative humidity, RH), or anhydrous CaCl₂ (45% RH). Humidities adjacent to the baskets were measured with a Lovibond humidity test kit using cobalt thiocyanate paper. Chambers remained sealed except for removal of baskets for weighing. There were no air currents adjacent to the fish except during weighing of the fish.

Fish were blotted dry for 10 seconds on tissue paper and weighed before placement in netting baskets. Fish within their baskets were weighed to the nearest mg on a Mettler LP200 top loading balance. Experiments were ended when fish had lost over 10% of their initial body weight. Lethal desiccatory tolerance was not tested.

Fish that had been depleted of 10-12% of their body weight by desiccation were placed on moist tissue paper while still within their netting baskets. Baskets were removed hourly, surface moisture removed and weighed to the nearest mg. This was to determine whether fish could regain body moisture by resorption from a moist substrate.
RESULTS.

6.3.1 Aquatic water balance

The total water content of 10 mudfish was 80.1 ± 0.4% of wet body weight. This was very consistent between fish.

Water turnover rates of mudfish totalled 49.7% of body water per hour or 0.4 ml g⁻¹ hr⁻¹ (see Table 6.1).

Partitioned water turnover rates indicated that water efflux occurred equally from both the head and body regions of mudfish. Partitioning significantly decreased total water efflux relative to that of unrestrained fish (Table 6.1; p<0.01). Exposure to hypoxic water did not affect water efflux rates. When the heads of mudfish were in air and bodies were in hypoxic water, the water efflux from the body was unchanged. This indicated either a net loss of water, or significant transcutaneous exchange of water. With the head emersed, the total water turnover of mudfish was significantly reduced (p<0.001).

Table 6.1 Water turnover rates of unrestrained mudfish and with mudfish partitioned in normoxic water, with the body in hypoxic water, and with the head emersed and body in hypoxic water. Units - % body water per hour.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>HEAD</th>
<th>BODY</th>
<th>TOTAL</th>
<th>BODY AS % OF TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrestrained</td>
<td>12</td>
<td></td>
<td></td>
<td>49.6</td>
<td>±2.5</td>
</tr>
<tr>
<td>Partitioned in normoxia</td>
<td>8</td>
<td>20.3</td>
<td>18.9</td>
<td>39.1**</td>
<td>48.3</td>
</tr>
<tr>
<td>Partitioned with hypoxic body</td>
<td>6</td>
<td>21.8</td>
<td>20.1</td>
<td>41.9</td>
<td>48.0</td>
</tr>
<tr>
<td>Partitioned with head emersed and body hypoxic</td>
<td>6</td>
<td></td>
<td>21.8</td>
<td>21.8***</td>
<td>100</td>
</tr>
</tbody>
</table>

Significance - **- p<0.01; ***- p<0.001.
6.3.2 Emersion water balance

Water efflux from mudfish during the first hour of emersion was significantly lower than the aquatic turnover rate (p<0.001), but was not significantly different from the partitioned body chamber rate (Table 6.2). Corresponding water influx during emersion was significantly lower than emersion efflux rate (Table 6.2, p<0.01). Mudfish may therefore suffer a net loss of body water upon emersion.

Table 6.2 Water fluxes of mudfish, following emersion compared to water turnover of mudfish in water.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>RATE CONSTANT</th>
<th>FLUX (ml/hr)</th>
<th>% BODY WATER PER HOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquatic water efflux</td>
<td>12</td>
<td>1.30</td>
<td>49.6 ±2.5</td>
<td></td>
</tr>
<tr>
<td>Emersion water efflux</td>
<td>8</td>
<td>0.220 ±0.013</td>
<td>0.95 ±1.2</td>
<td>21.1 ***</td>
</tr>
<tr>
<td>Emersion water influx</td>
<td>8</td>
<td>0.125 ±0.009</td>
<td>0.64 ±1.0</td>
<td>14.3 ***</td>
</tr>
</tbody>
</table>

Significance *-p<0.05; **- p<0.01; ***- p<0.001

Evaporative water loss rates of mudfish were high and linear in both relative humidities tested (Figure 6.1). The rate of water loss was 2.4 times greater at 48%RH than in 75%RH. Mudfish did not appear to be able to limit desiccatory water loss at any stage and lethal losses were therefore predicted to occur at 16 and 40 hours respectively for the two humidities (assuming death at 20% weight loss).

Dehydrated fish were able to regain up to 70% of their lost body water when placed in contact with a moist substrate (Figure 6.2). Rehydration occurred rapidly over the first hour, slowing to reach a stable hydration state by 5 hours that corresponded to having regained 70% of the water lost by desiccation. Predesiccation body weights were never achieved in these rehydration experiments.
Figure 6.1 Graph showing the cumulative evaporative water loss of mudfish at 45% and 75% relative humidities in still air.

Figure 6.2 Graph showing rate of rehydration of dehydrated mudfish placed on damp tissue paper.
DISCUSSION

The total body water content of mudfish was similar to the level seen in other freshwater fish (Love, 1980). This water volume is split between the intracellular and extracellular pools. If the sodium space calculated in the previous chapter is considered a good indication of extracellular water space then body water of mudfish is split 5:3 between intra and extracellular compartments respectively. The hydration and distribution of water within mudfish is therefore not unusual for a freshwater teleost (Evans, 1979).

The measurement of water turnover of mudfish from efflux rates assumes fish are in a state of water balance with influxes balancing effluxes and therefore water efflux is equivalent to water turnover. Using tritiated water efflux as a measure of turnover, the diffusional permeability to water of the whole fish was being estimated. As water is present at approximately equal concentrations on both sides of the skin and gills (55.5 moles l⁻¹), the major component of water turnover will be passive turnover between these two compartments. However, there is also an osmotic gradient (water entering the fish down an osmotic gradient) but the difference in water concentration due to this gradient is slight in relation to the passive diffusion component. Net flows are however, almost totally accounted for by the osmotic influx and are compensated for by urinary excretion, as there is little rectification of passive diffusional fluxes. However, since these net fluxes are small compared to passive diffusion, they are often considered insignificant in flux measurements (Adedire and Oduleye, 1984).

Measurements of passive water turnover denotes the ease with which water moves across the external epithelia of the fish. This has usually been considered to be gill surfaces but in skin breathers such as the mudfish it may include the skin surface and any water filled
air-breathing surfaces. The passive water turnover will be related to net water loading as it is a measure of permeability to water, and therefore is directly proportional to osmotic diffusibility.

As the mudfish skin is permeable to gases and ammonia it may also be highly permeable to water. The mean rate of water turnover was similar to many other fish species (Table 6.3) and therefore water permeability of mudfish may not be considered especially high. The water turnovers of other fish species are very variable and show no pattern with respect to euryhaline or stenohaline osmoregulatory abilities, or differences in body form. Fish size is important in considering passive water permeabilities because smaller fish will have proportionately greater exchange areas compared to body mass. The fish with the highest permeabilities in Table 6.3 are therefore the small intertidal and tropical species more comparable to mudfish.

External calcium concentration can greatly affect permeabilities as shown by Gregory and Macfarlane (1981; Table 6.3). This may not be a significant factor in this study because of the hardness of the water, but could explain some of the variability between and within species in Table 6.3.

The total water permeability of partitioned fish was significantly lower than that of the unrestrained fish. This is opposite to the effect seen with sodium fluxes and supports the view that ion loss and water turnover are essentially different processes. Increased urinary water flow due to a diuresis may be insignificant in relation to total water exchange but highly significant in ion loss.

Adedire and Oduleye (1984) found that handling stress and recovery from anaesthetics caused significant increases in water permeability of a cichlid fish species. Why these factors should decrease permeability in mudfish is uncertain. Adedire and Oduleye also described reduced
Table 6.3  Unidirectional water flux across the body surfaces of the Canterbury Mudfish and several other freshwater teleost fish.

<table>
<thead>
<tr>
<th>Species</th>
<th>Osmoreg. ability</th>
<th>Water Flux (% body water/hr)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canterbury mudfish Neochanna burrowsius</td>
<td>S?</td>
<td>49.6</td>
<td>This study</td>
</tr>
<tr>
<td>Tilapia mossambica</td>
<td>E</td>
<td>186</td>
<td>Potts et al (1967)</td>
</tr>
<tr>
<td>Platichthys flesus</td>
<td>E</td>
<td>11</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Gasterosteus aculeatus</td>
<td>E</td>
<td>11</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Anguilla anguilla</td>
<td>E</td>
<td>6.0</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Salmo trutta</td>
<td>E</td>
<td>27</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Salmo gairdneri</td>
<td>E</td>
<td>25</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Salmo salar</td>
<td>E</td>
<td>48</td>
<td>Potts et al (1970)</td>
</tr>
<tr>
<td>Aphanius dispar</td>
<td>E</td>
<td>68</td>
<td>Lotan (1969)</td>
</tr>
<tr>
<td>Fundulus kansae</td>
<td>E</td>
<td>138</td>
<td>Potts &amp; Fleming (1970)</td>
</tr>
<tr>
<td>Phoxinus phoxinus</td>
<td>S</td>
<td>139</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Carassius auratus</td>
<td>S</td>
<td>92</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Cottus morio</td>
<td>S</td>
<td>16</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Rutilus rutilus</td>
<td>S</td>
<td>13</td>
<td>Evans (1969b)</td>
</tr>
<tr>
<td>Clarias mossambicus</td>
<td>S</td>
<td>86.6</td>
<td>Eddy et al (1980)</td>
</tr>
<tr>
<td>Cyprinus carpio Low Ca++</td>
<td>S</td>
<td>60.5</td>
<td>Gregory &amp; MacFarlane (1981)</td>
</tr>
<tr>
<td>Cyprinus carpio High Ca++</td>
<td>S</td>
<td>36.0</td>
<td></td>
</tr>
</tbody>
</table>

* - S = Stenohaline freshwater; E = Euryhaline regulatory ability.
permeabilities in fish exposed to low constant levels of anaesthetic. Over the short periods of the experiments, mudfish may retain levels of anaesthetic and may therefore be more relaxed or inactive.

The equal water turnover rates of heads and bodies of partitioned fish suggest that cutaneous water turnover may be appreciable. The body turnover cannot be described by a diuresis as the accompanying ion loss would be many times greater than that measured in the previous chapter and far beyond the possible rates of urine formation. Since cutaneous water turnover appears to be appreciable but overall turnover of mudfish is not especially high compared to other fish, either the gill water turnover of mudfish may be lower than in other species or cutaneous water permeability may be more widespread in other fish species. This may be a fruitful line of research in other species.

A hypoxic stimulus had no effect on permeability of either the head or body compartments of mudfish. Adedire and Oduleye similarly found no significant effect of hypoxia on water permeability in cichlid fish. From the previous discussions, where cutaneous vasoconstriction under hypoxia was suggested to limit bloodflow adjacent to the skin surfaces, a decrease in body water permeability was expected upon exposure of the body to hypoxia. Therefore, either there was no vasoactive control of skin perfusion or water loss is relatively independent of blood flow. Appreciable water flux may continue across the epidermis to the interstitial water in skin tissues in the absence of blood flow, but for significant equilibration with the rest of the body water over the duration of the experiment, rapid mixing via the blood must take place.

When fish were placed with their heads in air and bodies in hypoxic water, the body water turnover remained at control levels. Since water loading via the gills could not then take place, the body efflux could only be matched by a cutaneous influx or lead to a rapid
dehydration of the fish. The latter did not occur as weight changes of the necessary magnitude never occurred. Such water turnover must therefore be transcutaneous and again was not modulated. Therefore, the data on hypoxic partitioning might be used as evidence for lack of perfusion control of cutaneous permeability, in contradiction to previous findings.

Clearly then, the skin of the mudfish has further effects on the physiology of the mudfish via water balance. An interesting point is the permeability of the skin to water but not ions. This point has been noted in freshwater snakes (Duns on, 1978) and may be an important property of cutaneous skin function. Ion permeability may serve no advantageous function, while water fluxes may be advantageous in several situations, especially in air in the maintenance of moist surfaces and in water uptake from moist surfaces.

During the first hour of emersion, water efflux remained at 43% of the total aquatic rate even though gill ventilation had ceased. Although the efflux was similar to the aquatic body compartment efflux, it cannot be readily explained solely as a continuing cutaneous water efflux, as only the ventral body surface was in contact with the moist substrate. However, during this initial period out of water, water may also be lost as water volumes retained around the gills and oral surfaces drain away. Urinary flow may also occur for some period after emersion. However, these would only be temporary losses and the major route of water flux, especially at longer periods, would be cutaneous fluxes.

Corresponding water influx rates were significantly lower than effluxes indicating an initial net loss of water upon emersion. This may be in the form of the previously mentioned gill and urinary water losses, drying of the dorsal surface and loss of hydrophilic mucus during movement. Gravitational effects may also cause a slight rectification of
water exchange through ventral skin. However, the water influx was still at an appreciable level, being 70% of the aquatic partitioned body rate. Even though the ventral skin and fins are in contact with the moist surface, less than 70% of the total skin area would be positioned to exchange water with the substrate. On an area basis, cutaneous fluxes therefore appear to be somewhat enhanced.

On a moist surface, cutaneous water exchanges may remain at high levels leading to changes in the state of water balance of emersed fish. However, the rates measured here will be lower once mudfish reach a stable state in air. The time course to achieve a relatively stable state may be related to respiratory and diuretic responses so may take from 4 to 24 hours. Influxes and effluxes must also match at some stage as fish did survive 85 days in air so must have achieved a stable state of water balance.

The mudfish appear to have a skin that is as permeable to water as the gill area is but the permeability is not modulated as was expected. Therefore mudfish might be vulnerable to water loss due to desiccation in air.

The evaporative water loss rates of mudfish in the two humidities were linear through to 10-12% loss of body weight. They were therefore not able to control desiccatory water loss. Comparison of water loss rates with other fish and amphibians is difficult, due to the varying conditions of air flow, lighting, temperature, animal activity, animal size, surface area to volume ratios and experimental humidities. Ventilatory mechanisms and frequencies will also affect water loss. At temperatures up to 15°C the ventilation rate of mudfish is low (Fig. 3.5), so ventilatory water loss may be discounted compared to the total water loss. Since the gill areas may be as great as the skin (section 7.3), then at higher temperatures when ventilation becomes more frequent ventilatory water loss may be as or more important than skin water loss.
The ventilation pattern may also be important to consider as tidal rather than flow through patterns may reduce ventilatory evaporative water loss. If the desiccatory weight loss is related to skin surface area (section 7.3) then mudfish had surface area specific weight losses of 1.0 and 2.43 mg cm\(^{-2}\) hr\(^{-1}\) respectively in the two humidities. This loss rate may be a slight overestimate due to whole body being exposed to the air whereas the ventral skin is usually obscured by contact with the ground. Also behavioural and social factors may also effectively decrease evaporative water loss rates. Since mudfish are often found in high densities in drought prone areas, mudfish might be expected to clump or group in the favourable microhabitats during aestivation. Such social clumping has often been described in aestivating amphibians (Reno et al, 1972) and has been suggested as advantageous in reducing desiccation and maintaining humidity in microhabitats. Coiling of mudfish upon themselves or around objects may similarly reduce areas at risk of evaporative loss. In the experimental treatments these mechanisms were not available to mudfish so loss rates may be considered a possible maximum rather than a routine rate. However, desiccatory water loss rates in mudfish are still high and unrestricted and therefore will severely limit the terrestrial potential of mudfish.

The lack of antidesiccatory mechanisms is expected for a fish with an extensive mucus covering and a high surface area to volume ratio. Since the skin has been shown to be permeable to water, water lost from the hydrophilic mucus will be rapidly replaced by a transcutaneous flux of body water. With a high skin permeability a constant rate of water loss might be expected up until death. Mudfish have not evolved cutaneous barriers to water loss as many amphibians have done and therefore the skin may not have been modified from its aquatic role.

Lethal desiccatory limits of mudfish were not tested but may
exceed a 20% body weight loss if their tolerance is similar to other small amphibious fish (Gordon et al, 1978). Their salinity tolerance and ability to cope with acid-base disruption and toxic accumulations suggest that mudfish should have a high tolerance to disruptions such as concentrating of body fluids. Thus, in the experimental humidities mudfish may be expected to survive for at least 16 and 40 hours respectively.

Many tropical aestivating fish and amphibian species form cocoons from dried mucus to seal themselves into a more favourable microclimates (Lee and Mercer, 1967; Reno et al, 1972; Smith, 1929). In this study mucus never dried to this stage nor detached from the fish, but did revert from a smooth slippery surface to a non-reflective, tacky surface, i.e. the mucus became dehydrated. Meredith (1981) noted several mudfish that jumped from disease treatment tanks and spent 18-20 hours on a dry concrete floor. The same conditions, measured several months later over several days, showed relative humidities of 58 ± 6% (4) RH. Under such conditions, I would predict from the experimental loss rates, that mudfish would have died long before rescue. However, although these mudfish were rigid, coiled, and covered with a thin dry flaky covering of mucus, 60% of the fish recovered upon reimmersion and shed the mucoid sheath. Most of these individuals subsequently survived. Thus, beyond a certain level of desiccation, the mucus layer may dry and detach to become cocoon like. This mucoid parchment has in other species been shown to be resistant to water diffusion and would therefore restrict water loss from the area within the cocoon (Reno et al, 1972). This mechanism may increase longevity of desiccated mudfish but water loss by this stage may be so extreme, as in the described fish, that many may be dead and others may have little reserve water before lethal limits are reached. Thus, the high surface area to volume ratio and small body size, of advantage in cutaneous exchange, are disadvantageous in
desiccatory mechanisms. Although the cocoon formation mechanism is available to mudfish, it may form too late to aid survival in all but the largest specimens. It is therefore unlikely to be a significant mechanism in naturally aestivating mudfish.

Since conditions in terrestrial environments are rarely constant, mudfish may survive brief periods of desiccation if dehydration is later relieved by access to water. If a large amount of free water is available, direct hydration through aquatic gill ventilation may take place. If only surface moisture such as dew or condensation is available mudfish may be able to rehydrate their body through the skin surface. Desiccated fish were able to regain 70% of lost body water through absorption from a damp surface. This rehydration may initially be via hydration of the hydrophilic mucus layer followed by osmotic influx through the skin itself. The initial high rate of weight gain over the first hour of rehydration may be a rapid rehydration of body mucus while over the next 5 hours rehydration of the body is slower.

Mudfish may not have been able to achieve predesiccation body weights for several reasons. These may have included inability to replace water held around the gills and in the mouth, lack of hydration of dorsal surface mucus, and possibly significant metabolic weight loss over the desiccation and rehydration period.

To prevent harmful degrees of desiccation, a favourable microclimate/microhabitat is necessary for the long aestivation periods described for the mudfish (Eldon, 1979c). This would include conditions of ground moisture, a humid atmosphere, lack of strong air currents, limitation of maximum temperature and shielding from direct sunlight. A lack of any of these would lead to death through desiccation. Several intertidal amphibious fish species will survive under adverse conditions but are able to retreat to water periodically or are covered during the
next tidal cycle (Gordon et al., 1968, 1969). Direct sunlight not only enhances desiccatory water loss but also heat stresses fish and may destroy the epidermis. Even short periods of exposure to ultra violet light may destroy teleost epidermis (Bullock, 1981). Eldon (1979c) stated that he considered mudfish selection of microhabitat as random, with many fish dying in unfavourable sites during droughts. Thus the environment itself may be the determining factor on survival of mudfish during droughts.

The degree of permeability of mudfish skin, and the extensive hydrophilic mucus layer are the only outstanding features of mudfish water balance that allow survival in air. Mechanisms to allow varying water permeability do not appear to occur. The skin is therefore not greatly modified for roles in water balance and functions to maintain a moist surface for cutaneous exchange mechanisms rather than limiting desiccatory water loss. Survival in air must therefore be limited to favourable conditions and microenvironments to overcome the limitations in water balance.
CHAPTER 7

STRUCTURE OF EXCHANGE SURFACES.

INTRODUCTION

This section describes the histology of the skin, gills and buccal regions of mudfish and relates this structure to previously described functions in gas exchange, excretion and other homeostatic processes. The exchange surfaces of most other air-breathing fish species have been studied with respect to their respiratory function. In the opinion of Carter and Beadle (1931b), to be a gas exchange organ, a surface must have air passed in and out of it, must possess a rich blood supply and it must be shown to take up and release gases. These criteria are also valid in confirming the multiple exchange functions of the surfaces utilised by mudfish.

The structure and modification of teleost skin for cutaneous respiration has been described for several amphibious fish (Schottle, 1932; Mittal and Munshi, 1971). These authors highlighted many structural modifications which increase the efficiency of the skin as an exchange organ. Its mucogenic nature, uniformity, thickness and area may all affect exchange efficiency.

The gills of fish are constructed so as to function as a countercurrent gas exchanger in water when constantly irrigated (Hughes, 1972). Their function in air may, however, be governed by the extent of modification to prevent them collapsing, with the subsequent loss of functional surface area. Modifications to the basic teleost gill pattern have been described in several air-breathing fish (Schöttle, 1932; Hughes, 1972; Hughes and Morgan, 1973). The structure, area and potential for collapse may, therefore, determine the effectiveness of the
gills of mudfish in aerial respiration.

Other areas of possible respiratory significance in mudfish include the buccal and opercular epithelia. These surfaces have been extensively modified as exchange surfaces in a wide range of amphibious species and the evolutionary advancement of the different modifications has been discussed (Schöttle, 1932; Munshi, 1976; Satchell, 1976).

Many other structures have been modified or developed to act as gas exchange organs in air-breathing fish. These include lungs, the gas bladder, suprabranchial organs, highly modified gills and the gut (Carter and Beadle, 1931b; Munshi, 1976; Johansen, 1970; Kramer et al, 1978). None of these has either developed or appears likely to possess significant respiratory function in mudfish (Davidson, 1949).

