Live Trout, Not Dead
Enhancement of ship-board survival of Coral trout destined for the live fish market

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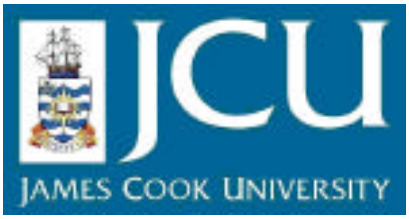

FISHERIES
RESEARCH &
DEVELOPMENT
CORPORATION

Project No. 97/341

National Library of Australia Cataloguing-Publication entry

Enhancement of ship-board survivorship of Coral trout destined for the live fish market.

Bibliography.

Includes index.

ISBN 0 86443 702 1

This publication should be cited as Anderson *et al*. (2003).
Enhancement of ship-board survivorship of Coral trout destined for the live fish market. James Cook University, Townsville. Pp.

Published by James Cook University 2003.

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LIST OF ABBREVIATIONS

ACTH	Adrenocoticotrophic Hormone
AVT	Arginine vasotocin
CPM	Counts per minute
DO	Dissolved oxygen
$g.L^{-1}$	Grams per litre
GBR	Great Barrier Reef
GH	Growth hormone
Hb	Haemoglobin
Hct	Haematocrit
HPI	Hypothalamopituitary interenal axis
IGF	Insulin-like growth factor
ISO	International Standards Organisation
kg	kilograms
L	Litres
M	Molar
MCH	Melanin concentrating hormone
$mg.L^{-1}$	Milligrams per litre
min	Minute
mM	millimolar
MSH	Melanophore stimulating hormone
MT	Metric tonnes
ng	Nanogram
$ng.ml^{-1}$	Nanograms per millilitre
NSB	Non specific binding
POMC	Propiomelanocortin
ppt	Parts per thousand
PRL	Prolactin
RIA	Radioimmunoassay
SC axis	Sympathetico-chromaffin axis
sec	Second
T	Testosterone
µl	Microlitre
µm	Micrometre

NON TECHNICAL SUMMARY

97/341	**Enhancement of ship-board survivorship of Coral trout destined for the live fish market**

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OBJECTIVES:

1. To increase fish survival in the live Coral trout fishery.
2. To identify practices in the harvest, ship-board transport and holding of live Coral trout that are the major stressors.
3. To identify the impact of these stressors on survival and disease resistance.
4. To develop benchmark practices for the