Both perfusion and ventilation of gas exchange areas are important in maintaining rates of exchange. Ventilation has already been discussed but perfusion parameters are more difficult to isolate. It is not the intention of this work to map out the arterial and venous connections to respiratory exchange surfaces in _Neochanna burrowsius_ as this has already been carried out by Davidson (1949) on the closely related _Neochanna apoda_. She described many branches of major vessels that anastomose to produce possible respiratory exchange surfaces. It is the purpose of this section merely to confirm the existence of capillaries and the likelihood of their use as part of an exchange surface, not to determine their origin.

**MATERIALS AND METHODS**

7.2.1 General Methods

The structures, dimensions and areas of different tissues from the Canterbury mudfish were examined by light microscopy of intact, dissected
and sectioned material, and by scanning electron microscopy.

Healthy mudfish were sacrificed using lethal doses of anaesthesia (0.1% benzocaine) followed by dissection and fixation in appropriate fixatives. Fish carcases were stored in 70% ethanol after fixation. Fish that had previously died from disease or experimental manipulation and showed little external damage were used for analysis of skin area, and skin and gill dry weights. Any fish with clearly visible gill or skin damage was discarded.

7.2.2 Skin

General skin structure was studied from tissue dissected from freshly killed fish and prepared as paraffin sections for viewing under light microscopy. Fish were killed by immersion in 0.1% benzocaine and fixed in Bouins fluid for 24 hours. Small (5x5x2mm) blocks of skin and muscle from three regions of the fish body (Fig. 7.1) were dissected from the fish, dehydrated in alcohols, cleared in terpineol and embedded in 58°C paraffin wax. Sections were cut at 8μm with a steel blade on a rotary microtome and fixed to glass slides. Slides were stained with either Ehrlich's haemotoxylin and eosin (Pearse, 1968) or Goldner's trichrome stain (Pearse, 1968).

Sections were viewed with a Nikon photomicroscope and measurements made with a precalibrated linear ocular micrometer. Measurements were taken at regular intervals along a randomly selected transverse section. All sections were assumed to have been cut perpendicular to the skin surface.

The structure of the different elements of the skin were described and relative thicknesses of the different elements compared between the skin from the three body regions. Presence of skin capillaries was described from the presence of orange stained erythrocytes. Other methods attempted for determination of capillary density included
staining for alkaline phosphatase in fresh frozen sections (Winkelman 1961) and intravascular injections of a gelatin/ink preparation (Rahr, 1979), but both proved unsuccessful.

![Figure 7.1](image)

**Figure 7.1** Body regions from which skin areas were removed for description and comparison of skin structure.

The relative numbers and sizes of mucous cells in the epidermis in the three regions of the body of mudfish were examined in excised sheets of skin using a method modified from Pickering (1974). From specimens fixed in 10% formalin in saline, one centimeter square sheets of skin were dissected free from the underlying muscle layer. These were rinsed in Cortland saline and then immersed in 1% alcian blue in 1% acetic acid (pH=3.0) for 8 minutes. Stained skin was rinsed in tapwater and mounted on glass slides in glycerol jelly. Counts of numbers of mucous cells per 0.06mm² quadrat in ten random quadrats and maximum diameters of 30 mucous cells along a random transect were measured for each skin mount using an ocular micrometer. This gave measurements of mean mucous cell density, mean mucous cell diameter and the maximum cross sectional area of skin taken up by mucous cells. The mucous cell parameters were compared between the three body regions and with studies of other fish species.

Sections of dorso-lateral skin were used to examine the histochemical properties of surface and goblet cell mucus. Paraffin
sections were prepared as above and replicate sections stained with PAS (Periodic acid/ Schiff reagent) and alcian blue (pH 2.8), or alcian blue (pH 1.0) and alcian yellow (pH 2.8), following methods modified from Blackstock and Pickering (1974). From the different staining properties of the mucous cell contents, the predominant mucus types were determined and compared with the mucus produced by other fish species.

The structure of the surface of mudfish skin was examined by use of scanning electron microscopy of blocks of skin and muscle. Small blocks of muscle were dissected from freshly killed fish and immersed in 2% gluteraldehyde in 0.1M cacodylate buffer (pH 7.2) for 24 hours at 4°C. Fixed tissues were rinsed in 0.1M cacodylate buffer, dehydrated in an alcohol series and then an alcohol-amyl acetate series. Dehydrated samples were critical point dryed in liquid CO₂ in a critical point dryer, mounted on stubs with copper powder cement and splutter coated with gold. Tissue was viewed on a Cambridge stereoscan electron microscope. Several tissue blocks were also fixed in 20% gluteraldehyde, prior to preparation, to yield mucus free surfaces. Increased fixative concentration has been previously shown to remove mucus coatings (Olson and Fromm, 1973). Areas were photographed with Ilford FP4 film. Surface sculpturing of the skin epithelial cells was compared between the different skin areas and with studies of other fish species.

The skin area of mudfish was measured by fixing fish in 10% formalin in saline and dissecting areas of skin free from the underlying muscle. All of the skin was removed as 6-8 sheets from each fish. The skin was spread out on constant density graph paper, equivalent areas drawn and cut out and the area determined gravimetrically against a known area after drying. The excised skin and remaining fish body was dried at 80°C for 24 hours to determine the skin dry weight and proportion of total dry body weight.
7.2.3 Gills

Three dimensional structure of mudfish gills was examined by scanning electron microscopy. The left hand side gill basket was removed from freshly killed fish and prepared for SEM as for the previously described skin samples. General structure and orientation of the gills was described, as was the sculpturing of the surfaces of both the primary and secondary lamellae.

Morphometric measurements and measurement of total gill area were made following the method of Hughes (1972), using gills removed from the left hand branchial basket of fourteen individuals. The right hand gill arches were presumed to be of similar dimensions to the left hand side. The individual gill arches were immersed in alcoholic borax carmine for 48 hours (Humason, 1979), rinsed in 50% ethanol and mounted on glass slides in glycerol jelly. Measurements were made of the total number of filaments per arch, mean filament length (from measurement of every fifth filament), and mean number of secondary lamellae per mm (measured along every fifth filament). However, secondary lamellar area could only be satisfactorily determined in these preparations from large specimens. Therefore, the right hand gill baskets of the sampled fish were removed and prepared for SEM as above. Secondary lamellae areas were calculated from measurements of height and width of secondary lamellae viewed along selected filaments and assuming a regular triangular shape. Total gill area was therefore calculated from the equation:

\[ A = L \times n \times b_l \]

where \( A \) = area in \( \text{mm}^2 \); \( L \) = total length of filaments; \( n \) = number of secondary lamellae per mm; and \( b_l \) = mean bilateral area of a secondary lamella.

Relationships of the different gill dimensions to body weight were calculated using a log/log relationship and the allometric equation
Huxley, 1932:

\[ \text{dimension} = a.W^b \]

Width to height ratios of secondary lamellae were calculated from micrographs and whole mounts. The true secondary lamellar shapes along primary lamellae were determined and representative lamellar shapes drawn.

To examine internal structure of the gill arches, gills were fixed in Bouins fluid, dehydrated in an ethanol series and embedded in 58°C paraffin wax. Sections were cut at 5μm with a steel blade rotary microtome and fixed to glass slides. Sections were stained with Goldner's trichrome stain and internal structure and measurements of blood to water distance determined. Blood/water distance was determined by the measurement of arithmetic mean distances from the capillary to the nearest outer margin using an optical micrometer.

The position and histochemistry of mucous cells on the gills was investigated in parafin sections and whole mounts. Stains used were the same as for skin mucus.

Corrosion casts of gill lamellae were made using Batesons #17 corrosion casting fluid (Polysciences Inc., USA.). Fish were heavily anaesthetised in 0.1% benzocaine and placed ventral side up on a dissecting board. The heart was exposed by ventral dissection and a heat flared vinyl cannula inserted into the bulbus arteriosus and attached with cotton thread. Heparinised saline (0.9% NaCl) was passed through the cannula to clear the blood system. The atrium was pierced to allow drainage of the blood and saline. After 15 minutes of saline flush, premixed casting fluid was introduced via the cannula using a 5ml syringe. Fluid was injected by pulsed pressure on the syringe by hand. Corrosion cast carcases were stored in saline at 4°C for 48 hours before digestion at room temperature in 10% KOH. After digestion, gill arches
were dissected free, rinsed in distilled water, dehydrated and prepared for SEM as for intact gill arches. Corrosion casts were examined at 25Kev and vascular patterns photographed.

Right hand gill arches were removed from the fish that had been used for analysis of skin area, and these were dried at 80°C for 24 hours. Gill dry weight and the percentage of total dry body weight were calculated.

7.2.4 Buccal and opercular epithelium

A further group of mudfish were killed, decapitated anterior to the pectoral fins and whole heads fixed in Bouins fluid for 48 hours. Heads were rinsed in 70% alcohol and decalcified for 72 hours in 3 changes of 5% trichloroacetic acid in 70% alcohol. Decalcified heads were dehydrated and embedded in 58°C paraffin wax. Sections were cut at 10μm on a steel blade rotary microtome and mounted on glass slides. Sections were stained with Goldner's trichrome stain and general structure and capillarity of tissues examined. The dorsal and ventral buccal epithelium and the inner opercular epithelium were examined as possible respiratory exchange surfaces.

7.2.5 Other structures

Anatomical evidence for a possible respiratory function of the swimbladder was investigated by dissection and examination of the structures in freshly killed fish. The swimbladders were inflated with a fine cannula for examination of the bladder surface for areas of vascularity.
RESULTS

7.3.1 Skin

The skin of mudfish, as with other teleost fish was easily divisible into an epidermis, a dermis and hypodermis (Fig. 7.2). The epidermis (Fig. 7.3) was an unkeratinised stratified squamous epithelium ranging from 5 to 12 cells in thickness and was readily discernable into several separate layers. Immediately above the basement membrane was the stratum germinativum layer comprising a continuous layer of germinal columnar cells. This layer comprised over half the epidermal thickness but was only one cell thick. The stratum germinativum is usually thought to give rise to all of the epidermal cells in the higher levels of the epidermis (Henrickson and Matoltsy, 1968a). In the middle layer, above the stratum germinativum, were cuboidal and polygonal cells with prominent spherical or oval nuclei. These cells have alternatively been called filament containing cells (Henrickson and Matoltsy, 1968a), epidermal cells (Kitzan and Sweeny, 1968), squamous cells (Brown and Wellings, 1970), keratinocytes (Parakkal and Alexander, 1972), malphigian cells (Bullock and Roberts, 1975) and epithelial cells (Whitear, 1970). They are undifferentiated cells that become the mucous goblet cells and cuticular cells of the higher layers. Many of these cells stained heavily with PAS (periodic-acid-Schiff) indicating that at a later stage these cells may be concerned with synthesis, storage and secretion of mucus. The true polygonal cells varied greatly in shape the closer they were to the surface. The outer layer consisted of cells with various degrees of flattening to form a semi-squamous outer layer, and large oval goblet cells. Only a single layer of large mucus or goblet cells were discernible adjacent to the outer surface with only an occasional small goblet cell beneath or adjacent to the base of the larger goblet cells. Immature goblet cells were therefore rarely seen. The mature goblet
Figure 7.2 Transverse section of the dorso-lateral skin of the Canterbury mudfish. Goldner's stain, x100.

O - Outer mucous layer
g - Goblet cell
C - Columnar germinal epidermal layer
d - Dermis
h - Hypodermis
m - Muscle blocks

Figure 7.3 Transverse section of the dorsal epidermis of the Canterbury mudfish. Goldner's stain, x200.
For key to structures see previous figures.

Figure 7.4 High power of transverse section of goblet cell and outer epithelial cells of mudfish dorsal epidermis. Note external mucus layer. H. and E. (x 860.)

cells had large oval bases with a tapering apical tip or neck that often breached the outer surface of the epithelium and was sometimes seen to be connected to an exudate of mucus (Fig. 7.4). The basal end of the goblet cells contained a thickening of the cell margin that contained the nuclear material. The goblet cells, although only one cell thick, comprised the majority of the volume of this outer layer. The outer margin of the epidermis was a relatively smooth surface with a variable thickness of cuticular secretions. The mucus and cuticular layer was variable in thickness and often slightly displaced from the epidermis in the sections. This layer often measured up to 10μm or 10% of the epidermal thickness. Measurements of the secretion layer were, however,
unreliable due to the action of fixatives and solvents on secretion and swelling or shrinking of the mucoid mass.

The entire epidermis was uniform in thickness with no areas of regional thinning or regions of dermal evaginations. The epidermis was avascular in all areas examined.

The basement membrane was thin and not well defined, but was a regular demarcation between the dermis and epidermis. The dermis was a relatively thick (25 \( \mu \text{m} \)) layer of vascular and connective tissue containing pigment cells. The dense regular connective tissue stained green in the Goldner's stain and and was composed of regular bundles of collagenous fibres. Directly beneath the basement membrane lay variable distributions of pigment cells or chromatophores. Another layer of pigment cells was found in the lower layers of the dermis adjacent to the hypodermis. An extremely variable density of capillaries, as shown by orange staining erythrocytes (Fig. 7.5), occurred throughout the dermis. In the lower levels of the dermis and the hypodermis, larger blood vessels were sometimes apparent (Fig. 7.6). These probably supplied the dermal capillaries. The dermis lacked the layer of overlapping scales found in most teleost skin, and did not show any other forms of calcareous or rigid support as evidenced by the staining methods used. The dermis was not readily divisible into separate stratum compactum and stratum laxum layers.

The hypodermis was usually only 20-30% of the thickness of the dermis, and spanned the distance between the muscle blocks and subdermal pigment cells. It often appeared more diffuse than the dermis but this may have been due in part to the tendency for the skin to pull away from the muscle layer in many sections. The hypodermis contained dense irregular connective tissue, assemblages of nerves, capillaries and larger vessels and many unfilled spaces that may hold tissue fluids.
Figure 7.5 Transverse section of dermal and hypodermal capillaries in the dorso-lateral skin of the Canterbury mudfish. Goldner's stain, x 400.

C - Capillaries
E - Epidermis
d - Dermis
h - Hypodermis
V - Large dermal vessel

Figure 7.6 Transverse section of mudfish skin showing large dermal blood vessels. Goldner's stain, x60
* Recent evidence summarised in Vogel (1985a,b) suggests that a network of secondary vessels of capillary dimensions and containing only plasma, may be an important feature of the skin of fish. This possibility was not considered at the time the thesis was written.


The dimensions of the different skin layers did not vary greatly between the three body regions studied (Table 7.1). The dermis and hypodermis were not significantly different between any of the areas. The layer of mucous cells appeared to be thinner in the dorso-lateral region but was not significantly different from the other two body regions. The apparent decrease in thickness was also reflected in a lower dorso-lateral epidermal thickness but this was also not significant.

Table 7.1 Thicknesses (µm) of the different elements of the skin of mudfish from three body regions. Wt= 6.80 ± 0.50g (n=6).

<table>
<thead>
<tr>
<th>BODY Region</th>
<th>Mucous cells</th>
<th>Epidermal layer</th>
<th>Mucus cell as % Epi.</th>
<th>Dermis</th>
<th>Hypodermis</th>
<th>Total skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal head</td>
<td>±5.1</td>
<td>±8.2</td>
<td>±1.8</td>
<td>±1.1</td>
<td>±10.3</td>
<td>130.1</td>
</tr>
<tr>
<td>Dorso-lat abdomen</td>
<td>28.5</td>
<td>77.7</td>
<td>36.7</td>
<td>25.8</td>
<td>8.7</td>
<td>112.2</td>
</tr>
<tr>
<td>Ventro-lat abdomen</td>
<td>36.5</td>
<td>95.1</td>
<td>38.4</td>
<td>25.0</td>
<td>10.7</td>
<td>130.8</td>
</tr>
<tr>
<td>Mean</td>
<td>±4.6</td>
<td>±8.8</td>
<td>±2.0</td>
<td>±1.8</td>
<td>±12.1</td>
<td>121.4</td>
</tr>
</tbody>
</table>

All non significantly different.

Erythrocyte presence was considered to be an adequate indicator of capillary presence, but did not allow accurate quantification of capillary density and therefore potential skin perfusion. However, none of the regions showed especially high levels of capillarity, so no area was considered to be consistently more heavily vascularised than any other.

The relative numbers and sizes of mucous cells in mudfish epidermis are shown in Table 7.2. A surface view of the mucous cell distribution is shown in Fig. 7.7.
Table 7.2 Mucous cell density and dimensions of superficial mucous cells in the epidermis of three regions of mudfish. Wt= 5.84 ± .63 (12)

<table>
<thead>
<tr>
<th>Body Region</th>
<th>Density #/mm²</th>
<th>Mean cell diameter µm</th>
<th>Mean cell area µm²</th>
<th>% of total skin area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal head</td>
<td>3535 ± 232</td>
<td>7.18 ± .91</td>
<td>192 ± 22.7</td>
<td>67.7 ± 5.6</td>
</tr>
<tr>
<td>Dorso-lateral abdomen</td>
<td>3026 ± 176</td>
<td>7.23 ± .86</td>
<td>164.3 ± 20.0</td>
<td>49.7 * ± 6.5</td>
</tr>
<tr>
<td>Ventro-lateral abdomen</td>
<td>3106 ± 216</td>
<td>6.94 ± .57</td>
<td>151.4 ± 11.4</td>
<td>47.0 * ± 6.7</td>
</tr>
<tr>
<td>Mean values</td>
<td>3223</td>
<td>7.33</td>
<td>169.1</td>
<td>52.7</td>
</tr>
</tbody>
</table>

Significance * p<0.05.

Figure 7.7 Surface of skin from dorsal head region of a mudfish showing staining of superficial mucous cells. x400.
If the cross sections of mucous cells are considered to be circular, as most are, the percentage of the cross sectional area of the epidermis taken up by mucous cells can also be calculated (Table 7.2). The dorsal head surfaces appeared to have both larger and a greater density of mucous cells compared to the dorso-lateral and ventro-lateral areas. However, due to the variability between fish only the calculated total cross-sectional area of mucous cells was significantly greater \((p<0.05)\) than the other regions. The anterior dorsal regions are therefore possibly an area of increased mucus production. The skin of the whole body surface appeared to have a high mucus content with a mean maximum cross-sectional area of mucous cells exceeding 50\%. Since 40\% of the epidermal thickness is also spanned by mucous cells, the total mucous cell contents may represent a major transcellular diffusive pathway through the upper layers of the skin.

Since the skin structure, diffusion distances, capillarity and epidermal composition do not vary greatly between the skin regions studied, the whole external fish surface may be considered as a uniform exchange surface with regard to its exchange functions.

The histochemical staining properties of mudfish mucus and mucous cells indicated that several mucus types were present. With PAS/ alcian blue (pH 2.8) goblet cells stained predominantly dark blue, although some stained pale magenta. Thus, contents were predominantly acidic glycoprotein. The alcian blue (pH 1.0)/alcian yellow (pH 2.8) stains showed an even pale green staining indicating that both sulphated (blue) and carboxylated (yellow) glycoprotein were present. The mucus contents were thus a mixture of acidic mucoid substances with both carboxyl and sulphate groups.

The cellular sculpturing of the skin surface of mudfish is shown in Figures 7.8 and 7.9. Superficial epidermal cells were covered with a
Figure 7.8 Scanning electron micrograph of the dorso-lateral skin surface of a mudfish showing even mucus covering and cellular margins. x5000, bar=4 μm.

Figure 7.9 Scanning electron micrograph of the dorso-lateral skin surface of a mudfish with the mucus removed to show surface sculpturing of epithelial cells. x 5000 bar=4 μm.
thick coating of mucous secretions that obscured all but the cellular margins and pits of the mucous cell openings. With the mucus removed, the epithelial cell surfaces consisted of long interconnected whorls of microridges running longitudinally and in circular patterns. There was no obvious differences in the mucus covering or the surface sculpturing from any of the different skin regions.

Skin areas of mudfish are presented in Table 7.3 and Figure 7.10, and show a relationship of weight (grams) to area (mm$^2$) of $\text{Area} = 830.W^{0.763}$. This is similar to the equation $A = 103.W^{2/3}$ often used in the absence of empirical data (Gray, 1953). Since the exponent is less than 1.0, the ratio of skin area to weight will decrease with increasing weight.

The proportion of skin dry weight to total dry body weight, 6% (Table 7.3), is relatively low and shows that the skin itself does not make up a large proportion of the body mass.

7.3.2 Gills

The general structure of the gill arches, filaments and secondary lamellae and the relationships between them are well shown with scanning electron microscopy (Figures 7.11, 7.12, 7.13). All fish examined had four complete gill arches on each side with large numbers of paired filaments arising from the entire length of each arch. All filaments had variably shaped secondary lamellae arising regularly along the entire length of the filaments. Secondary lamellae occurred as separate plates with no fusion between successive plates or filaments. None of the structural components of the gill appeared visibly reduced or modified in any area relative to the rest of the gills.
Table 7.3 Dry weights of gill and skin tissue and proportion of total body weight, and skin area of 14 Canterbury mudfish.

<table>
<thead>
<tr>
<th>No.</th>
<th>Wet Wt (g)</th>
<th>Dry Wt (g)</th>
<th>Dry Wt % Wet</th>
<th>Skin (mg)</th>
<th>Skin % Total</th>
<th>Gills (mg)</th>
<th>Gills % Total</th>
<th>Skin Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.528</td>
<td>0.091</td>
<td>15.6</td>
<td>9.1</td>
<td>10.00</td>
<td>2.06</td>
<td>2.26</td>
<td>5.55</td>
</tr>
<tr>
<td>2</td>
<td>1.358</td>
<td>0.232</td>
<td>17.1</td>
<td>18.8</td>
<td>7.84</td>
<td>4.14</td>
<td>1.78</td>
<td>9.85</td>
</tr>
<tr>
<td>3</td>
<td>2.180</td>
<td>0.412</td>
<td>18.9</td>
<td>29.6</td>
<td>7.18</td>
<td>3.52</td>
<td>0.85</td>
<td>14.6</td>
</tr>
<tr>
<td>4</td>
<td>2.453</td>
<td>0.583</td>
<td>23.8</td>
<td>37.3</td>
<td>6.39</td>
<td>4.07</td>
<td>0.70</td>
<td>16.5</td>
</tr>
<tr>
<td>5</td>
<td>2.894</td>
<td>0.596</td>
<td>20.6</td>
<td>35.4</td>
<td>5.93</td>
<td>5.52</td>
<td>0.93</td>
<td>17.3</td>
</tr>
<tr>
<td>6</td>
<td>3.259</td>
<td>0.619</td>
<td>19.0</td>
<td>42.5</td>
<td>6.87</td>
<td>7.12</td>
<td>1.15</td>
<td>18.0</td>
</tr>
<tr>
<td>7</td>
<td>4.160</td>
<td>0.846</td>
<td>20.3</td>
<td>50.5</td>
<td>5.97</td>
<td>8.41</td>
<td>0.99</td>
<td>23.6</td>
</tr>
<tr>
<td>8</td>
<td>4.631</td>
<td>0.863</td>
<td>18.6</td>
<td>47.9</td>
<td>5.55</td>
<td>6.87</td>
<td>0.80</td>
<td>26.6</td>
</tr>
<tr>
<td>9</td>
<td>4.788</td>
<td>0.880</td>
<td>18.4</td>
<td>43.2</td>
<td>4.91</td>
<td>8.84</td>
<td>1.01</td>
<td>27.1</td>
</tr>
<tr>
<td>10</td>
<td>5.166</td>
<td>0.926</td>
<td>17.9</td>
<td>58.4</td>
<td>6.31</td>
<td>8.53</td>
<td>0.92</td>
<td>29.1</td>
</tr>
<tr>
<td>11</td>
<td>5.791</td>
<td>1.086</td>
<td>18.4</td>
<td>52.9</td>
<td>4.95</td>
<td>14.05</td>
<td>1.32</td>
<td>28.8</td>
</tr>
<tr>
<td>12</td>
<td>7.102</td>
<td>1.300</td>
<td>18.3</td>
<td>101.2</td>
<td>7.78</td>
<td>16.89</td>
<td>1.30</td>
<td>38.8</td>
</tr>
<tr>
<td>13</td>
<td>7.752</td>
<td>1.379</td>
<td>17.8</td>
<td>65.7</td>
<td>4.76</td>
<td>10.86</td>
<td>0.79</td>
<td>33.0</td>
</tr>
<tr>
<td>14</td>
<td>8.141</td>
<td>1.704</td>
<td>20.9</td>
<td>97.2</td>
<td>5.71</td>
<td>14.40</td>
<td>0.85</td>
<td>40.7</td>
</tr>
</tbody>
</table>

X = 19.0 ± 0.5

Figure 7.10 Log/log plot of the skin area versus wet body weight of 14 Canterbury mudfish.
Figure 7.11 Scanning electron micrograph of the second gill arch of a 6g Canterbury mudfish. x200, bar=400um.

- g - Gill bar
- f - Filament
- S - Secondary lamellae

Figure 7.12 Scanning electron micrograph of the distal portions of gill filaments. x1000, bar=100um.
The epithelial cell surface sculpturing of primary and secondary lamellae is similar to the patterns found in many teleost fish (for review see, Whitear, 1970). The primary lamellae were covered with tightly packed microridges, while the secondary lamellar surfaces were covered with short microridges and microvilli (Figures 7.14, 7.15). Mucus covering was generally very thin and varied over all of the surfaces.

Gill morphometric data (Table 7.4; Appendix 5) show how the components of the gill structure make up the total gill area. Gill area was increased with increasing body size by increasing individual and total filament length, and mean secondary lamella area but with a decrease in number of secondary lamellae per mm. Filament length and mean secondary lamellar areas were also greater on the first and second gill arches and successively reduced on the third and fourth arches.
Figure 7.14  Scanning electron micrograph of the surface sculpturing of the primary gill filament epithelial cells. x 10000. bar = 2 µm.

Figure 7.15  Scanning electron micrograph of the surface sculpturing of the secondary lamella epithelial cells. x 10000. bar = 2 µm.
Table 7.4  Relationship of the different gill dimensions to body weight of the Canterbury mudfish. Values for the intercept (a) and slope (b) for the allometric equation $Y = aW^b$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intercept a</th>
<th>Slope b</th>
<th>Correlation coeffic. r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total filament length</td>
<td>291.5</td>
<td>0.383</td>
<td>0.959</td>
</tr>
<tr>
<td>Secondary lam. /mm (per side)</td>
<td>23.3</td>
<td>-0.108</td>
<td>0.954</td>
</tr>
<tr>
<td>Bilateral area of sec. lamellae</td>
<td>0.0507</td>
<td>0.501</td>
<td>0.950</td>
</tr>
<tr>
<td>Total gill area.</td>
<td>660</td>
<td>0.795</td>
<td>0.955</td>
</tr>
</tbody>
</table>

Fig 7.16 Log/log plot of gill surface area versus wet body weight of 14 Canterbury mudfish.
The relationship of gill area (mm$^2$) to wet body weight (grams) gives an equation of Area = $661. \ W^{0.795}$ (Fig. 7.16). The exponential function is similar to that derived for other fish species (Hughes, 1972) and to that derived for skin surface area. Total gill and skin areas are therefore equivalent over the normal size range of adult mudfish.

The proportion of gill dry weight to total dry body weight shows that the gills, like the skin, are only a small proportion (1.13%) of the total weight of metabolically active tissue (Table 7.3).

The actual secondary lamellar shape, width to height ratios and lamellar spacing may give an indication of the susceptibility of the gill to collapse in air. A range of representative lamellar shapes along a filament are shown in Figure 7.17. The lamellae are roughly triangular in shape with a steeply rising afferent margin, and an efferent margin with a step in an otherwise regular but more gentle slope. The shape of the lamellae resemble those of eels (Byczkowska-Smyk, 1958) more than any other species described (Hughes and Morgan, 1973). Lamellae varied from the distal to proximal ends of a filament with the distal lamellae being smallest, and having the greatest height to width ratio. The central lamellae were the largest followed by a subsequent decrease in size proximally to yield broad and low lamellae. All of the lamellae could be described as triangular rather than trapezoid and height to width ratios ranged from 1.6 - 0.4 ($\bar{x} = 0.92$). This mean ratio is greater than for any of the species presented by Hughes and Morgan, (1973).

Secondary lamellae are thin structures comprising a sheet of capillaries separated by pillar cells and covered with a variable thickness of squamous epithelial cells. The epithelium was generally two cells thick and thinnest directly adjacent to the capillaries. However, the thickness varied greatly. Arithmetic mean blood water distances were $6.3 \pm 3.1 \mu m$, which are similar to distances measured in other aquatic fish species (Hughes and Morgan, 1973).
Figure 7.17 Shapes of secondary lamellar plates along a representative gill filament of a Canterbury mudfish.

Apart from width height ratios, the relative dimensions of mudfish gills were not appreciably different from other aquatic teleost fish species. Therefore, they may not be considered specially adapted for air-breathing or for prevention of gill collapse or coalescence in air.

Gill mucous cells were found predominantly on the afferent margin of the primary lamellae and in the interlamellar space regions. They were never observed on secondary lamellar plates. High densities of mucous cells also covered the gill arch and gill raker epithelium.

The analysis of gill mucus showed dark blue and green staining mucous cells with the two stain combinations respectively. Thus gill
mucus contains both carboxylated and sulphated acid mucopolysaccharides, as in the skin. The mucous coating on the gills and skin may therefore be considered to be made up of the same mucoid substances.

Corrosion casts of gill vasculature showed the arrangements of the gill vessels in relation to one another (Figures 7.18, 7.19). The secondary lamellae had a uniform dense vascular network over the entire lamellar surface except for larger basal and apical margin channels, which were approximately twice the diameter of the inner channels. Spaces corresponding to pillar cells appeared to be randomly arranged across the face of the lamellae rather than in any regular pattern. Efferent and afferent arterioles connecting the lamellae to the efferent and afferent filament arteries were short and set deep into the primary lamellae. Secondary lamellae were also all individually perfused by individual arterioles rather than perfused as groups from one arteriole. The non-respiratory central venous sinus circulation did not cast in the preparations (Fig. 7.19).

7.3.3 Buccal epithelium

The structure of the buccal surfaces in the Canterbury mudfish are different to that of the external body skin (Figure 7.20, 7.21). The epidermis was irregular in form and thickness. The stratum germinativum layer had much shorter columnar cells or in some areas was composed completely of polygonal cells. The middle layer was variable in thickness and was composed of an assemblage of polygonal cells which were similar to those in the external skin. In the upper layer, a single layer of small spherical mucous cells were sparsely distributed. The surface of the epithelium was undulating with variable thicknesses of mucus and accumulating debris adhering to it. Epidermal thickness varied from 32 to 60μm (x = 43 ± 6.2μm). The dermis varied greatly in
Figure 7.18 Scanning electron micrograph of corrosion casts of gill filaments. Note secondary lamellae but lack of filling of central venous sinus. x200. bar=100um

a - Afferent filament artery
l - Lamellar arteriole
m - Marginal channel

Figure 7.19 Scanning electron micrograph of corrosion cast of secondary lamellae and connections to afferent filament arteries. x500 bar=40um.
thickness depending on the epithelial region and underlying material (16 to 120µm). Capillary density was also very variable both between fish and different areas. A high capillary density could be described in some fish in the dorsal regions although this was variable and hard to quantify.

None of the areas of the buccal epithelium appeared to be structurally specialised for respiratory or regulatory functions. There were no areas of decreased epidermal thickness or mechanisms to increase functional surface area.

The inner opercular epidermis was much thinner than the outer epidermis (32 ± 5 compared to 85 ± 8 µm) and possessed numerous capillaries, so may contribute to the overall respiratory surface area when the opercula are inflated. Opercular epidermis was otherwise structurally very similar to external skin but reduced in total thickness.

7.3.4 Other structures

The swimbladder in mudfish is a physostomous structure with a 15-20mm long narrow connection to the anterior dorsal region of the stomach. The length and diameter of the conduit may limit the swimbladder from functioning in any respiratory capacity. Also, the swimbladder is an extensive thin walled structure with no obviously visible vascularised regions. It is therefore unlikely that the swimbladder has an appreciable function in respiratory gas exchange.
Figure 7.20 Transverse section of buccal surfaces of the Canterbury mudfish. Goldner's stain. x40

C - Cartilage
b - Buccal cavity
e - Epidermis
d - Dermis
g - Goblet cells

Figure 7.21 Transverse section of buccal epidermis in ventral floor of the buccal cavity. Goldner's stain, x400.
DISCUSSION

7.4.1 Skin

The skin of the mudfish resembles that of many other aquatic and amphibious fish species. In recent years, the fine structure of the skin of many species of fish has been described. These have included both marine and freshwater species from widely divergent habitats (Roberts, 1971; Whitear, 1970; Bullock and Roberts, 1975). They have also included several species of air-breathing and amphibious species (Liem, 1967a; Mittal and Munshi, 1970, 1971). The majority of these species possess a mucogenic epidermis with a diversity of mucus producing cells, a metabolically active outer layer of cells, and a mucoid extracellular coat or cuticle. Recently, however, several intertidal fish species have been shown to have keratinised rather than mucogenic skin, with areas of dead cornified cells in the outer epidermis (Mittal and Banerjee, 1980). The Canterbury mudfish possesses a well developed mucogenic epidermis with no areas of keratinization. The mucous cell proliferation therefore appears central to the structure and function of the skin of mudfish.

The thickness of the epidermis for most of the more recently studied teleost species, range from 34μm to over 500μm but is usually in the range of 100-200μm. The epidermis ranges from 2-3 cells thick (in the guppy) to a more common 7-12 cells thickness (Henrickson and Matoltsy, 1968a; Liem, 1967a; Mittal and Munshi, 1971; Roberts, 1971). The total thickness and cell density of mudfish epidermis is therefore comparable with the majority of teleosts. Since the epidermal thickness of mudfish may be considered 'normal', the permeability of the epidermis might be expected to be similar to other fish species.

The actual structure of the mucogenic teleost epidermis is very similar throughout all groups. Most species possess a columnar germinal layer as in mudfish but several species also possess a cuboidal basal
germinal layer. However, the morphology of the germinal cells does not correlate with their habitat as both occur in different air-breathing and cutaneous respiring fish (Mittal and Munshi, 1971).

Even though they have been called by such a diversity of names the polygonal, cuboidal or filament containing cells that make up the bulk of the epidermis are similar throughout all teleost groups. They usually contain filaments and are attached by tight junctional desmosomes in the upper layers. They are usually regarded as the elements that restrict skin permeability. At the outer surface these may become squamous, semi-squamous or remain essentially polygonal. The form of these cells at the surface, similarly does not appear to correlate with fish habitat or skin function (Mittal and Munshi, 1971). The polygonal cells of the mudfish epidermis do not deviate from usual patterns or indicate any special arrangement or function.

The unicellular mucous or slime cells in fish take several forms. These are flask or pear shaped 'goblet' or 'mucous' cells containing glycoproteins, but club cells (= giant cells) and sacciform granulated cells also occur (Henrickson and Matoltsy, 1968b,c; Mittal and Banerjee, 1980). The latter two are also often referred to as serous or albuminous cells. While goblet cells are the most commonly described slime cell, the club and sacciform cells are frequently described in amphibious species (Mittal and Munshi, 1971) but may be absent in other amphibious species (Liem, 1967a). Their function is not well understood and contents may be very variable from proteinaceous and lipoid compounds to carbohydrates and glycoproteins (Roberts and Bullock, 1980).

Mudfish only possess the goblet type mucous cell. The secretory content of mudfish mucus may therefore be limited in composition relative to the fishes possessing a range of slime cell types. Since the functions of the other slime cell types are poorly understood, the implications of
the lack of them in mudfish is unknown. The mudfish mucous cells were smaller than those found in many other species but more numerous. However, they appear very similar in general structure and position to those found in all fish species.

The dermis is similar to that described in other species. Jakubowski (1958, 1960) described ratios of epidermal to dermal thicknesses in three teleost species and related these to skin function. He found that the loach had an epidermis to dermis ratio greater than 1.0 while the eel and blenny had a ratio much less than 1.0. He related the dermal thickness to its protective function and the presence of underlying bone and scales. Since the mudfish is scaleless, the dermal layers might be expected to be greatly developed. However, in mudfish the dermis is only one third the thickness of the epidermis so the correlation does not appear to hold.

In general, all of the skin areas retained a similar structure and total thickness. Thus, all areas may have similar diffusive permeability. Several other studies have shown the whole body surface of fishes to be relatively uniform in thickness (Jakubowski, 1958, 1960; Pickering, 1974). In water there may be little advantage in differential thickening of different surfaces. However, in air, changes in skin structure or thickness could be advantageous. There are well described differences in dorsal and ventral skin thickness in the Amphibia (Feder and Burggren, 1985), but such an arrangement has not been developed in any of the air-breathing fish so far examined.

Since neither the skin structure nor dimensions were unusual relative to other species, investigation of the ultrastructure of skin was not undertaken. For descriptions of teleost skin ultrastructure, reference to Henrickson and Matoltsy (1968a,b,c) and Hawkes (1974) are recommended.
Fish scales are an inert inorganic layer, so may be relatively impermeable and not allow the unrestricted passage of substances through the skin. In most fish they lie as overlapping plates and appear to form a complete barrier between the inside and outside of the fish (van Oosten, 1957). Amphibious scaled fish may, however, possess regular arrangements of dermal capillaries adjacent to the overlap or scale margins, or have deep set scales with overlying capillaries (Mittal and Munshi, 1971). Mudfish are however, a member of a scaleless family of fishes. The complete lack of scales is not unique and occurs in several groups of fish. Also, many scaled fish possess scaleless areas that may retain more permeable properties (Marshall, 1977), or vestigial scales that may have little effect on skin permeability (Pankhurst and Lythgoe, 1982).

Rahn and Howell (1976) suggested that scalelessness and skin breathing were precursors of the primitive adaptations that enabled fish and amphibians to leave the water. In a simplified scheme, possession of a permeable skin was considered a first step that allowed CO₂ excretion and therefore acid/base regulation in air where gills may collapse and be functionally lost. However, desiccation effects still left fish with a reliance on water. Later, specialised air breathing organs took over O₂ uptake. CO₂ excretion from primitive lungs was accompanied by loss of cutaneous permeability in the higher vertebrates. This scheme holds well for amphibians, but many fish have developed airbreathing organs without development of skin respiration, due to a stimulus of hypoxia avoidance rather than for amphibiousness. The mudfish is only at the lower end of this evolutionary scheme towards amphibiousness possessing the first step with cutaneous permeability and cutaneous mechanisms predominating.

There have been many descriptions of the modifications of the skin of fish species for respiratory functions. These range from areas of
reduced thickness, invaginations of the dermis into the epidermis to allow closer capillary contact and capillary loops flowing close to the outer surface (Jakubowski, 1958, 1960; Mittal and Munshi, 1971; Schottle, 1932). It is surprising that none of these modifications are found in the mudfish, considering that the skin appears to have so many physiological functions. On closer scrutiny, the species possessing these various modifications are almost exclusively marine or euryhaline species where cutaneous permeability may not present as great an osmotic and ionic problem as in freshwater habitats.

The mudfish, being small, has a relatively large skin area with the potential for significant transcutaneous fluxes. The total epidermal thickness and permeability might therefore be structured as a compromise, balancing detrimental against beneficial exchange fluxes. The greatest detrimental losses might be ion and water fluxes.

There does not appear to be any region that, from a capillarity viewpoint, could be considered more permeable than others. However, the estimate of capillarity was only subjective and therefore may prove only that all areas were perfused to some extent and therefore all areas may potentially contribute to cutaneous gas exchange.

The relative perfusion of the different areas of fish skin have not been extensively studied. Jakubowski (1958, 1960) has presented capillary densities and origins of dermal capillaries in three fish species. In the species he studied, the blood supply arose from lateral and dorsal segmental vessels and intercostal vessels and formed a diffuse capillary network. Capillary density did not vary greatly over the entire body surface but did vary significantly between species. He presented data showing that some species possessed capillary densities greater than many amphibians but also possessed much greater epidermal thicknesses. He emphasised the uniformity of capillary distribution over
the whole surface.

Distribution of cutaneous blood flow in amphibians has been studied more extensively and more recently. Moalli et al (1980) studied the variability of the perfusion of the different skin areas of the frog *Rana catesbeiana* and found blood flow was from two sources and varied over the body in source and density of perfusion. Blood flow was from both a cutaneous arterial supply from the pulmocutaneous trunk and a diffuse systemic arterial supply from the skeletal muscle blood supply. While the latter supply was relatively evenly distributed the former varied in different regions and was more highly regulated. However, the pulmocutaneous trunk is a feature of amphibians and has not been developed in teleost fish. Therefore, only the relatively even and lesser regulated supply is analagous to fish.

Mosse (1980) described the blood supply of the trunk musculature of the flathead and branches of the lateral line arteries that gave rise directly or by successive branching to capillaries at the interface between red muscle and the skin. Extensive skin circulation may arise from such existing circulatory arrangements. Systemic vessels such as these would arise from the dorsal aorta and be connected in series with the gills and would be supplied with arterial blood. Such an arrangement emphasises that mudfish aerial respiration is not 'bimodal' as there can be no shunting of blood away from gills to 'other' gas exchange organs. A diffuse systemic origin of cutaneous vessels would also explain the regular distribution over the whole body surface of fish. Davidson (1949) also found diffuse dermal capillaries in the skin of *Neochanna apoda* but no obvious direct vascular connection to them. Her finding suggests they may be derived from a general systemic source such as that described by Jakubowski (1960), and Mosse (1980) rather than any major vascular modification. Such a supply would therefore be expected to encompass most regions of the fish body and be relatively uniform.
If skin permeability and perfusion are considered uniform then there would be no advantage in exposing specific skin areas during emersion. Therefore, the changed orientations and rolling behaviours of emersed mudfish, previously described, are unlikely to be mechanisms to expose differently perfused areas and the alternative explanation of tissue hydration and exchange, or excretion of metabolites appears more reasonable.

The distribution and relative density of mucous cells in mudfish is high compared to most other fish and supports the argument that, with the scaleless condition, increased mucus concentrations gain a protective function. Certainly mucus appears to be central to many of the abilities of mudfish to cope in air and to prevent abrasion and damage in its aquatic habitat.

The quantification of mucous cell parameters has been broadly surveyed in a few fish species. Studies have predominantly been carried out on salmonid species. Investigations include general histochemical analysis (Harris et al., 1973), mucous cell distribution throughout different skin areas (Pickering, 1974), changes with season (Pickering, 1977), changes with growth and development (Blackstock and Pickering, 1974), the effects of handling (Pickering and Macey, 1977), and the effects of salinity transfer (Solanki and Benjamin, 1982).

The immediate observation of mudfish mucous cells is that they are on average 5–7 times more numerous than any of the regions of trout epidermis. The mucous cell diameters are, however, smaller than in trout (Pickering, 1977). This reflects a 2–3 fold increase in epidermal mucus contents relative to trout. This is not surprising considering the initial proposal was that increased mucus density was a strategy to allow for the scaleless condition. The body distribution of mucous cell density in mudfish is similar to Pickering's (1974) finding of increased
concentrations anteriorly and dorsally. Therefore, increased anterior-dorsal mucus production allows more mucus to spread back over the body surface under the effects of water flow and abrasion. Other species generally have larger mucous cells than the mudfish (Mittal et al., 1980; Mittal and Munshi, 1970). The size and distribution of mucous cells are, however, very variable between species and groups so the measure of crossectional area may be the most valid comparison of mucus density. It is the overall production of mucus rather than the method of production and delivery that is of the greatest importance to the physiology of the fish. Analysis of size and density serve to describe the strategy of mucus production of the species which can be of interest in comparative studies. Mudfish are therefore highly mucoid, with high concentrations of small mucous cells.

The density of mucous cells in the epidermis means that the outer epidermis may function as a thick mucoid mass for over a third of the epidermal thickness. Gases and water will diffuse through mucus almost as readily as through water so this component of the skin would not represent a significant diffusive barrier. Charged particles and larger compounds may diffuse slowly or be bound within the matrix of the mucus molecules. This is due to the viscosity of mucus making it act as an unstirred layer and charge densities on the mucus components binding or inhibiting ion movement. The degree of disruption of ionic movements is controversial, as by virtue of its polyionic nature it will tend to concentrate cations (Kirschner (1978), but Marshall (1978) demonstrated in vitro that the ability of mucus to impede ion diffusion was not impressive and that it had permeation characteristics similar to water. Shephard (1982) has shown, in oesophageal epithelium, that the mucus layer acts as an unstirred layer and supports a stable portion of the transepithelial ionic gradient. Therefore, this layer could be a significant selectively permeable barrier to exchanges through the
mudfish skin and increased concentrations would enhance the effects. The degree to which the mucous cells will aid diffusion will depend upon the major diffusive pathway through the epidermis. Compounds may move through transcellular or paracellular routes. Compounds moving transcellularly through the cell cytoplasm will have less cell membranes to cross if diverted through the mucous cell contents. Compounds moving by the paracellular route, between cells, may be little affected by mucous cell density. If, however, they can cross cell membranes then the mucous cell contents would be a route through the dense array of desmosomes connecting the outer array of epithelial cells. The mucous cells may, therefore, short circuit many of the permeability reducing features of skin. The greater the concentration of mucous cells the greater this effect would be.

The enhanced mucous concentration will also enhance the other functions proposed for fish skin mucus. These include chemical and immuno-chemical reactions, lubricating properties, protective roles and osmoregulatory roles (Mittal and Banerjee, 1980). The composition and quantity of mucus is therefore selected for on many grounds and any change in composition or quantity would have to be compatible with all of the above mentioned functions. The implications of the high mucus density to emersion in mudfish involve its action as a barrier, initially isolating the fish from the environment. Mucus is hygroscopic so would hold a store of water and would therefore maintain a relatively thick moist layer. This layer is crucial to cutaneous respiration and exchange processes. However, the hygroscopic nature of mucus would, under desiccatory conditions, draw water from the body as it was lost from the surface mucus layer. This was seen in a previous chapter. It may also have a role in drawing and trapping water from the ground in a moist microhabitat. These water balance functions are the major factors in air, but many of the other factors such as abrasion resistance and as a
pathogen barrier are of undiminished importance in air.

The mucus composition of goblet cells and surface mucus may determine the effectiveness of some of the properties discussed above. The types of mucoid fractions in fish mucus vary. Predominant mucus fractions in teleost fish tend to be highly acidic sulphated glycoprotein but weakly acidic sulphated, neutral, and carboxylated glycoprotein have been described (Mittal and Banerjee, 1980). Sulphated glycoprotein might also be expected to be most common in freshwater fish due to the high charge density of this form and therefore its probable greater effectiveness in reducing ion movements. Families of mucous cells containing different mucus types have also been described within individuals so generalisations are far from clear.

Roberts and Bullock (1980) subdivided three groups of marine fishes on predominant glycoprotein type and related this to their need for mucus and mucus function at different depths in the ocean. Blackstock and Pickering (1974) found the alevin and fry stages of brown trout changed from predominantly sulphated mucins to carboxylated mucins as they changed from a benthic to the free swimming fry stage. This was also accompanied by a reduction in mucous cell densities and reflected the necessary degree of a protective mucus covering in benthic habitats. A decrease in the proportion of carboxylated mucins was also noted in larval plaice as they made the opposite transition from being pelagic larvae to benthic adults (Roberts et al., 1973). These trends led Blackstock and Pickering to suggest that there was a correlation between sulphated mucins and benthic habitats, and carboxylated mucins and a pelagic or free swimming habit. This is supported in several recent studies of fish mucus histochemistry. The benthic or burrowing fishes, Heteropneustus fossilis, Amphiopnous cuchia, Mastacembelus panculas
Mittal and Munshi, 1971), *Pleuronectes platessa* (Roberts et al., 1973), *Channa striata* (Mittal and Banerjee, 1975), *Notopterus notopterus* (Mittal and Banerjee, 1974), *Monopterus cuchia* (Mittal and Agarwal, 1977), and *Anguilla anguilla* (Archer, 1979; cited in Blackstock and Pickering, 1974) all have predominantly sulphated mucins. Many of these benthic species are also air-breathing or amphibious species, so the correlation cannot be easily related to amphibious habits alone. Examples of free swimming fish with carboxylated mucins include most of the salmonid species (Harris and Hunt, 1973; Harris et al., 1973; Pickering and Macey, 1977).

The predominant mucus fractions in mudfish, a mixture of highly acidic sulphated and carboxylated glycoproteins do not allow the mudfish to be placed in either group, but may be considered more generally adapted for either habitat. The finding of occasional weakly acidic mucous cells may be related more to the development of mucous cells. Since immature mucous cells were not obvious in the middle layers of the epidermis, they may develop close to the surface. Thus, immature mucous cells may stain differentially as mucus develops within them. The mucus types produced may be due to aquatic needs for osmoregulation and protection within their habitat, with the composition not greatly modified for amphibious conditions. The mucus components of aquatic species may therefore be adequate in dual aquatic and terrestrial situations and not require selection for a change in composition. Since the greatest function of mucus in air is the retention of water to maintain a moist surface, most glycoproteins could fulfil this function as they are all large hygroscopic macromolecules. If the mudfish were to rely on development of cocoons or other such structures, changes in mucoid fraction may indeed be necessary. Therefore, the mucus histochemistry supports the general trend that the mudfish does not need to change and is little modified for its terrestrial survival.
The uniform and unspecialised nature of mudfish skin is also born out by the appearance of the epithelial surface sculpturing. The skin surface is the first major barrier to invasion of the fish. It might therefore be expected to reflect any changes in skin function. Also, abrasion on land may be more extreme than in water so added mucus retention may be selected for. However, epithelial sculpturing is similar in all of the skin areas studied and to studies of other aquatic and amphibious fish species (Hawkes, 1974). Therefore, the existing aquatic sculpturing patterns are also adequate for amphibious survival.

Skin areas gave a power function for the variation of area with size that is comparable with other fish (Gray, 1953; Hughes and Munshi, 1979). The areas are more valuable as a comparison of relative respiratory surface areas within and between individual fish and fish species. Unfortunately, few studies have concentrated on the skin area of small fish species comparable to mudfish. Schottle (1932) obtained values of skin area similar to the mudfish from several species of amphibious gobiid fishes. These showed gill and skin areas were similar in these species, a feature also seen in the mudfish. Other species studied are generally much larger and therefore have unfavourable ratios of skin to gill area. Gray (1953) presented gill to skin ratios of up to 28:1 for different fish species and sizes. Therefore, this again shows the adaptiveness of small size in skin respiration efficiency.

Apart from aded mucification of skin and the scaleless condition, the skin of the mudfish appears unmodified for skin respiration and terrestrial habits. Thus, the basic structure of Teleost skin is adequate for the exchange mechanisms utilised by mudfish but are limited to within the constraints of small body size and overall permeability.
7.4.2 Gills

The gills of the mudfish, like the skin, appear to be relatively unspecialised for an aerial respiratory function, with no reductions or modifications to the basic teleost gill pattern. A common adaptation of air breathing fish is reduction of the fourth gill arch to form either an accessory breathing organ (Munshi, 1976), a blood shunt to bypass the gills (Satchell, 1976) or a general reduction of gill area to restrict gill collapse (Hughes, 1972). Schottle (1932) observed that *Periopthalmus schlosseri* had the first gill arch supporting a fold of vascularised epithelium. It, therefore, only had three functional pairs of gill arches. More simple adaptations to gill function in air are seen in many marine amphibious species and generally lead to a reduction in gill area. These normally include; increased lamellar spacing on the filament, increased lamellar thickness and changes in lamellar shape. The extent of the reduction can be correlated with the air breathing and amphibious ability (Graham, 1976). Adaptations which occur to prevent lamellae from collapsing have also included raised marginal ridges on the lamellae (Schmidt, 1942) and fusing of lamellae (Daxboeck et al, 1981). The possible modifications of gill vasculature for air breathing are, therefore, many and varied.

In mudfish, the fourth gill arch was not greatly reduced relative to the other arches and all arches contained full complements of primary and secondary lamellae. Lamellar spacing was comparable to other aquatic species so was not appreciably reduced. None of the previously described adaptations are, therefore, apparent in mudfish.

Total gill area calculated from morphometric data gave comparable gill areas to other teleost fish species (Hughes, 1972; Hughes and Morgan, 1973). The method by which the gill area increased with body weight was also similar to other fish species. Most of the structural components making up the gill area increased with body weight to yield
the increased gill area. Only lamellar density per mm decreased but this trend is found with most other species (Hughes, 1972). Therefore, there was no size related tradeoff of any component of gill structure (Hughes, 1972).

Comparison of gill and skin areas show that both surfaces are similar in total area in adult mudfish. The size at which areas of gill and skin of mudfish are equal is about 5g or 100mmTL. Below this size, skin areas may slowly exceed gill areas. Schottle (1932) presented data for comparable gill and skin areas in a range of marine amphibious species. She found that ratios (gill:skin) varied from slightly greater than one in primarily aquatic species to 0.7 and 0.35 in successively amphibious species. Since body form was similar, the change was due to successive reduction of gill area. Graham (1976) similarly described several studies where successive reduction in gill area correlated with air-breathing or amphibious habits. In this scheme, with a ratio of approximately 1.0, mudfish would be considered primarily aquatic with little adaptation to amphibiousness. It has also been suggested that the reduction in gill area correlates with an increase in development of vascularised buccopharyngeal or opercular epithelium (Graham, 1976). Possession of an unreduced gill area, comparable to skin area, may therefore suggest that buccopharyngeal vascular development in mudfish may not be very advanced. However, the mudfish do have amphibious abilities.

Although similar in total area, the gills and skin differ in their relative diffusion distances (6.8 and 96 μm respectively) to the air or water currents flowing over them. Whereas the gills are actively ventilated, having both a rapid flow across them and being set up for countercurrent exchange (Piiper, 1982), the skin has little flow over it and must passively exchange with the environment. The only fish showing
a deviation from this simple situation is larval *Monopterus albus* which Liem (1980) showed to direct a current of water across the body in an opposite direction to blood flow. Thus the whole body would function as a large simple gill. This is, however, unusual or unique in fish. Therefore, in air, skin ventilation is negligible and may not be interrupted.

The gills lose their efficient countercurrent exchange but are still ventilated by tidal breaths. They also have a much greater capillary density than the skin to carry and remove gases or metabolites and may therefore maintain higher diffusion gradients. Therefore, although gill and skin areas are similar, the gills may be a much more efficient exchange surface even in air and with variable ventilation parameters, the efficiency of the gill exchange can be increased and regulated.

The question of gill collapse and decrease in respiratory areas of the gill when exposed to air has been widely speculated on in teleost fish (Hughes, 1966; Steen, 1970). Although most species are thought to functionally lose most of their gill area, even the carp— a species not noted for its survival out of water— can derive significant amounts of oxygen from gulping air across its gills (Burggren, 1982). Therefore it is the degree of lamellar coalescence and the corresponding area loss, and not whether or not the gills collapse, that is important to consider. When large mudfish were breathing air the first and second, and third and fourth gill arches could sometimes be seen to lie close together against the cranial and caudal surfaces of the opercular cavity respectively, with a large air space between them. The pairs of gill arches appeared to have collapsed together but the functional loss of area could not be determined.

The triangular shaped secondary lamellae of mudfish cannot be
considered less prone to collapse than those of other species because they have a greater height to width ratio than other species. Therefore they might be expected to collapse more readily due to surface tension and gravitational effects. The trapezoid shaped and low broad lamellae of many active aquatic species could be argued to be less prone to collapse (Hughes, 1972). Similarly, lamellar spacing is not unusual relative to aquatic species. Mudfish gills are therefore not considered to be in any way specifically adapted for air breathing. However, the species with the greatest similarity to mudfish gill structures is the eel (Anguilla) (Byczkowska-Smyk, 1958), a fish also relatively unspecialised but able to survive in air and to pass air over its gills. Therefore, although the potential for gill collapse appears great, the similarity to eel gill structure may mean either that some other factor has not been considered or that both species can cope with functionally losing large gill areas in air.

Mucus secretions may play a role in the support of gills in air. Solanki and Benjamin (1982) have shown that many factors such as salinity and stress may affect production of gill and buccal mucus and mucous cells. Although copious quantities of mucus were seen to be lost from the opercular apertures when air exposed fish were reimmersed, gills of air exposed fish did not appear to have increased numbers of mucous cells or mucus secretion when dissected gill arches were examined. Gills from two air-exposed fish were indistinguishable from aquatic fish gills when examined in whole mounts and with SEM. Even so, mucous layers may keep gill surfaces moist and may have a role in positioning of gills in air and preventing them from drying out. The mucus secretion also aids the sealing of the margin of the opercula. This allows tidal ventilation and would therefore also restrict ventilatory desiccation rate as humid air is retained in the cavities. The mucus loss from the opercula after reimmersion may be from buccal and gillraker surfaces rather than the
gills as these surfaces may increase secretion on emersion rather than the gill filament mucous cells.

The mucus secreted around the gills is essentially similar to the skin mucus, being acidic sulphated and carboxylated glycoproteins. Thus, its primary function is probably similar to that of the skin in ion trapping and aquatic roles. Therefore, gill mucus does not appear to have undergone any adaptations or changes for air breathing.

The surface sculpturing of gill surfaces is similarly unchanged from that of other teleosts, indicating retention of the basic aquatic architecture of gill surfaces (Hawkes, 1974; Hughes, 1979). Therefore there are no obvious mechanisms to allow increased adhesion of mucus to gills during air-breathing.

Histological sections and corrosion casts showed that secondary lamellae are structured similarly to those of other species, being a sheet of interconnected channels. The enlarged inner and outer capillaries may have distinct advantages as blood shunts and for hydrostatic rigidity respectively, but are seen in most fish species. Tuurala et al (1984) discussed the use of the large basal channel in trout as an 'intralamellar non-respiratory pathway' or shunt to regulate gill surface perfusion. In the absence of any other shunts or gill bypasses, such a function may be important in mudfish during exposure to hypoxic water.

Although Munshi (1960) and Munshi and Singh (1968) have described evidence for alignment of pillar cells in air breathing fishes, the arrangement of pillar cells in mudfish lamellae appear completely random. Alignment of pillar cells across the lamellar was considered to aid laminar flow and therefore lower gill resistance and increase efficiency. There was also no evidence for vascular shunts to bypass the gills,
therefore, the gills would appear to be perfused in all conditions. Thus the mudfish cannot be described as a bimodal or trimodal breather as the gas exchange surfaces are in series. Also, the blood to water distance of mudfish gills falls in the middle of the range of published values for aquatic fish species (Hughes, 1972). Several amphibious species such as the climbing catfish (Hughes, 1966) show greatly increased blood to water distances associated with air-breathing habits. Clearly mudfish have not developed any of these adaptations for air-breathing.

Overall there is no departure in mudfish gill architecture from the usual teleost gill architecture. The lack of modification may either indicate the lack of importance of the gills in air breathing in mudfish or that the degree of collapse and loss of area are either not significant or not great enough to limit respiratory efficiency. The lack of any specific adaptations is consistent with all other findings for mudfish.

7.4.3 Buccal and opercular surfaces

The buccal and opercular epithelia are the only other surfaces that appear to have any appreciable respiratory function. Like the skin and gills they do not appear to be modified for this function other than in the possession of dermal capillaries. However, the buccal epithelium is very similar to that of Monopterus albus (Liem, 1967a), a fish described as breathing via a buccopharyngeal organ. The surface areas of these surfaces were not measured but would be low compared to the gill or skin areas since there are no structures to increase functional surface area as in other fish (Munshi, 1976). The diffusion distance across these epithelia are variable but consistently less than the skin and are much greater than the gill. Therefore, these surfaces may be more efficient at gas exchange than the skin due to the lower diffusion
distance, the active ventilation of them preventing unstimred layers and maintaining high partial pressures of oxygen, and the source of blood supply to them. Whereas the skin may be perfused by general systemic circulation arising from muscle blood supplies, Davidson (1949) described anastomoses of capillaries in the buccal regions arising from the external carotid arteries and draining via the superior jugular veins. She described:

"The main anterior branch of the external carotid has subdivided extensively in the pre-orbital region. Post-orbitally, the external carotid gives off a branch which anastomoses on the oral membrane beneath the adductor arcus palatini. In the branchial region the third efferent branchial artery gives off several small arteries which anastomose over the epiarccualia transversi and the underlying dorsal branchial epithelium. The posterior ventral ends of the efferent branchial arteries anastomose on the ventral branchial epithelium.

The superior jugular vein, ventrally usually, has in Neochanna a dorsal branch also, the two converging post-orbitally to form the main superior jugular. The dorsal and ventral superior jugular veins return blood from the anastomoses of their anterior tributaries on the oral membrane and post-orbitally, the dorsal branchial membrane. There is an anastomosis, not extensive, of tributaries of the inferior jugular vein on the ventral branchial epithelium."

Perfusion of the epithelia from these sources would allow greater capillary densities and total blood flow rates than in skin due to higher vascular pressures and greater potential for regulation of perfusion. Although this degree of capillarity was not observed in sections from Canterbury mudfish, similar circulation is probably present due to the similarities and close relationship between the Neochanna species. Opercular epithelium may add to total respiratory surface areas as it is a ventilated surface. It is of similar structure to external skin but is of much reduced thickness and does have a degree of capillarity that would indicate an exchange function. Graham (1976) noted several authors who have described vascularised areas on the opercular walls of gobiid species. As with buccal surfaces, capillaries arose from existing vessels of the head.
The buccal, opercular and gill surfaces may therefore all retain gas exchange functions in mudfish. Oral exchanges are a combination of exchanges from a series of surfaces but the relative roles of them cannot be separated individually. As a group they are effective at meeting the oxygen requirements of mudfish even though the level of development does not appear very great.

The swimbladder has been described as an accessory gas exchange organ in several air-breathing fish (Carter and Beadle, 1931b; Munshi, 1976), but from its structure, orientation and from fish air breathing behaviour it is unlikely to have a respiratory function in the Canterbury mudfish. This was also the conclusion of Davidson (1949).

Although the mudfish has a variety of surfaces which it uses for excretion and exchange of gases, it does not appear to have had to compromise the structure of any of them to increase aerial efficiency. Therefore, all of the surfaces retain their original structure and function well in both air and water. This feature is central to the physiology of air exposure in the mudfish as it means the fish does not have to switch from one organ system to another during acclimatisation to the new medium with the complications this would involve. The lack of change is reflected in the lower efficiency of the systems in air which limits the scope for activity relative to other more highly adapted species. The strategy of using an array of unmodified surfaces is only really feasible in small fish species that have a high surface area to volume ratio. Small size may therefore be considered adaptive and the main feature allowing terrestrial survival from a structural viewpoint.
CHAPTER 8

GENERAL DISCUSSION

There are a number of potential problems facing a gill breathing aquatic animal when it moves onto land. These include: movement without the support of water; sensory perception in a different medium and maintenance of metabolic rate without further feeding. One of the most potentially serious problems is the passive loss of body fluids across permeable surfaces. Being small fish with a high surface area to volume ratio, mudfish might seem to be potentially vulnerable to desiccation, particularly as there is no evidence that they depress their metabolic rate. However, in exploiting the thin skin as a significant gas exchange organ they place less reliance on gills for oxygen uptake. Reducing gill ventilation presumably reduces water loss from that source.

Use of a permeable skin appears to be one of the primary mechanisms enabling survival in air. Skin permeability has been approached from the cutaneous respiration viewpoint and although predominantly discussed from this stance here, most points apply equally well to the exchanges of other substances through the skin.

Cutaneous respiration and the design limitations of utilising a strategy of substantial cutaneous exchange have been reviewed by Feder and Burggren (1985). They discussed the limitations of cutaneous respiration, noting it to be highly diffusion limited and a relatively inefficient mechanism, which is nevertheless widespread in many vertebrate groups. In fish, it is widespread in both adult and larval stages, in many groups and in fish from widely differing habitats. Most air-breathing fish are now routinely assumed to possess some cutaneous respiratory component but it is seldom quantified. Feder and Burggren presented six categories of reliance on skin respiration which form a
continuum for reliance on cutaneous respiration. These are:

1. Negligible role in gas exchange.
2. Meets the needs of the skin and superficial tissues.
3. Accessory gas exchange under special circumstances.
4. Minor role in respiratory requirement.
5. Primary gas exchanger.

Kirsch and Nonotte (1977), Nonotte and Kirsch (1978) and Nonotte (1981) showed that many freshwater and marine fish show significant skin respiration, but few support a stable transcutaneous gas transfer, as in category 2 above. However, oxygen uptake of skin was assessed in vitro so its overestimation would mask any transcutaneous uptake. In the several species which do show transcutaneous oxygen uptake, this cannot be described as being greater than category 4 in any fish other than the larval Nonopterus albus (Liem, 1980) which may be a unique case.

The cutaneous respiratory component of mudfish was not shown to include transcutaneous flux, but from the high oxygen uptake and low skin mass the exchange was considered to be far beyond possible skin usage alone. The level of cutaneous ammonia excretion also suggests significant transcutaneous fluxes. In the mudfish, cutaneous respiration is not considered the major gas exchanger, as its proportional contribution will be limited at high temperatures, when the balance of oxygen demand must be supplied through the gill. However, at low temperatures the skin may be the major gas exchange organ.

Cutaneous respiration has been extensively documented in the amphibia, with many groups relying on the skin as the primary or even sole gas exchange organ. However, the amphibia possess specialised cutaneous blood supplies with potentially high capacitance deoxygenated blood being supplied to the skin. They have essentially two separate
circulations to skin including a general systemic and a pulmocutaneous supply that may be regionally different in perfusion and density of capillarity (Moalli et al, 1980). They also have active transport mechanisms in the skin and different skin structure from fish. They may therefore, be considered considerably different from fish.

Feder and Burggren suggested that the effectiveness of cutaneous respiration can be viewed from either an evolutionary or a functional viewpoint and that this distinction must be made when asking questions of the efficiency of cutaneous systems. From a functional viewpoint cutaneous gas exchange can be considered severely diffusion limited due to skin thickness. There is little scope for regulation via ventilation or perfusion control and it is also limited by the origin of the blood flow to the skin, especially in fish where cutaneous blood flow is essentially from the dorsal aorta. Thus, cutaneous exchanges may rapidly reach a ceiling level of effectiveness. Feder and Burggren suggested that cutaneous respiration should only be found in small animals, at low temperatures, low metabolic rates and in ideal conditions such as in normoxia. It may also only be effective as a supplementary gas exchange mechanism. The mudfish aptly fits most of these predictions except for the ideality of conditions in its environment.

From an evolutionary viewpoint, the functional effectiveness of cutaneous respiration is not examined. It must only be shown to be adequate with little cost to the animal. The first point is clearly demonstrated in the diversity of vertebrates that possess it. Secondly, ventilatory expenditure is absent or minimal and so long as other functions are not compromised there is no real cost to utilising cutaneous respiration. In the mudfish the use of cutaneous exchanges to cope with other emersion problems, such as ammonia excretion, shows that the skin is adequate for more than just respiration. The mudfish does
not actively ventilate its body surface, and as diffusive fluxes of ions and water do not appear to be unusually high, the second criterion of cost is also met.

In acknowledging the limitations of cutaneous respiration, the relative roles of the skin, and gill and buccal surfaces, in air, must be noted. For simplicity, the gill and buccal surfaces may be lumped together and considered as an "oral" exchange surface. Since the skin has limited scope for increased gas exchange, or regulation of gas exchange, these functions fall to the oral surfaces. When metabolic rate is increased, the extra respiratory load must be taken up by the oral surfaces unless the fish becomes anaerobic. Ventilation rate increased in mudfish as \( V_O^2 \) increased. The oral surfaces may therefore be considered to be the primary gas exchange surface and the skin as secondary or supplementary.

Rather than considering the skin and oral surfaces as separate systems they must be viewed as complimentary, and linked by an "in series" blood circulation. That is, blood must traverse the gills before reaching the skin. This arrangement can be considered in relation to the hypoxia partitioning experiments. In these experiments, it was shown that 80% of the skin could be functionally lost as a gas exchanger for several hours but mudfish still survived. The head was shown to be able to compensate for the loss of cutaneous respiratory or excretory exchange. It is unlikely that mudfish would survive the opposite situation, with the head immersed in hypoxic water. The circulation of the skin is poorly positioned to function as the sole oxygen uptake surface as blood from the skin would be collected at the heart and passed through the gills before reaching most of the body. Thus, the gills may effectively deoxygenate the blood before it ever reaches the body. Just as the gills may act as an effective oxygen uptake surface, they may work
equally well at removing oxygen under a reversed partial pressure gradient. The mudfish would, therefore, be in a functionally hypoxic state regardless of the skin circulation. There do not appear to be any well developed shunts to bypass the gill circulation in mudfish, as in other air-breathing fish (Wood, 1984).

With the loss of oral surfaces and only the skin functioning, the fish may have little active regulation or control over the efficiency of respiration or excretion, as blood gas levels would fluctuate with changes in metabolic rate (Boutilier and Toews, 1981). Amphibians relying solely on cutaneous respiration can regulate plasma pH (Forster and Shelton, Unpublished). However, as previously discussed, these animals have circulatory differences from fish, which may allow greater perfusion control.

The skin and "oral" surfaces must therefore be viewed as complimentary to one another in supporting aerobic metabolism and not as entirely separate and exclusive systems. One is necessary for maintaining the level of routine metabolism and the other for regulation and scope for increases.

As previously stated, cutaneous respiration should involve little cost to the fish. This cost analysis can be viewed as a balance of the advantages and disadvantages of using cutaneous exchanges in different circumstances. In both air and water, cutaneous gas exchanges are almost all advantageous as they allow exchange of O₂, CO₂ and ammonia at no cost, compared to active ventilation of the gills. However, does the possession of a permeable skin lead to a high rate of loss of water and ions? It seems that fluxes of water are within acceptable limits and ion loss is not appreciable. There is also potential for ion and water movements at the gill and buccal surfaces which may be reduced by much of the oxygen uptake function being taken by
the skin, as noted above. Thus, under favourable conditions in either air or water the advantages of cutaneous exchanges are obvious and the disadvantages few.

When the water becomes hypoxic, as it may frequently do in mudfish habitats (Eldon, 1979a), the mudfish may take three courses of action. They may remain breathing water, commence air breathing, or leave the water (Meredith, 1981). If they remain in the water they may become hypoxic. At a certain stage of hypoxia, the mudfish must resort to air breathing. Air-breathing in hypoxic water places conflicting demands upon the two exchange surfaces. Since the gills are in contact with a rich oxygen source (the air) adequate oxygenation of the blood will occur. Much of this oxygen may however be lost to the water as it passes through the skin. This has been shown to occur in tadpoles (Feder, 1983a,b). Meredith et al (1982), failed to show any O₂ loss from the skin of mudfish, and argued that cutaneous perfusion and therefore O₂ loss was restricted by cutaneous vasoconstriction. Vasomotor control was also indicated from the reduced ammonia excretion rates. In plaice, cutaneous oxygen uptake increased in proportion to gill uptake in hypoxia (Steffensen et al, 1981), probably as a consequence of deoxygenation of dorsal aortic blood.

In mudfish, cutaneous vasoconstriction may be a local response to hypoxia rather than under central control. The implications of a reduced perfusion are many. When the gills are emersed, their ion and acid/base balance functions are lost. Also CO₂ excretion to air may occur much more slowly than to water. These functions may take even more prominence in the skin. If local hypoxic vasoconstriction has abolished most of the cutaneous exchange function, in the situation where it is most needed, the fish may be in a serious state of imbalance. Thus, air breathing in hypoxic water may be a potentially hazardous situation if it occurs for long and cutaneous vasoconstriction is extensive. In practice, this
situation does not occur for long periods as mudfish resort to one of two strategies. They either periodically resort to aquatic ventilation which allows periods of regulation through gill exchanges, or they leave the water to avoid the hypoxic stimulus. Thus, while hypoxia may be a potential problem for mudfish associated with their habitat, they ultimately choose between either exclusive aquatic or aerial environments, and exploit cutaneous exchanges in both.

As cutaneous exchanges are important mechanisms allowing survival in both air and water, it might be argued that little change may be necessary in the transition from water to land. This view was also suggested by the lack of change in the gas transport characteristics of mudfish blood (Wells et al., 1984). However, the preceding physiological experiments and measurements of blood concentrations have shown that on emersion there are widely differing time courses for a variety of physiological functions to reach stable states. These range from several minutes to 24-48 hours or more and illustrate the multiplicity of processes being disrupted.

Over the first few minutes, behaviour patterns such as ventilation, locomotion and postures are modified. Changed orientations and rolling behaviours occur over longer periods. Changes in ventilation and oxygen consumption patterns, over the first few hours may also reflect experimental stress. By 24 hours, mechanisms of respiration and excretion stabilise, as evidenced from excretion patterns and blood concentrations. The suggested change in metabolism and accompanying change in nitrogenous excretion patterns is yet another example of a response to emersion, and here again cutaneous ammonia fluxes help to regulate internal body composition. Therefore, although mudfish cope well with the transition to air, it is a period of substantial change in their physiological processes. Thus, it is necessary to allow a minimum
24 hour period before it can be considered that mudfish have achieved a relatively stable state in air.

The comparisons of emersion physiology with that of fasting or starvation is also interesting. Bicudo et al (1979) suggested that in amphibious fish, emersion physiology was essentially that of starvation physiology. Fasting per se had little effect upon mudfish as it did not stimulate any obvious changes in respiration, excretion or metabolism, which might reflect the animals low metabolic rate. However, on emersion there were major changes in metabolism, nitrogen excretion and acid-base balance. Therefore, emersion physiology in mudfish is not essentially similar to starvation physiology.

Although the discussion of the adaptations of mudfish have centred largely on the skin, the tolerance of the mudfish to severe internal and external disruptions may be central to emersion survival mechanisms. Emerged fish have to initially survive varying blood and tissue levels of CO₂, ammonia, urea, osmolarity, ion levels and acid-base disruption. In the long term they have to change their metabolism and cope with levels of starvation involving metabolism of much of their own body tissue. In the external environment, they must tolerate the abruptly changing and widely varying temperatures that are a feature of terrestrial environments, as well as cope to some extent with desiccation and the accompanying concentration of the blood. In their aquatic environment they must be tolerant to hypoxia (Meredith, 1981), high levels of dissolved organic matter (Eldon, 1979c), hypercapnia and acidity and turbidity of the water. They have even shown high tolerance to salinity changes that may lead to 60% increases in plasma osmolality. This overall tolerance or hardiness may make them less susceptible to succumbing to hazardous conditions than most other species and gives them the potential to cope with severe disruptions whilst they adapt to
new conditions.

As well as the physiological mechanisms, developmental and habitat factors may play a role in mudfish survival under harsh environmental conditions. The concept of optimal size, or a refuge in size, has frequently been discussed in the preceding chapters. These have not only argued for an upper size limit restriction on mudfish but also a lower size limit for survival success during droughts. Strategies of rapidly achieving a minimum size and then remaining within a limited size range suggest differential growth rates and longevity may be important for success in their drought prone environments.

Miller (1979) reviewed the adaptiveness of small size in teleosts and noted that 10% of known teleost species are less than 10cm long or 15g body weight. He related adaptiveness of small size to efficiency of feeding on small prey items and exploitation of spatially restricted habitats and complex substrates. Certainly these considerations pertain to mudfish and may limit their effective size should they remain in their present habitats. The adaptiveness of small size in relation to emersion and drought survival are considered to be of equal or greater importance in this study. Small size allows favourable surface area to body weight ratios and, in combination with low metabolic rates, the necessary respiratory efficiency of skin gas exchange. Such a strategy is maintained with little compromise to the structure of any organ system, allowing mudfish to remain efficient at exploiting its aquatic habitat as well as surviving in air. Small size also confers the ability to burrow or negotiate small cracks, fissures, root holes and thus the ability to find and fit into naturally occurring microhabitats favourable for survival out of water. Drought survival, might, therefore, also lead to selection of a relatively small size. The degree of drought susceptibility of the habitat might determine the limitation of species size. This is also illustrated by the observation that the mudfish lives
for several years but retains a considerably smaller maximum body size relative to other closely related galaxiid species (McDowall, 1978b).

Other physiological factors may have limited the body size to that presently encountered. Although satisfying the respiratory requirement is the most important immediate factor in air, survival for the complete duration of the drought will be equally important. Over long periods, if a fish were too small then desiccatory rates or starvation may cause the fish to perish before the water reappears. Thus, too small a size may be deleterious. I have already predicted a size selective mortality during long droughts. Therefore, to optimise survival, most of the population should achieve an adequate size before the time of greatest drought susceptibility. This accords with the growth rates described by Eldon (1979c); hatching of eggs in post drought periods, maximum growth in the first year and a much slower subsequent growth over several years, especially in males. In different latitudes and temperature and climatic regimes, selection for different optimal sizes or strategies such as dormancy may need to be developed (see Chapter 9).

Dehadrai and Tripathi (1976) also noted poor growth in terms of size in most Indian air breathing fish and attributed it to the stress they are subjected to in the adverse environments of derelict and swampy waters. The comparable growth of mudfish during their first year, to the other galaxiid species suggests this may not be a significant factor for mudfish. Thus, the interplay of all of these size related factors may have led to selection of the present optimal sizes of Canterbury mudfish.

In considering the critical habitat requirements of the mudfish, one important point to consider is whether the present habitat is representative of that in which the mudfish developed its amphibious potential. Since the mudfish is now rare and confined to limited populations in widely spaced localities, certain factors must be
contributing to its decline. Observations of the three habitats mudfish were sampled from revealed that these are not as they would have been even one hundred years ago, as they have been extensively modified by man. This has changed not only the vegetation patterns but also the climatic and hydrological features. Therefore, although the need for mudfish to survive three month drought periods is envisaged (Eldon, 1978a), such conditions may bear little relation to the conditions in which they developed. The swamp forest conditions described for the Brown mudfish (Eldon, 1978b) may be more representative of early Canterbury conditions. Canterbury was once covered in lowland Kahikatea forest, few remnants of which remain today. Thus it may be misleading to consider adaptations of mudfish in relation to their present habitat, even though past conditions may be hard to predict.

The microhabitats within the mudfish habitats are considered of most importance for success of drought survival as these shelter fish from the extremes of the environment. Thus, it is the conditions within these that are crucial in deciding whether mudfish can survive in air. Cover and shelter must therefore be abundant. On a gross level, forest canopy or vegetation cover produce indirect shelter, but the shelter afforded by stumps, fallen timber, debris and irregularities in the ground are more important. A fairly complex or cluttered habitat is therefore necessary to provide an abundance of favourable positions for mudfish to survive in. Unfortunately both of these features are relatively incompatible with current agricultural practices, so the potential for attainment of favourable microhabitats becomes more restricted. This may have led to the extinction of many populations. Skrzynski (1968) considered mudfish may have been in decline for the past 1000 years due to climatic and vegetation changes. The recent changes, however, must have had far greater impact.
An optimal size, and ability to find or utilise favourable features of the environment, allow mudfish to survive perturbations that would kill most other fish species. They are therefore found, and have probably always existed, only in drought prone or poor water quality areas that exclude most other species (Eldon, 1979a). Their only intolerance is coping with other species or possible lack of competitiveness. They are not an aggressive species and with a small mouth are not equipped for aggression, often being pursued and intimidated by other native fish species in aquarium tanks. Their low metabolic rate, which allows significant use of cutaneous respiration, may also reduce their potential for active competition. They are also a generalist feeder (Eldon, 1979b), and therefore may not cope well competing with specialist fishes for the food resources in flowing waters. Finally, evolving in an environment relatively free of aquatic predators may mean they are devoid of antipredator avoidance mechanisms and easily preyed upon. In the Oxford habitat small numbers of mudfish were caught in many branches of Washpen stream but only in the one small drought prone area were mudfish numerous. Thus, although they were often being washed into the surrounding water system, as fry, and during floods, they were not successful at exploiting that habitat and building up stable populations. This aptly illustrates their probable lack of competitiveness with other species.

Mudfish may have remained relatively unspecialised compared to other fish species facing similar emersion problems, due in part to the environment they have developed in. Graham (1976) compared the morphological and physiological adaptations of air breathing in marine and freshwater fish with their habitat, requirements and ecological basis for air breathing. He suggested that marine species are largely amphibious rather than strictly air breathing and upon exposure air breathing is continuous. It is also a requirement from early on in a
fishes' ontogeny, as the fish make complete transitions between air and water on most tides. He suggested that air breathing organs are usually only the gills, buccopharyngeal epithelium and skin, because their aquatic habitats are usually air saturated and when in air CO₂ excretion is important. Thus, they have evolved as a species adapted to terrestrial life in order to avoid competition in the abundant normoxic waters and to exploit an open habitat.

Freshwater species normally cope with stagnation and anoxia during annual dry seasons (Carter and Beadle, 1931a; Dehadrai and Tripathi, 1976; Kramer et al., 1978) and therefore facultative airbreathing and amphibiousness is a seasonal occurrence. Obligatory air breathing is an extension of this. Graham noted that airbreathing in the majority of species either supplements aquatic respiration or enables a species to carry on a normal existence in a hypoxic habitat. Therefore, extensive supplementary oxygen uptake organs have developed and are usually sequestered away from the gills to allow these to excrete CO₂ to the water. Under these different requirements and ecological bases, freshwater species are not as highly adapted for terrestrial life as marine species but are more highly adapted for poor water conditions.

How these schemes relate to mudfish is intriguing. Superficially the respiratory mechanisms and strategy of mudfish resemble that of the marine group and clearly they have a substantial terrestrial capability. They also remain potentially active with a normal metabolic rate when emersed unlike most freshwater fish. However, they are a freshwater fish subjected to seasonal conditions. The seasonal conditions may, however, not be as extreme as in tropical dry seasons as lack of extremes of temperature may not generate the stagnant, hypoxic and hypercapnic conditions for long periods. This may not necessitate long periods of supplementary airbreathing. Eldon (1979c) considered air breathing in
mudfish to be a response to hypoxia, but the prevalence of airbreathing compared to total emersion is worth considering. Hypoxia may certainly be a feature of mudfish habitats both diurnally in densely weed choked waters, and in the latter stages of water drying up during droughts. However, as discussed previously, mudfish showed a readiness to leave the water rather than remain air breathing in water. Physiological reasons for this have already been presented, but other reasons may allow emersion to be favourable for mudfish but not for tropical species. In the essentially island environment of New Zealand the paucity of freshwater species (31 endemic species) means the mudfish may have had little interspecific competition in their habitats and therefore be under low selective pressure from other species, whereas there is a diversity of tropical airbreathing species. Similarly, leaving the water in poor water conditions is possible due to the paucity of amphibian competitors or predators compared to tropical regions. It simply may not be a viable option in tropical areas.

Finally, the habitat the mudfish chooses to live in is usually adjacent to permanent flowing waters so mudfish are actively choosing the marginal habitat over a normoxic habitat rather like littoral marine species. The development of marine type strategies and adaptations in mudfish, rather than the classical freshwater strategies, may be due largely to the less extreme environment in New Zealand and an essential strategy of avoidance of competition.

Mudfish have an effective series of mechanisms to allow survival in air during droughts as a strategy which allows them sole access to a range of habitats that exclude other fish species. With the encroachment of man their habitat has been one of the first to be disrupted or "reclaimed". Protection of their remaining habitats and habitat values are therefore necessary for their continued existance.
CHAPTER 9.

EMERSION OF GALAXIID FISHES

INTRODUCTION

Although the Neochanna species possess abilities to survive emersed for considerable periods of time, New Zealand also possesses ten other galaxiid species found in widely differing habitats. To what extent these species might be similar to mudfishes in their potential to survive in air, may be interesting to consider.

The New Zealand galaxiid species fall easily into 3 groupings; the mudfishes, the whitebait (diadromous) species, and the subalpine species. The development of these groupings has generated much speculation on the phylogeny of the family and the New Zealand piscine fauna. They have been examined taxonomically via meristic characters (McDowall, 1970) and electrophoretic polyphenotypic characters (Mitchell and Scott, 1979). However, a scheme of phylogenetic development of several species is still controversial. Have the exclusively freshwater species, epitomised by the mudfishes, developed from dispersed diadromous species or do they stem back to the fragmentation of Gondwanaland? Such speculation cannot be readily settled, but evidence of similarities can be collected in support of either notion. Cadwallader (1975b) presented an analysis of brain morphology and brain area of the galaxiids and suggested that such comparisons, although not a rigid taxonomic tool, could be used to discuss similarities in groupings of fish. Similar affinities may be observed in relation to emersion tolerance or other physiological parameters.

The origin of the emersion tolerance of mudfish may be viewed from this comparative approach, to assess whether it may stem from ancestral diadromous species, like those today, or was developed in freshwater.
swamp habitats. Many clues to such a premise may be gained from looking for diadromous characters such as larval stage similarities and salinity tolerance in mudfish, as well as emersion tolerance in diadromous species. Clues may also be obtained from seeking similarities in the many Australian species.

The physiological affinities of the mudfishes to other members of the family may not only place the adaptations in ontogenetic and phylogenetic perspective, but also supply information on the other galaxiid species that may aid or lead to consideration of related aspects of their ecology and development.

MATERIALS AND METHODS

Specimens of nine of the thirteen species of galaxiid fish in New Zealand were collected or acquired from the localities presented in Table 9.1; Fig. 9.1. Fish, of comparable size to the adult mudfish previously studied, were selected and acclimated to laboratory aquaria. After fish had been acclimated at 15°C for at least 7 days and had resumed feeding, they were fasted for 24 hours, removed from holding tanks, and placed in perspex lunchboxes. They were maintained in the lunchboxes on damp cotton wool and stored at 15 ± 1°C under a 12 hr light/dark cycle. Survival of individuals was monitored hourly for the first day and then daily for one week. The different species were scored as either not being able to survive in air, or being able to survive for 1 day or 1 week in air. The potential of the other New Zealand species that were not collected, and some Australian galaxiid species, to survive in air was estimated from similarity to other species and references to aestivation or emersion in the literature.

During monitoring of survival in air, respiratory and locomotory patterns and behaviour of the different species was recorded.
Differences from the patterns exhibited by mudfish were noted.

Table 9.1 Areas galaxiid species were collected from for comparative studies. Numbers refer to map in Fig. 9.1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection Locality</th>
<th>Map Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neochanna apoda</td>
<td>Hari Hari, Westland</td>
<td>1</td>
</tr>
<tr>
<td>Neochanna burrowsius</td>
<td>Oxford, Canterbury.</td>
<td>2</td>
</tr>
<tr>
<td>Neochanna diversus</td>
<td>Whangamarino swamp, Waikato.</td>
<td>3</td>
</tr>
<tr>
<td>Galaxias argenteus</td>
<td>Hokitika, Westland</td>
<td>4</td>
</tr>
<tr>
<td>Galaxias fasciatus</td>
<td>Hokitika, Westland</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>South Westland.</td>
<td>6</td>
</tr>
<tr>
<td>Galaxias brevipinnis</td>
<td>Lake Coleridge</td>
<td>7</td>
</tr>
<tr>
<td>Galaxias maculatus</td>
<td>Taumutu stream, Canterbury</td>
<td>8</td>
</tr>
<tr>
<td>(Whitebait)</td>
<td>Rakaia River, Canterbury</td>
<td>9</td>
</tr>
<tr>
<td>Galaxias vulgaris</td>
<td>Ashley River, Canterbury</td>
<td>10</td>
</tr>
<tr>
<td>Galaxias paucispondylis</td>
<td>Wilberforce R. Canterbury</td>
<td>11</td>
</tr>
</tbody>
</table>

The basal aquatic oxygen consumption of several of the species was measured by respirometry. This was carried out by use of a closed jar and oxygen electrode as described for mudfish (section 3.2.1). Mean ventilation frequencies were also measured during respirometry measurements.

The partitioning of $\dot{V}O_2$ between the head and body of three of these species in water was carried out according to the method used for mudfish (section 2.5). Partitioning of $\dot{V}O_2$ in air was also attempted.

For the species unable to survive for one day in air, mortality curves were constructed. Fish were observed every 15 minutes and percent mortality calculated. Respiratory patterns were also monitored to determine any factors that may have contributed to the mortality. Oxygen
Figure 9.1 Map of New Zealand showing the sites from which different galaxiid species were collected. Numbers refer to species on Table 9.1.
consumption in air was also measured hourly in several fish. The oxygen consumption of these fish was determined in a Gilson differential respirometer by the method previously described (section 3.2.2).

Juvenile or 'whitebait' stages of Inanga (*Galaxias maculatus*) were captured in seawater during their upriver migration phase. They were maintained in a recirculating seawater aquarium system. Several whitebait were also placed into freshwater aquaria but otherwise fed and maintained identically to the marine whitebait. The presence and appearance of blood pigments was observed daily and related to the period in captivity. The behaviour and development of the two groups was also regularly observed.

Since the level of cutaneous respiration is dependant upon skin structure, skin thickness and skin area, these parameters were examined in several specimens of different galaxiid species. Specimens were all of similar size to adult mudfish (4-7g). Sections of skin and muscle from the dorso-lateral trunk regions of fish were dissected free and paraffin sections prepared as for mudfish (section 7.2.1). Sections were stained with haematoxylin and eosin or Goldner's stain and viewed with a Nikon photomicroscope. Any differences from the structure of mudfish skin were noted. Several fish significantly larger than the size range of mudfish were examined to determine whether skin thickness increased appreciably in large individuals.

**RESULTS**

The results of aerial survival of the nine galaxiid species are presented in Table 9.2. All of the species surviving for one day also survived for at least one week. Only the Inanga, *Galaxias maculatus* was unable to survive at least one day of emersion.

References to several Australian galaxiid species indicates that
several of these species may also survive long periods in air (Table 9.2). Thus, the family Galaxiidae is composed predominantly of species tolerant to emersion.

Table 9.2 Survival periods of galaxiid species in air at 15°C.

<table>
<thead>
<tr>
<th>Species</th>
<th>Survival period</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>&gt;1 day</td>
</tr>
<tr>
<td><strong>New Zealand</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neochanna apoda</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Neochanna burrowsius</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Neochanna diversus</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxias argenteus</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxias fasciatus</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxias postvectus</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Galaxias brevipinnis</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxias maculatus</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Galaxias vulgaris</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxias gracilis</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Galaxias paucispondylis</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxias prognathus</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Galaxias divergens</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td><strong>Australia</strong> **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepidogalaxias salamandroides</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxiella pusillus</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxiella nigrostriatus</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Galaxias cleaveri</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Note:
? species not obtained and survival not mentioned in literature.
** This is only a small number of the total number of Australian species
All species, except the Inanga, managed to remain upright during emersion. The mudfish species remained in a coiled or 's' shaped position which facilitated maintenance of an upright position. When moving around they used anguilliform sinusoidal movements although they were prone to a lack of grip due to their mucoid surface.

The other species had ventrally displaced pectoral fins and maintained position with outstretched pectoral and pelvic fins. Their body movement was also sinusoidal with the outstretched fins preventing slipping. They were capable of rapid and coordinated movement on most surfaces. These species also have good climbing ability on smooth surfaces using adherence of the outspread fins.

Most Inanga remained stretched out on their sides, although several managed to remain in a dorsal stance for periods by lying in a semi-coiled position. They are neither ventrally flattened nor have ventrolaterally displaced fins so are poorly adapted to maintain orientation on land. Movements were generally by active flips of the tail which propelled the fish in random directions. Unlike the other species their movements were random and uncoordinated on land.

Aquatic respiratory patterns were consistent throughout the species, varying only in ventilation frequency and oxygen consumption. All of the Galaxias species had significantly higher oxygen consumption than the mudfish, the highest being the Inanga which ventilated 2-3 times more rapidly and consumed 3-4 times more oxygen than mudfish (Table 9.3).

During aerial respiration, all of the species actively inflated their buccal and opercular cavities by movement of the floor and lateral surfaces of the buccal cavity. All Galaxias species ventilated their gills 2-3 times more rapidly than the mudfish species at all stages (mean rates ranged from 3.0-8.5 per minute at 4 hours after emersion). The Inanga was the only species which did not show a progressive decrease
in ventilation rate over the first few hours of emersion similar to the pattern of mudfish ventilation.

Table 9.3 Aquatic ventilation rates and oxygen consumption of several Galaxiid species at 15°C (ventilation rate, breaths per minute; $\dot{V}O_2$, $\mu$10$_2$ g$^{-1}$ hr$^{-1}$).

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>size (g)</th>
<th>n</th>
<th>Aquatic Ventilation</th>
<th>$\dot{V}O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neochanna burrowsius</td>
<td>5.59</td>
<td>15</td>
<td>51.9</td>
<td>54.2</td>
</tr>
<tr>
<td></td>
<td>±.61</td>
<td></td>
<td>± 9.0</td>
<td>±9.8</td>
</tr>
<tr>
<td>Galaxias maculatus</td>
<td>5.59</td>
<td>6</td>
<td>109 **</td>
<td>140.9 **</td>
</tr>
<tr>
<td></td>
<td>±.61</td>
<td></td>
<td>±14</td>
<td>± 9.8</td>
</tr>
<tr>
<td>Galaxias brevipinnis</td>
<td>4.78</td>
<td>4</td>
<td>100.8 **</td>
<td>102.1 **</td>
</tr>
<tr>
<td></td>
<td>±.37</td>
<td></td>
<td>± 5.6</td>
<td>±12.7</td>
</tr>
<tr>
<td>Galaxias vulgaris</td>
<td>4.97</td>
<td>3</td>
<td>114.3 **</td>
<td>117.3 **</td>
</tr>
<tr>
<td></td>
<td>±.90</td>
<td></td>
<td>± 8.0</td>
<td>±14.2</td>
</tr>
<tr>
<td>Galaxias fasciatus</td>
<td>5.44</td>
<td>6</td>
<td>98.4 **</td>
<td>94.4 **</td>
</tr>
<tr>
<td></td>
<td>±.81</td>
<td></td>
<td>±10.6</td>
<td>±10.7</td>
</tr>
</tbody>
</table>

** p<0.001.

The partitioning of aquatic $\dot{V}O_2$ between the head and body of the Galaxias species is shown in Table 9.4. All of the Galaxias species had similar rates of oxygen consumption from the body, but with the higher total $\dot{V}O_2$s compared to mudfish, the body accounted for much lower percentages of total $\dot{V}O_2$. All of the Galaxias species had significantly higher cutaneous total $\dot{V}O_2$ than mudfish (p<0.001).

None of the Galaxias species survived the stresses of partitioning in air for long enough to adequately measure partitioned aerial respiration.

A mortality curve for the emersion of Inanga, Galaxias maculatus, is presented in Figure 9.2. This shows that all Inanga survived for at least 1.5 hours but none survived greater than 18 hours. Values for LT50 and LT80 were 5.2 and 8.6 hours respectively. The differential mortality
Table 9.4 Partitioned oxygen consumption between head and body of several Galaxiid species in water at 15°C.

\[
\dot{V}O_2, \mu lO_2 g^{-1} hr^{-1},
\]

<table>
<thead>
<tr>
<th>Species</th>
<th>Size (g)</th>
<th>n</th>
<th>Head $\dot{V}O_2$</th>
<th>Body $\dot{V}O_2$</th>
<th>%Body $\dot{V}O_2$</th>
<th>Total $\dot{V}O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. burrowsius</em></td>
<td>§ 7.22</td>
<td>6</td>
<td>151.2**</td>
<td>29.6**</td>
<td>16.4</td>
<td>180.8**</td>
</tr>
<tr>
<td></td>
<td>±.77</td>
<td></td>
<td>±12.7</td>
<td>±1.2</td>
<td></td>
<td>±13.6</td>
</tr>
<tr>
<td><em>G. maculatus</em></td>
<td>6.48</td>
<td>4</td>
<td>121.8**</td>
<td>26.2**</td>
<td>17.7</td>
<td>148.0**</td>
</tr>
<tr>
<td></td>
<td>±.98</td>
<td></td>
<td>±10.9</td>
<td>±1.8</td>
<td></td>
<td>±11.4</td>
</tr>
<tr>
<td><em>G. brevipinnis</em></td>
<td>6.91</td>
<td>4</td>
<td>109.8**</td>
<td>25.9**</td>
<td>19.1</td>
<td>135.4**</td>
</tr>
<tr>
<td></td>
<td>±.89</td>
<td></td>
<td>±11.5</td>
<td>±1.4</td>
<td></td>
<td>±11.6</td>
</tr>
</tbody>
</table>

§ From Meredith et al (1982).

** p<0.001

---

Figure 9.2 Graph of % mortality versus time out of water for a group of 50 Inanga emersed at 15°C.
of individuals was not correlated with either size or sex.

The emersed Inanga had two ventilatory patterns. The first was a regular ventilation of the oral cavities with both inspiration and expiration by active movements through the mouth. The second pattern, superimposed on this, was a very rapid movement of the buccal muscles generating an intra-oral air movement. Although the mouth was held partially open during this latter pattern, it probably involved little inspiration or expiration but served to move air within the buccal and opercular cavities. Unlike the other galaxiids high ventilation rates were maintained until shortly before death.

Inanga were deemed to be dead when they ceased ventilating and buccal and opercular cavities remained collapsed. Tissue opaqueness and rigidity accompanied cessation of breathing, although the heart continued to beat for a further 20 minutes or so.

The oxygen consumption of Inanga in air is presented in Figure 9.3. Aerial \( \dot{V}O_2 \) never exceeded 60% of the aquatic \( \dot{V}O_2 \) and slowly decreased with increasing periods in air. Fish death could not be determined from \( \dot{V}O_2 \) data beyond 4 hours, as both dead and living fish had similar \( \dot{V}O_2 \) s. Inanga were therefore presumed to be in an anoxic state at all times during emersion.

Marine whitebait stages of Inanga are active surface schooling fish. They are transparent and show no blood pigmentation in head, gill or body areas. They actively and rapidly ventilate their gills. Whitebait transferred to freshwater behaved similarly and showed no ill effects due to the rapid transfer. Both groups of whitebait developed red pigment in the spleen a number of days after capture (Figure 9.4). Freshwater whitebait developed spleen pigmentation an average of 2 days earlier than seawater fish. Both groups showed circulation of blood pigment in the gills and head several days after spleen pigment
Figure 9.3  Graph of aerial oxygen consumption of Inanga until seven hours after emersion at 15°C

Figure 9.4  Graph of percentage of whitebait developing visible blood pigments against time after capture and placement in either freshwater or seawater.
developed. However, freshwater whitebait showed much greater blood pigment development than seawater whitebait at all stages.

After the increase in blood pigments, freshwater whitebait developed body 'colour' and pigmentation and descended to school in mid and lower tank levels. Seawater whitebait, however, failed to 'colour' and remained schooling near the surface. Apart from development of blood pigment, development of whitebait was slowed or halted by remaining in seawater.

Skin structure of all the galaxiid species examined was similar to that described for the Canterbury mudfish. All of the species had a uniform skin thickness with an external layer containing a large number of mucous cells. The middle and basal epidermal layers were also very similar to that of mudfish. Dermal capillaries were present in all species but distribution and density of them were not remarkable in any species. Several large galaxiid specimens had a thick skin with a greatly enlarged middle layer in the epidermis. This middle layer was composed of uniform small squamous epithelial cells separating the outer mucoid and inner columnar layers. The skin from a 350g giant kokopu (Galaxias argenteus) is shown in Figure 9.5. The basal and outer layers of the epidermis are similar to mudfish but the middle is greatly extended so as to account for over 70% of the total epidermal thickness. In a 50g banded kokopu specimen, the middle layer was well developed but much less extensive than in the larger fish (Fig. 9.6). Such thickening of the skin was found to be present in all galaxiid fish that exceeded 30g wet body weight. This included G. argenteus, G. fasciatus, and G. brevipinnis, in this study. Presumably any galaxiid species achieving these sizes would develop the squamation.
Figure 9.5 Transverse section of the skin of a 350g giant kokopu showing the greatly increased skin thickness.
H & E, x40.

.g- Goblet cells
e- Epidermis
c- Columnar layer
s- Squamous layer
d- Dermis
m- Muscle blocks

Figure 9.6 Transverse section of the dorso-lateral skin of a 50g banded kokopu, showing middle squamous layer.
H & E, x100.
DISCUSSION

The possible reasons for large numbers of galaxiid species possessing the potential to survive emersion are many. The mechanisms allowing survival may be an ancestral condition (see later section) with the underlying mechanisms being present in all species. This may indicate the three groupings of galaxiid species have stemmed from a diadromous stock rather than developed emersion tolerance independently. However, the reasons for the retention of the ability to survive in air can be related to several behaviours of different species at different life stages. These include aestivation abilities, climbing abilities of migratory stages and terrestrial spawning behaviour. The occurrence of these behaviours in the different species is presented in Table 9.5.

The Neochanna species, possibly some of the forest species \( \text{(G. fasciatus)} \) and several of the Australian species are known to 'aestivate'. Thus, there is selection in these groups for their ability to survive droughts. Emersion tolerance would, therefore, be expected to be most highly developed in these groups.

Many of the galaxiid species possess migratory whitebait stages that penetrate long distances up rivers to reach their adult habitat. During these journeys, several species are known to negotiate high waterfalls or velocity barriers by climbing damp walls or rockfaces in the splash zone (McDowall, 1978). Such climbs may take several hours or days and functionally leave the fish emersed for long periods. These fish will also have a high metabolic rate while emersed due to the considerable activity involved in climbing. In the laboratory even 5g specimens of \( \text{G. fasciatus} \) were able to climb vertically up smooth surfaces, showing that climbing abilities are retained into adulthood. Thus, survival abilities during emersion and efficiency of air-breathing may increase the effectiveness of this climbing ability and the attainment of adult habitats. This may promote retention or development
of emersion abilities.

Several galaxiid species are terrestrial spawners. That is, they emerse themselves to spawn on marginal vegetation above the mean water or tidal level (McDowall, 1978; Mitchell and Penlington, 1982). Eggs are therefore only immersed and hatched at high tidal or flood conditions when fry will be effectively dispersed. Survival ability of adults in air would therefore enhance the active scope and longevity of spawning fish and possibly spawning success by their increased ability to achieve or select spawning sites. However, compared to the former two situations terrestrial spawing may only necessitate short periods out of water. Some species, such as the Inanga, generally die after spawning and only limited survival abilities may be necessary. The 1.5 hour minimum survival of Inanga in air may be all that is necessary to achieve successful spawning, so this behaviour may not lead to selection for terrestrial survival. However, in long lived species that spawn terrestrially, such as G. fasciatus (Mitchell and Penlington, 1982), regaining the water and post-spawning survival are important. Thus in these species increased terrestrial ability may be developed.

It is not surprising that under these pressures many species retain aerial survival abilities. The extent of their abilities may differ depending upon how extensive the behaviours discussed above are used by each species and in what ways the different species have developed to live in different habitats. That is, the strategies must still be essentially similar to those of the mudfish discussed previously and any departures from these, such as increased metabolic rate, may decrease potential for survival in air.

Most galaxiid species are sluggish or benthic and live in slow flowing or still habitats, or shelter from strong currents, only making brief forays into the current. They might, therefore, have lower basal metabolic rates than more active fish species. This may be
Table 9.5 Occurrence of terrestrial behaviours of New Zealand and Australian Galaxioiroid fishes, and presence of experimental emersion survival ability.

<table>
<thead>
<tr>
<th>Species</th>
<th>Aestiv.</th>
<th>Climbing</th>
<th>Terr.</th>
<th>Spawn</th>
<th>Air Surv</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New Zealand</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neochanna apoda</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neochanna burrowsius</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neochanna diversus</td>
<td>X</td>
<td>?</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Galaxias argenteus</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Galaxias fasciatus</td>
<td>?</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Galaxias postvectus</td>
<td>X</td>
<td>?</td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>Galaxias brevipinnis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Galaxias maculatus</td>
<td></td>
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<td></td>
<td>X</td>
</tr>
<tr>
<td>Galaxias vulgaris</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Galaxias gracilis</td>
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<td>Galaxias paucispondylis</td>
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</tr>
<tr>
<td>Galaxias prognathus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>Galaxias divergens</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Australia</strong></td>
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<td></td>
</tr>
<tr>
<td>Lepidogalaxias salamandroides</td>
<td>X</td>
<td></td>
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<tr>
<td>Galaxiella pusillus</td>
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</tr>
<tr>
<td>Galaxiella nigrostriatus</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Galaxias cleaveri</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

**NOTE:** Information on many species is incomplete.

**This is only a small number of the total number of Australian species**
related to their habitat requirements and basal activity levels. This can be seen in both the oxygen consumption and gill ventilation rates of the Galaxias species in water. The different species can be graded into a continuum ranging from the most sluggish to the most active species on these features. The mudfish species are the most sluggish, being cryptic nocturnal swamp dwellers, while the Inanga is the most active being a riverine active schooling fish.

Since all of the species examined were long slender fish and had similar skin structure and aerial respiratory behaviour to mudfish, the physiological mechanisms allowing survival during emersion were considered to be essentially similar. Higher basal metabolic rates would reduce the scope for activity and temperature tolerance in air. Poole (1974) showed that survival of Galaxias brevipinnis in air was dependant on air temperature. While they survived several days in air at 15°C or below, they would only survive for several hours at 25°C. Such temperature dependance would be expected in the more active species. Many species may show a tradeoff between emersion ability and the metabolic rate necessary for exploitation of their habitat. Most species showed much higher aquatic oxygen consumption and gill ventilation rates relative to mudfish species and are therefore less well adapted to air than mudfish. At the extreme of this was the Inanga which had treble the metabolic rate of the mudfish. It is therefore not suprising that this species was the least successful in air.

Partitioned oxygen consumption in water showed the presence of cutaneous respiration in all of the species examined. It may therefore be a feature of this family. Although the level of cutaneous oxygen uptake was similar in all of the Galaxias species, it was also related to the total oxygen consumption of the fish species, in that the Inanga with the highest oxygen consumption had the highest cutaneous oxygen
consumption. Body form and size were similar, so relative skin area was unlikely to be significantly different. The diffusive properties of the skin are also likely to be similar. The actual differences in oxygen uptake are probably related to oxygen transport properties. The level of deoxygenation of cutaneous blood, or possession of haemoglobins of higher affinity may cause greater oxygen gradients across the skin. However, since cutaneous blood is of dorsal aortic origin it is unlikely to be significantly deoxygenated before arriving at the skin unless gill uptake is compromised and blood is insufficiently oxygenated. Capillary density or perfusion may vary in different species leading to different cutaneous respiratory efficiency. There was no evidence of this.

The Galaxias species all possessed higher cutaneous uptake than the mudfish. The reasons for this may be many but cannot be readily isolated. As discussed above, the more active species may have increased perfusion and ventilation of skin surfaces, tissue oxygen consumption or different blood properties, all of which may increase O₂ uptake. However, the more important point to note is the contribution cutaneous uptake makes to total oxygen uptake. In this respect, the cutaneous exchange is far more important in the mudfish than in the other species.

The implications of limited cutaneous uptake for the more active species is great. At higher metabolic rates, cutaneous uptake becomes a progressively smaller portion of the total oxygen uptake. Almost all increases in metabolic rate must therefore be met by the respiratory organs of the head. This in turn would lead to reduced scope for increases in metabolic rate and potential for survival at increased activity or temperatures in air. This further shows how low metabolic rates are necessary for survival in air using a cutaneous strategy.

Cutaneous respiration in air was unable to be measured in any of the Galaxias species because of mortality of partitioned fish. The high respiration rate of restrained fish, due to the stress effects of
partitioning and the high routine metabolic rates, may have made the fish unable to derive sufficient oxygen from the air when emersed. They may therefore have died of anoxia. I would expect that the level of cutaneous oxygen consumption in air is probably similar to that in water, as with mudfish. This further supports the premise that cutaneous mechanisms may not be adequate for supporting emersion of active fish unless alternative gas exchange organs are present. The level of efficiency of the gill and buccal organs of galaxiid fish for gas exchange in air may therefore be limited.

Since the Inanga was the only species not able to survive in air, the differences between them and the other Galaxias species may highlight what limits emersion survival. Inanga are a whitebait species but are not a 'climbing' species. Similarly they do not aestivate or live in drought prone conditions. However, they do exhibit terrestrial spawning but die soon after spawning. Extended survival for two hours in air may therefore aid spawning success but further potential may be unnecessary. They may therefore have little use for well developed aerial survival mechanisms. Inanga also differ from most other galaxiid species in being an active midwater schooling fish and therefore maintain a high metabolic rate. They are therefore functionally and behaviourally quite different from the other species. These differences are highlighted by their inability to efficiently move on land. Their high metabolic rate is exhibited as the high ventilation rate in air and lack of any decrease in ventilation rate while in air. The lack of modulation of the ventilation rate in air suggests that Inanga are either barely receiving enough oxygen from maximal ventilation or are not managing to achieve necessary oxygen uptake levels. The latter situation is born out by the oxygen consumption values where in air Inanga can barely maintain 50% of their aquatic uptake rate. Since this total $\dot{V}O_2$ was lower than the scope for aerial respiration shown by mudfish, and the cutaneous
respiration was probably at a similar level, the gill and buccal surfaces in Inanga may be less developed for aerial respiratory functions than mudfish.

The gills of Inanga are superficially similar to those of mudfish (Peacey, 1982) so it may be that buccal and opercular respiratory development or capillary density is less in Inanga than in mudfish. Also, Inanga often lay on their sides in air. This would bring pressure to bear on one opercular cavity and may therefore prevent ventilation of half of the gill and opercular area.

Although no other species failed to survive in air several other species may be considered similar to Inanga. The dwarf Inanga, *G. gracilis*, is a schooling species, thought to be derived from Inanga (McDowall, 1978), so may also be similarly poorly suited for emersion. Australia possesses active and schooling species such as *G. truttaceus*, that may have little use for emersion survival mechanisms. Thus Inanga may not be the only galaxiid species unable to survive in air.

The skin structure and cutaneous respiration has been suggested earlier to reflect a development of the marine developmental stages of diadromous galaxiid fish. Many marine larval fish rely on cutaneous permeability for most physiological regulatory functions. The skin is important because gills are generally poorly developed, the kidney is a simple pronephros and the gut may not be open (Holliday, 1968; Roberts et al., 1973). Larval eels and yolksac larvae of herrings have mitochondria rich cells present in the skin which have been suggested to be analagous to 'chloride cells' (Lasker and Threadgold, 1968). However, with adult development, body organs develop, the skin increases in thickness and scales become prominent, preventing many of the skin exchange functions. Roberts et al. (1973) described the development of the skin of the larval plaice. They noted that skin thinness for gas exchange took precedence over structural strength during the first 60
days after hatching. The skin was a delicate epidermis overlying a fluid filled dermal space. At day 60 extensive metamorphosis took place, including obliteration of the dermal space, degeneration of chloride like cells and increases in skin thickness. Such a metamorphosis was assumed to parallel changes in the ecology and physiology of the fish and changes in other organs.

"Whitebait" on the South Island coast are generally the common Inanga and are as an example of a marine larval galaxiid. Whitebait appeared undeveloped, with a transparency showing little body organ development. At this stage, they are on an upstream migration and presumably on a course of rapid development. They lack any visible blood pigments. In the larval condition such pigments may be unnecessary as cutaneous diffusion may supply most of the body, much like primitive invertebrates and the previously described larval fish. The migratory fish rapidly developed blood pigments, presumably haemoglobin, a number of days after capture. This may be to improve scope for respiration and potential for activity as fish will from there on need to swim against river currents rather than drift in the sea. The development of haemoglobin, in whitebait remaining in seawater, may indicate that such development is stimulated by some factor other than exposure to freshwater, such as size, daylength, or age and has stimulated an active migratory urge. However, the whitebait were caught adjacent to a rivermouth and so may have been exposed to diluted seawater. Poor development of whitebait in seawater suggests that they are primarily a freshwater species requiring development in freshwater to achieve adult size.

Whitebait may, therefore, be analagous to typical marine larval fish with a predominantly cutaneous exchange strategy in early life.

In a scaleless family, limited metamorphosis of the skin during adult development could lead to a whole group of cutaneously respiring
fish. Skin exchanges may therefore be considered a larval characteristic which is retained in the adult galaxiid fish. In this regard it might be considered a neotenic character.

The mudfish species and several other galaxiid species are exclusively freshwater fish, lacking the marine whitebait stage in their life histories. However, McDowall (1978) considered the family to be derived from diadromous species. This is seen not only in the broad salinity tolerance of adult G. maculatus (Chessman and Williams, 1974, 1975), but also in the osmoregulatory abilities of the Canterbury mudfish (section 5.3.3).

Larval stages of exclusively freshwater fish have not been studied to the same extent as marine larval fish. Therefore, the extent to which they rely on or use cutaneous exchanges is unknown. However, the juvenile stages of the exclusive freshwater galaxiid species have many similarities with the diadromous species. The freshwater whitebait are small and transparent. They school at the water surface for a period of their development before developing adult traits and behaviours such as cryptic and benthic habits (McDowall, 1978). The freshwater whitebait may, therefore, be considered not far removed from the diadromous whitebait.

A final feature of the galaxiid family to be reconciled with emersion tolerance is the effect of size. I have already commented on how the mudfish species are probably restricted within a limited size range by their survival mechanisms. Both smaller and larger sized species may therefore be interesting to consider.

The smallest galaxiid species are the Galaxiella species, and Lepidogalaxias salamandroides (of Family: Lepidogalaxidae; Superfamily: Galaxiodea) of western Australia. Several of these are described as 'aestivating' species (see Table 9.5). The small sizes of these species (<2.5g; McDowall and Frankenberg, 1981) may be related to the much higher
temperatures exhibited in that region compared to New Zealand and different habitats. Aestivating species at higher temperatures may require a smaller size to increase the surface area to body weight ratio and cutaneous efficiency. This assumes that the same basic strategy has developed for emersion in these species. Pusey (pers comm) has found the salamanderfish, Lepidogalaxias salamandroides to be essentially similar to the Canterbury mudfish in most aspects of its physiology. Therefore, the comparison and possible reasons for smaller size may be valid. An extension of this is seen in the size of the other Australian 'aestivating' species, the Tasmanian mudfish, Galaxias cleaveri. This species is superficially very similar to the Canterbury mudfish in that it still retains pelvic fins. It exists at a similar latitude to the Canterbury mudfish and has a very similar size range (Merrick and Schmida, 1984). The shift south relative to the other Australian aestivating species may have allowed development of a greater body size. Such a trend is not so obvious in the three New Zealand mudfish species as the latitudinal variation is not great and the species' latitudinal range, especially of the Brown mudfish, is considerable. However, the northern black mudfish, Neochanna diversus is the smallest of the three species (normally <90mm; McDowall, 1978).

On the other extreme, several of the New Zealand galaxiid species grow to large sizes compared to mudfish. Survival of these large specimens and whether they correspond with the previous results, is of interest in confirming the size restriction on emersion survival. Several specimens of the banded kokopu, G. fasciatus in the size range 20-50g were able to survive up to a week in air at 15°C. They were able to orientate themselves and move around effectively like the small specimens. They were however, 2-5 times greater in size than mudfish. However, at higher temperatures they may rapidly succumb to emersion as they ventilated frequently and showed little reduction in ventilation.
rate during emersion. A large (350g) specimen of a giant kokopu, *G. argenteus*, could also orientate itself and move around on land. It also survived at least 8 hours of emersion, although longer periods were not tested in order to conserve the specimen. There are reports of the hardiness of giant kokopu when occasionally encountered on eeling trips and their tenacity for life when left in air. Therefore, even large galaxiids have potential for survival during emersion at least at lower temperatures. Eldon (pers comm) has observed relatively large kokopu specimens to voluntarily leave experimental ponds presumably in search of better conditions. Woods (1963) also curiously commented on a 'foreign galaxias', kept in an ornamental pond, that was in the habit of taking frequent "walks" in the garden! Woods also noted that galaxiid fish transport best in containers of damp moss. Thus, the general ability of galaxiid fish to survive in air has been appreciated for some time.

In laboratory aquaria most of the 'climbing species' would leave their tanks and disperse throughout the aquarium room system unless the lids of aquaria were tightly sealed. Galaxiid fish treated for disease would similarly leap from tanks or climb and adhere to the sides of tanks in air. Thus, galaxiid fish may consider leaving the water to be a alternative to many changes in aquatic conditions. They might therefore be expected to move overland between bodies of water as described for eels. The family could, therefore, almost be generalised as an amphibious family of fishes.

The skin thickness of the larger fishes bears no relation to the small specimens. Above 30g bodyweight most galaxiids appeared to possess greatly increased skin thickness. This is first exhibited in the dorsal regions and with further size increase, increases over the entire body surface. The increase in thickness is brought about by an increase in the
middle layers of the epidermis which make up a squamous layer. The inner and outer layers remain unchanged in form or thickness. It is probably this skin development that led McDowall (1978) to describe galaxiids as possessing a 'thick leathery skin'.

Since I have already commented on skin structure possibly being a restriction of further development of larval skin, this development may be viewed as a 'delayed' metamorphosis or development of the skin. Thus, it may only be in the larger fish that the larval condition or mechanisms are totally discarded in further development. This could therefore be viewed as a major change of strategy in galaxiid fish. The distinction of fish size may therefore be of extreme importance when generalising on the cutaneous respiration and survival abilities of galaxiid fish.
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Oxygen uptake by the skin of the Canterbury mudfish, *Neochanna burrowsius*

A. S. MEREDITH1
P. S. DAVIE2
M. E. FORSTER1*

1Department of Zoology
University of Canterbury
Christchurch 1, New Zealand
2Department of Physiology and Anatomy
Massey University
Palmerston North, New Zealand

Abstract Canterbury mudfish (*Neochanna burrowsius*) were held in small respirometer chambers with a rubber dam separating head and body. Oxygen uptake by the head and by the body were determined in water and in air. In both media cutaneous \(O_2\) uptake is a significant component (>43%) of total \(O_2\) consumption (\(V_{O_2}\)). In air, more carbon dioxide was lost to the body compartment than to the head compartment. With the head in air and the body in hypoxic water (PO\(_2\) < 20 mm Hg) there was no loss of \(O_2\) to the water over 2 h, and \(V_{O_2}\) was maintained by an increase in \(O_2\) uptake by the head.

Keywords *Neochanna burrowsius*; Galaxiidae; air-breathing fish; respirometry; cutaneous oxygen uptake; oxygen consumption (\(V_{O_2}\)).

INTRODUCTION

The Canterbury mudfish *Neochanna burrowsius* (Phillips) is found in slow-flowing swampy streams and ponds—habitats that can become hypoxic (Eldon 1979a). Mudfish are able to survive periods when their habitat becomes dry, and can breathe air when water becomes hypoxic (Eldon 1979b, Meredith 1981). Cutaneous oxygen \(O_2\) uptake has been described for numerous teleost fish species, in both air and water (Krogh 1904, Nonnotte & Kirsch 1978). As with other galaxiids, *Neochanna burrowsius* lacks scales (McDowall 1978); its slender, tubular shape gives it a high surface area/mass ratio.

In this paper we describe experiments designed to measure cutaneous \(O_2\) uptake by *N. burrowsius* held both in water and in air. In a further experiment we tested the possibility of net \(O_2\) loss via the transcutaneous route when the fish is breathing air with the body in hypoxic water.

MATERIALS AND METHODS

Experimental animals
Canterbury mudfish ranging in weight from 4.17 g to 10.02 g were supplied by Mr G. A. Eldon (Fisheries Research Division, MAF) from breeding stocks held in ponds. Before use they were maintained for up to 9 months in running dechlorinated tapwater at 16–17°C under a 12:12 h photoperiod on a diet of tubificid worms.

Respirometer chambers
Respirometer chambers were constructed from the barrels of 50 ml plastic syringes, over one end of which were fitted equivalent lengths of plastic tubing. Rubber dams were constructed from the fingers of surgical gloves ('Regal'; Batavian Rubber Co., Featherston). Each mudfish was lightly anaesthetised in a 0.01% solution of benzocaine, and a dam was slipped over its head to a position just caudad of the pectoral fins. Denture adhesive ('Super Polygrip'; Frank Stevens Ltd, Auckland) was used to cement the dam to the skin. The outer margin of the dam was folded over the end of one half of the respirometer chamber, and the end of the other half of the chamber was slipped over the dam to create a tight seal. Thus, the mudfish was held in place in the chamber with head and body in separate compartments. The ends of the chamber were sealed with rubber bungs through which passed inlet and outlet tubes for water circulation and hypodermic needles for water sampling. Small, glass-coated iron rods were placed in each compartment, and by means of a magnet these were used to stir the water before samples were taken. The residual volumes of the head and body compartments were measured for each fish at the end of each experiment.

Experimental protocol
After the rubber dams were fitted, the fish were left for at least 2 h before head, body, and (by addition) total \(O_2\) consumption were measured. The respirometer chambers were submerged in a water bath maintained at 17°C.
Table 1. Oxygen uptake and carbon dioxide excretion by the head and body of Neochanna burrowensis in water and air, and by the head alone when the body was in hypoxic water; units (STPD) ml kg\(^{-1}\) h\(^{-1}\) at 17°C, 100% relative humidity. Carbon dioxide excretion in water was not measured.

<table>
<thead>
<tr>
<th></th>
<th>Water VO(_2)</th>
<th>Water VO(_2)</th>
<th>Head VO(_2)</th>
<th>Head VO(_2)</th>
<th>Air VO(_2)</th>
<th>Body VO(_2)</th>
<th>Air VO(_2)</th>
<th>Body VO(_2)</th>
<th>Air VCO(_2)</th>
<th>Body VCO(_2)</th>
<th>Head in air, body in hypoxic water VO(_2)</th>
<th>Body VCO(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20.93 ± 1.71</td>
<td>15.73 ± 1.87</td>
<td>32.80 ± 2.42</td>
<td>12.85 ± 0.96</td>
<td>24.58 ± 1.53</td>
<td>17.48 ± 1.32</td>
<td>51.69 ± 2.91</td>
<td>16.65 ± 1.32</td>
<td>15.73 ± 1.40</td>
<td>16.65 ± 1.32</td>
<td>32.80 ± 1.40 (n = 11)</td>
<td>16.65 ± 1.32</td>
</tr>
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</table>

In the first experiment water was circulated through the respirometer chambers, using separate pumps for head and body compartments (modified Cole Palmer ‘Masterflex’ and Watson Marlow ‘H. R. Flow-Inducer’ respectively). Flow rates were set at approximately half the volume of each compartment per min. Water was recirculated through 500 ml reservoirs gassed with ambient air. To measure oxygen consumption (VO\(_2\)) the appropriate pump was stopped and the chamber inlet and outlet were clamped shut. The fish depleted the O\(_2\) in the water, and the partial pressure (P\(_{O_2}\)) was measured at intervals over a 1 h period. P\(_{O_2}\) was not allowed to fall below the critical oxygen tension (Pc\(_{O_2}\)) of 52 mm Hg* determined by Meredith (1981).

In the second experiment VO\(_2\) was determined with the head and body in air. The experimental animals had not been exposed to air in the days before use. The chambers were refilled with ambient air just before the experiment, and were then sealed off. A few drops of water remaining in each chamber maintained saturated vapour pressure. The animals were left for an average of 4.5 h, to allow significant depletion of O\(_2\).

In the third experiment the P\(_{O_2}\) of the water in the body compartment was reduced to less than 20 mm Hg by gassing the reservoir with O\(_2\)-free nitrogen. The flow was then stopped, and the P\(_{O_2}\) of the hypoxic water was measured while the head of the mudfish was in air.

**Gas analysis**

The P\(_{O_2}\) of the water in the circulating systems was monitored using Beckman Type 39533 O\(_2\) electrodes with Fieldlab analysers. Partial pressures of O\(_2\) in air and water and of carbon dioxide (P\(_{CO_2}\)) in air were measured in 400 l samples of water or air using a blood gas analyser (‘Micro 13’; Instrumentation Laboratories). The blood gas analyser was calibrated at 17°C using 2 humidified gas mixtures, 20% O\(_2\) / 5% CO\(_2\) / 75% N\(_2\) and pure N\(_2\).

Results are expressed as ml O\(_2\) (STPD) consumed per kilogram of fish weight per hour at 17°C, and are given as mean ± 1 sample SEM. Student’s t-test was used to establish significant differences (P < 0.05).

**RESULTS**

All animals appeared to be in good condition after the experiments. Mudfish were normally quiescent while in the chambers, but occasionally struggled when exposed to air.

In both water and air the body contributed more than 43% of the total VO\(_2\) (Table 1). Total VO\(_2\) was significantly less in water-breathing mudfish than in air-breathing specimens (P < 0.001). VO\(_2\) values for both head (P < 0.001) and body (P < 0.001) were smaller in water (Table 1). Meredith (unpubl. results) has measured total VO\(_2\) of unrestrained mudfish in water and in air, and the values are similar to those reported here. Thus, attachment of the rubber dam did not affect total VO\(_2\).

When the body was surrounded by hypoxic water, head VO\(_2\) increased significantly (P < 0.001) but total VO\(_2\) was not significantly different from total VO\(_2\) with both head and body in air (Table 1). While the head of the mudfish was in air there was no significant loss of O\(_2\) from the animal to the hypoxic water over a 2 h period. The average rise in P\(_{O_2}\) of the water was 1.0±1.4 mm Hg (n = 12).

In air, mudfish lost more CO\(_2\) through the body than through the head (P < 0.01; Table 1). The respiratory quotient (R) for the body was significantly greater than that for the head (R = 0.71±0.06 and 0.39±0.03 respectively; P < 0.001, n = 11).

We performed several checks on the competence of the partition between head and body. In one experiment the body compartments of 3 fish were perfused with water that had been gassed with pure O\(_2\) to a saturation of greater than 80%, while the head compartments were perfused with air-saturated water. The pumps were stopped and the compartments were sealed off. After 30 min there was a fall in the P\(_{O_2}\) of water in the head.
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compartment. In a similar experiment using rubber dams without fish there were no changes in the PO₂ of the head compartment.

It should be noted that when the head compartment was air-filled and the body compartment was filled with hypoxic water (PO₂ < 20 mm Hg) there was no rise in PO₂ in the body compartment, despite the partial pressure gradient across the dam. Our method of separating the head and body forms an effective barrier to the movement of oxygen, therefore. In addition we measured biological oxygen demand (BOD) of the water in the absence of fish. Since the volume of water used was small in relation to the mass of the mudfish, and BOD was low, we did not find it necessary to subtract these small blank values from the measured VO₂.

DISCUSSION

When head and body are externally partitioned, head VO₂ will include O₂ uptake by the skin of the head region in addition to branchial uptake. We consider that body VO₂ will underestimate total cutaneous uptake by 15–20%. The results show that, at 17°C, O₂ uptake across the skin of the body is greater than 43% of total VO₂ of N. burrowsi, in both water and air. A similarly high cutaneous VO₂ uptake has been reported for the estuarine mudskippers Periophthalinus sobrinus (Teal & Carey 1967) and P. cantonensis and Boleophthalmus chinensis (Tamura et al. 1976). There are no reports of such a high cutaneous component of total VO₂ for freshwater teleosts, though Sacca & Burggren (1982) report that in the paleoniscid Calamoichthys calabaricus cutaneous uptake accounted for 32% of total VO₂ in water. Relative to VO₂ in water, mudfish showed increased VO₂ when in air; this was perhaps associated with greater activity. Misgurnus fossilis (Jeuken 1957, cited in Kirsch & Nonnotte 1977) and Anguilla anguilla (Berg & Steen 1965) showed a reduction of VO₂ during air exposure. Berg & Steen (1965) have also shown the cutaneous component to become relatively more important in air-exposed eels. By contrast, VO₂ in air was higher than in water for P. sobrinus (Teal & Carey 1967), for Monopterus albus (Liem 1967), for Synbranchus marmoratus (Bicudo & Johansen 1979), and for Calamoichthys calabaricus (Sacca & Burggren 1982), all of which are facultative air breathers. Not only does N. burrowsi maintain the same proportion of cutaneous VO₂ when in air, but when O₂ uptake by the body is prevented, the head's VO₂ can be increased in compensation.

Kirsch & Nonnotte (1977) found a significant cutaneous O₂ uptake in 3 species of freshwater teleost, but demonstrated that isolated patches of skin had high O₂ consumption, and suggested that there was no net transcutaneous movement of O₂. However, these authors presented evidence for the transcutaneous uptake of O₂ in fishes adapted to sea water (Nonnotte & Kirsch 1978). The skin of N. burrowsi is thin, and an extremely high O₂ consumption would be required to account for all cutaneous uptake. In one fish that died, skin represented 4% of total body weight.

When the body was in hypoxic water there was no loss of O₂ from the blood to water over a 2 h period. Thus, in the short term, O₂ loss to hypoxic water is not a problem when the fish is breathing air at the water's surface. Can mudfish reduce cutaneous gas exchange, possibly by vasomotor control? The skin is an important site of CO₂ loss in air, the R value for the body being higher than that for the head. Over longer periods CO₂ may accumulate in the blood when the fish is airbreathing in hypoxic water.

When Meredith (1981) exposed mudfish to water made progressively hypoxic, 3 fish left the water at a PO₂ of less than 21 mm Hg, and did so repeatedly when returned to it. Over periods in excess of 2 h, increased skin conductance associated with CO₂ loss by the cutaneous route may lead to O₂ loss to the water. Our observations raise interesting questions about gas exchange in mudfish which survive long periods of drought.

REFERENCES


BLOOD OXYGEN AFFINITY IN THE AMPHIBIOUS FISH NEOCHANNA BURROWSIUS (GALAXIIDAE: SALMONIFORMES)

R. M. G. WELLS, M. E. FORSTER,* AND A. S. MEREDITH*

Department of Zoology, University of Auckland, Auckland, and *Department of Zoology, University of Canterbury, Christchurch, New Zealand

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The erythrocytes of the amphibious mudfish, Neochanna burrowsi, contained 6.8 μmol ATP/ml red cells and 4.4 μmol GTP. Trinucleotide concentrations were not significantly changed in fish acclimated for 3 wk in moist air. The oxygen affinity, defined by the half-saturation oxygen tension (P(O2)) of whole blood at 12°C was 11 mm Hg at pH 7.8 and 18 mm Hg at pH 7.4 in water-breathing mudfish. No appreciable Root shift was observed. O2 affinity decreased in air-acclimated fish at low pH (25 mm Hg at pH 7.4). This corresponded to an increase in the Bohr effect (∆log P(O2)/∆pH) from −0.6 in water to −0.8 in air. The similarity between oxygen equilibrium curves and other hematological parameters from the two groups of fish reflects the common mode of gas exchange, with more than 50% of oxygen taken up by skin in either air or water.

INTRODUCTION

Fish living in slow-moving or temporary bodies of water which periodically become depleted of oxygen may have to breathe air in order to survive. Fish from a great number of families use atmospheric air as an oxygen source on occasion (Johansen 1970), and where air breathing is habitual it is often accomplished with a specialized air-breathing organ (Dehadrai and Tripathi 1976; Randall et al. 1981). However, a number of fish which can move onto land have no accessory air-breathing organs and rely on gills and skin for aerial gas exchange (Berg and Steen 1965; Johansen 1966; Tamura, Morii, and Yazurih 1976).

The prevailing view that the blood of air-breathing fish has a higher oxygen capacity, P50, and Bohr shift and greater red cell organic phosphate concentrations than that of water-breathing fish has been challenged (Powers 1980) but, when closely related species are compared, holds true (Johansen, Mangum, and Lykkeboe 1978; Riggs 1979). It is not clear whether a change in respiratory properties of the blood should be expected where the predominant site of gas exchange is unchanged in air or water.

The Canterbury mudfish, Neochanna burrowsi (Phillips), inhabits slow-moving streams and ponds, which it leaves to live among moist vegetation or under stones when the oxygen tension in the water falls below 20 mm Hg or in drought (Eldon 1979). The mudfish has a cutaneous oxygen uptake rate in both air and water, which is higher than that reported for other freshwater fish (Meredith, Davie, and Forster 1982), and lacks accessory air-breathing structures. In this study, the transport properties of mudfish blood are described for fish acclimated in air and water.

MATERIAL AND METHODS

The mudfish used in this study had been held for several months in aerated aquaria with running fresh water. Since the species is rare and endangered, only small numbers were used in the study and none could be sacrificed. The experimental animals were divided into two groups of similar mean weight, and half the fish were placed for 3 wk into plastic food boxes lined with moist cotton wool. Light regimes and temperature (12°C) were identical for both groups.

The weight range of the fish was 7–12 g and precluded the possibility of cannulation for blood sampling. Instead, blood
was taken by acute puncture of the bulbus arteriosus or ventricle, using a heparinized 1-ml tuberculin syringe and 27-gauge needle. In this way, 30–60 μl of blood was taken within a minute of first handling a specimen.

Hemoglobin (Hb) concentration was estimated spectrophotometrically from 2-μl samples using the met-cyanide derivative (Dacie and Lewis 1975). As an added precaution, the met-cyanide solutions were centrifuged to remove red cell debris prior to optical determination. Hematocrit (Hct) was estimated by centrifugation of 3-μl blood samples at 8,000 g for 5 min in glass capillary tubes.

Red cell trinucleotides were resolved by thin layer chromatography (TLC) on cellulose plates impregnated with polyethyleneimine (Cashel, Lazzarini, and Kalbacher 1969) with technical modifications by Johansen et al. (1976). Protein precipitation of 20-μl blood samples with trichloroacetic acid provided clear supernatants which were applied to TLC plates. Following one-dimensional development in phosphate buffer, pH 3.5, we identified and circumsected "spots" under UV light and eluted them with Tris-HCl-Mg²⁺ buffer, pH 7.5. Adenosine triphosphate (ATP) and guanosine triphosphate (GTP) concentrations were ascertained from absorption maxima using the appropriate extinction coefficients. Relative mobilities of trinucleotide compounds obtained from Sigma (USA).

Continuous, whole blood oxygen equilibrium curves were obtained from thin blood films (~1 μl) using a Hemoscan instrument (Aminco, USA) which was modified to reduce dynamic error (Lapennas, Colacino, and Bonaventura 1981; Wells and Weber 1983). Equilibration gases were forwarded by Wösthoff gas mixing pumps (Bochum, FRG) arranged in series to cascade CO₂ into the mixes. The possibility of a Root effect was investigated by running continuous curves to P₀₂ > 150 mm Hg and then switching out the CO₂. Any stepped increase in maximal saturation (P₁₀₀) was thus taken to represent a Root shift. This procedure assumes that the Root effect is negligible at pH > 8. Lengthy exposure to hyperbaric oxygen (P₀₂ > 160 mm Hg) was precluded by significant oxidation of the hemoglobin. Oxygen affinity was evaluated by P₅₀ as the Po₂ at which half the hemoglobin was saturated with oxygen. At the conclusion of a run, blood films were scanned in a Unicam SP-1800 recording spectrophotometer to discern the extent of oxidation to methemoglobin.

Blood pH was measured with an IL pH electrode and Micro-13 Blood Gas Analyzer following equilibration with the equilibrium gas mixes in an IL-213 tonometer (Instrumentation Laboratories, USA).

Freshly taken blood was used in all analyses and placed on ice prior to equilibration. Exposure of red cells to anoxic gas mixtures was avoided where possible in order to minimize trinucleotide depletion (Tetens and Lykkeboe 1981).

RESULTS
Trinucleotide concentrations and hematological indices from Novacanna blood were not significantly different for fish living in air or in water (P > 0.2) (table 1). The mean molar ratio of nucleoside triphosphate (NTP) to hemoglobin for all fish was 2.31 ± 0.99 mol NTP/mol Hb⁻¹.

The relationship between Pco₂ and pH for blood equilibrated in vitro suggested that the buffer capacity for both air- and water-acclimated fish could be described by the same regression line (fig. 1). Bicarbonate concentration was calculated from these data using the Henderson-Hasselbalch equation: HCO₃⁻ mmol liter⁻¹ = 10^pH-pK⁺ α·PCO₂, with solubility coefficients α and pK' from Severinghaus

---

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Hb (g liter⁻¹)</th>
<th>Hct (%)</th>
<th>ATP (μmol ml RBC⁻¹)</th>
<th>GTP (μmol ml RBC⁻¹)</th>
<th>NTP (Total) (μmol ml RBC⁻¹)</th>
<th>NTP/ATP mol mol⁻¹</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>59.1 ± 16.8</td>
<td>24.4 ± 5.7</td>
<td>6.77 ± 2.26</td>
<td>4.19 ± 1.53</td>
<td>11.16 ± 3.33</td>
<td>1.57 ± 0.14</td>
<td>9.58 ± 1.74</td>
</tr>
<tr>
<td>Air</td>
<td>84.8 ± 11.6</td>
<td>24.9 ± 4.0</td>
<td>5.72 ± 1.41</td>
<td>4.19 ± 1.12</td>
<td>9.91 ± 0.85</td>
<td>1.47 ± 0.66</td>
<td>9.10 ± 2.40</td>
</tr>
</tbody>
</table>

*Note:* Results expressed as mean ± SD for two groups of fish.
BLOOD OXYGEN AFFINITY IN AN AMPHIBIOUS FISH

At pH 7.65 and 8.20, HCO₃⁻ concentrations would be 17.6 and 6.2 mmol liter⁻¹, respectively. From these figures, buffer capacity, ΔHCO₃⁻/ΔpH, would be 19 mmol liter⁻¹ per pH.

The oxygen equilibrium curves of whole blood typically show a high affinity for oxygen at 12 C with half-saturation tension, $P_{50}$ = 11 mm Hg at pH 7.8 and 18 mm Hg at pH 7.4 (fig. 2a). No appreciable Root effect was observed in the pH range examined. Double logarithmic transformations of these equilibrium data, shown in figure 2b, estimate Hill's coefficient, $n$, the measure of cooperative oxygen binding. The mean value of $n$ was 1.64 ± 0.16 (32 curves), indicating a moderate degree of cooperative oxygen binding. Cooperativity did not appear pH dependent.

The sensitivity of the equilibrium parameter $P_{50}$ to pH is given in the Bohr plots of figure 3 and shows a small increase in the Bohr slope, $\delta$ (= $\Delta \log P_{50}/\Delta \text{pH}$), from −0.6 in water to −0.8 in air. The range of $P_{50}$ values is also indicated in figure 3, and the data are fitted by least squares regression. The small number of individuals examined precluded further statistical analysis.

Methemoglobin did not exceed 5% of total hemoglobin throughout the pH range examined.

DISCUSSION

Neochanna burrowsii does not possess an exceptionally high oxygen carrying capacity or blood oxygen affinity. Other air-breathing fish are reported to have high

![Graph](image-url)

**Fig. 1.** $PCO_2$-pH diagram for *Neochanna* blood at 12 C. $\bigcirc$ = air-breathing fish, $\bullet$ = water breathers.

**Fig. 2.** Representative whole blood oxygen equilibrium curves for (a) blood from water-breathing *Neochanna* at 12 C, and (b) log-transformed data depicting the corresponding Hill plots.

**Fig. 3.** Bohr plots for *Neochanna* blood at 12 C for air-exposed (O-O) and water-breathing ( Jebb) fish. Each data point represents one $O_2$ affinity determination, and lines were fitted by least squares regression.
hemoglobin concentrations and thus high oxygen carrying capacities (Johansen 1970; Johansen et al. 1976, 1978; Lomholt and Johansen 1976). Moreover, oxygen carrying capacity in the catfish Hypostomus rose after a few minutes of air exposure (Farvareto, Petenuscii, and Lopes 1981). Since we did not limit oxygen as an environmental factor during acclimation of the mudfish, we surmise that the responses noted by previous authors are principally responses to compensate for hypoxia and hypercapnia which are also manifested by water-breathing fish (see reviews by Wood and Lenfant [1979]; Powers [1980]; Weber [1982]). Powers (1980) has surveyed the blood oxygen transport properties of numerous air- and water-breathing fish and found considerable overlap in the range of oxygen affinities. He attributed this to relative efficiencies of gas exchange in different species rather than the breathing medium per se. As Neochanna takes up oxygen at similar rates in air and water (Meredith et al. 1982) and over half that oxygen crosses the skin in both media, it is perhaps not surprising that there are no significant differences between the bloods of air- and water-breathing fish.

Carbon dioxide loss from the specialized air-breathing organs of fish tends to be low, and blood carbon dioxide tensions are elevated above those of water-breathing fish (Randall, Farel, and Haswell 1978; Randall et al. 1981). As diffusion limits cutaneous gas exchange and calculated diffusion constants for carbon dioxide in skin are about 20 times higher than those for oxygen (Piiper 1982), we would not expect carbon dioxide to accumulate in the blood of air-breathing mudfish, unless the dehydration of bicarbonate is limiting. Over the first 4–5 h of air exposure, the gas exchange ratio (R) was only 0.52 in Neochanna (Meredith et al. 1982), and in Synbranchus, where the gills and skin are used in bimodal breathing, Heisler (1982) found that arterial carbon dioxide tensions rose more than fourfold over 10 h and plasma pH fell by 0.6 units. Neochanna blood showed an increased sensitivity to acid on air exposure, but the blood has a high buffering capacity, intermediate between that of the air-breathing fishes Hypostomus (Wood, Weber, and Davis 1979) and Amphipnous (Lomholt and Johansen 1976). It is possible that, at least in the short term, the in vivo oxygen-binding properties of blood and/or cardiac output are very different from those in the water-breathing mudfish. A study of the modulation of the oxygen transport functions by different hemoglobins or by urea might be rewarding.

When a wide range of air-breathing species is studied, one might expect a correlation between total red cell trinucleotide concentration and the ability to breathe air because these phosphate compounds are modulators of blood oxygen affinity. A survey of trinucleotides in fish erythrocytes does not, however, suggest a correlation (Bartlett 1980). Nonetheless, there is a tendency for air-breathing fish to show a high concentration of GTP relative to total NTP (Issacks, Kim, and Harkness 1978; Bartlett 1980), and this is also the case in Neochanna (table 1).

A study of the responses of Neochanna to hypoxia should confirm our contention that the breathing medium in itself is not a sufficient cause for modulation of the oxygen transport properties of blood.

LITERATURE CITED

BLOOD OXYGEN AFFINITY IN AN AMPHIBIOUS FISH


BASIC program for Apple IIE microcomputer for calculating oxygen consumption from Gilson respirometry data.

1 CLEAR
10 REM PROGRAM FOR RESPIROMETER DATA
20 REM CALCULATING O2 CONSUMPTION RATES FROM RESPIROMETRY DATA
25 V$ = CHR$(4)
30 DIM WEIGHT(20), D(2), TH(30), R2(30), G(30), Gl(30)
40 PRINT "INPUT NO OF VESSELS EXCLUDING THERMOBURETTES, NO OF READINGS, TIME INTERVAL BETWEEN EACH READING IN MINS, AND NO OF THERMOBURETTES"
50 INPUT F, B, C, D
60 PRINT "DO YOU WANT TO TEST FOR SIGNIFICANCE AND CORRECT FOR THERMOBURETTE DATA, ANS Y OR N"
80 IF S$ = "N" THEN 595
70 T = T + 1
140 IF T > D THEN 425
150 PRINT "INPUT X VALUES (O2 USAGE) FOR THERMOBURETTE FLASK NO";
160 FOR I = 1 TO B
170 INPUT TH(I)
180 S1 = S1 + TH(I)
190 S2 = S2 + ((I - 1) * C)
200 NEXT I
210 REM ALL THERMOBURETTE DATA FOR THIS VESSEL HAS BEEN ENTERED
220 REM REGRESSION TO BE CALCULATED
230 M1 = S1 / (I - 1)
240 M2 = S2 / (I - 1)
250 FOR I = 1 TO B
260 S3 = S3 + (TH(I) - M1) ^ 2
270 S4 = S4 + (((I - 1) * C) - M2) ^ 2
280 S5 = S5 + (TH(I) - M1) * (((I - 1) * C) - M2)
310 NEXT I
320 T(T) = S5 / S4
345 T1 = T1 + T(T)
350 T1(T) = M1 + (T(T) & M2)
360 REM CALCULATING COEFF OF DETERMINATION
370 R(T) = S5 ^ 2 / (S3 * S4)
380 REM TESTING SIGNIFICANCE OF REGRESSION FROM ZERO
390 SD = (1 / (B - 2) * (S3 - (S5 ^ 2 / S4))) ^ 0.5
400 TS = (T(T) - O) / (SD / (S4 ^ 0.5))
421 GOTO 135
422 MT = T3 / D
425 PRINT V$"PR#1"
430 PRINT TAB(10)"THERMOBURETTE DATA"
440 PRINT TAB(10)"**COEFFICIENT**"
450 PRINT TAB(2)"No" TAB(8)"EQUATION OF" TAB(25)"SIGNIFICANCE" TAB(3)"9"
460 PRINT TAB(11)"LINE" TAB(28)"OF LINE" TAB(40)"DETERM"
470 PRINT TAB(11)"****" TAB(28)"OF LINE" TAB(40)"DETERM"
480 PRINT "**COEFFICIENT**"
490 FOR K = 1 TO D
495 Q$ = "$n$
500 IF T2(K) < 1.960 THEN Q$ = "NS"
520 PRINT TAB(2)K TAB(6)"Y=" TAB(2)K TAB(6)"T1(K)" "TAB(2)K TAB(6)"T2" TAB(2)K TAB(6)"M" TAB(43)R(K)
530 NEXT K
540 PRINT "**COEFFICIENT**"
545 PRINT "SIGNIFICANCE OF LINE CALCULATED AT 5% BY REGTEST AGAINST ZERO"
546 PRINT "**NS** INDICATES THERMOBURETTE DATA IS NOT SIGNIFICANTLY DIFFERENT FROM A LINE OF"
547 PRINT "ZERO SLOPE"
548 PRINT
549 PRINT
550 REM PRINTER OFF
560 PRINT V$"PR#0"
565 PRINT " DO YOU WANT TO DELETE ANY OF THE THERMOBURETTE DATA, ANSWER Y OR N "
566 INPUT T$
567 IF T$ = "N" THEN GOTO 585
568 E = D
569 PRINT " INPUT THERMOBURETTE NO TO BEDELETED"
570 INPUT S$
571 T(S) = 0
572 E = E - 1
573 PRINT " DO YOU WANT TO DELETE ANOTHER? ANS Y OR N"
574 INPUT S$
575 IF S$ = "Y" THEN 669
576 FOR K = 1 TO D
577 TI = TI + T(K)
578 NEXT K
579 MT = TI / E
580 REM FINDING REGRESSION FOR EACH VESSEL
581 G = G + 1
582 PRINT " INPUT MEAN TEMPERATURE TAKEN WITH READINGS IN DEGREES C"
583 INPUT TME
584 PRINT " INPUT BAROMETRIC PRESSURE LESS SATURATED VAPOUR PRESSURE IN MM HG"
585 INPUT P
586 PRINT " INPUT X VALUE FOR VESSEL NO.";G"ONE PER LINE."
587 FOR I = 1 TO B
588 INPUT RESP(I)
589 CC = CC + RESP(I)
590 NEXT I
591 PRINT " INPUT WEIGHT OF ANIMAL IN VESSEL";G"IN GRAMS"
592 INPUT WEIGHT(G)
593 ME = CC / B
594 MF = C1 / B
595 FOR I = 1 TO B
596 C3 = C3 + (RESP(I) - ME) ^ 2
597 C4 = C4 + (((I - 1) * C) - MF) ^ 2
598 C5 = C5 + (RESP(I) - ME) * (((I - 1) * C) - MF)
599 NEXT I
600 REM NOW DO REGRESSION
601 G(G) = C5 / C4
602 G1(G) = MF - G(G) * ME
603 R2(G) = C5 ^ 2 / (C3 * C4)
604 G = G + 1
605 IF G > F THEN 890
606 GOTO 600
607 REM PRINT " RESEARCH THERMOBURETTES"
608 INPUT T$
609 IF T$ = "N" THEN 960
610 REM CORRECT SLOPE FOR THERMOBURETTES
611 FOR J = 1 TO F
612 G(J) = G(J) - MT
613 NEXT J
614
960 FOR J = 1 TO F
970 REM SLOPE TO BE PLACED IN THE FORM 60/C 02 USED/VESSEL/HR
980 G(J) = G(J) * 60
990 REM NOW PUT SLOPE IN THE FORM 02USED/CM/HR
1000 G(J) = G(J) / WEIGHT(J)
1010 NEXT J
1020 PRINT "DO YOU WANT TO CORRECT FOR STF?'ANS Y OR N"
1030 INPUT S$
1040 IF S$ = "N" THEN 1081
1050 ST = (273 * P) / (760 * (TEMP + 273))
1060 FOR J = 1 TO F
1070 G(J) = G(J) * ST
1080 NEXT J
1081 PRINT "INPUT DATE IN FORMAT DAY,MONTH,YEAR AND I.D. NUMBER"
1082 INPUT X,Y,Z,ZI
1083 PRINT "PRINTER ON"
1085 PRINT V$"PR#1"
1090 PRINT TAB(10)"RESPIRATION RESULTS"
1100 PRINT TAB(10)"************ **********"
1110 PRINT
1120 PRINT "VESSEL" TAB(7)"SAMPLE" TAB(16)"RATE02USAGE" TAB(33)"COEFF"
1130 PRINT TAB(2)"No" TAB(7)"WEIGHT" TAB(16)"MICRO2/2/HR" TAB(34)"DETERM"
1140 PRINT "****************************
1150 PRINT
1160 FOR I = 1 TO F
1170 PRINT TAB(2)I TAB(6)WEIGHT(I) TAB(18)G(I) TAB(33)R2(I)
1180 NEXT I
1190 PRINT
1200 PRINT "***********************
1210 PRINT "THE TEMPERATURE OF THE SYSTEM WAS ";TEMP;"DEGREES C"
1220 PRINT "RUN TERMINATED ON";X"";Y"";Z;";ZI".DATA IDENTIFICATION NUMBER
1225 PRINT V$"PR#0"
1226 PRINT "DO YOU WANT ANOTHER RUN, ANSWER Y OR N ?"
1227 INPUT W$
1228 IF W$ = "Y" THEN 1
1230 END
APPENDIX 4

DETERMINATION OF AMMONIA AND UREA

The phenyl-nitroprusside method for the determination of ammonia was employed (adapted from Trietz (1970)). Urea concentration was determined by digestion with a urease solution (Sigma Chemical Co.) and the liberated ammonia measured by the above method.

Reagents used were: As in Trietz, but with 1.0N sodium hypochlorite in 0.1N sodium hydroxide replacing 0.42g of sodium hypochlorite in step 3.

Procedure:

I: Ammonia Nitrogen

1. One test-tube was labelled "blank", one "standard" and one for each water sample.
2. Two ml of tap water was pipetted into the blank and 1 ml of tap water into each of the other tubes.
3. 1 ml of 5ug/l ammonia std solution was pipetted into the "standard" tube.
4. 1 ml of each water sample was pipetted into the correct tubes.
5. Adding rapidly and successively mixing after each addition, 5 ml of phenyl-nitroprusside solution and 5 ml of alkaline hypochlorite solution were added to each tube.
6. All tubes were placed in a water bath at 40 degrees C for 20 mins. with regular shaking.
7. The absorbance of each sample was measured using a Bausch and Lomb Spectronic 20 at 560 nm.
8. Ammonia nitrogen concentration was determined as:

\[
\frac{\text{ABS unknown}}{\text{ABS std}} \times 5 \times 0.2 = \mu gN/g/hr
\]

\[
\text{fish weight x time (hrs)}
\]

II : Urea Nitrogen.

1. Test-tubes were labelled similarly to ammonia determinations.

2. 1 ml of urease working solution was pipetted into each tube.

3. 1 ml of tapwater was pipetted into the "blank" tube,
   1 ml of 5µg/l urea std into the "std" tube and 1 ml of each water sample into the correct tubes.

4. All tubes were incubated in a waterbath at 40 degrees C for 20 mins with regular shaking.

5. Proceed as steps 5 to 9 for ammonia determination.

The value obtained for ammonia nitrogen is subtracted from this value to give the urea concentration.
## APPENDIX 5

### GILL MORPHOMETRIC DATA

1. Total number of filaments per arch.

<table>
<thead>
<tr>
<th>#</th>
<th>Weight (g)</th>
<th>Gill Arch 1</th>
<th>Gill Arch 2</th>
<th>Gill Arch 3</th>
<th>Gill Arch 4</th>
<th>Total per side</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>0.436</td>
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<td>60</td>
<td>48</td>
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2. Mean filament length (mm).

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<th>Gill Arch 3</th>
<th>Gill Arch 4</th>
<th>Mean</th>
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### 3. lamellae per mm (one side)

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### 4. Lamellar area (one side only). \( \text{mm}^2 \times 10^{-2} \)

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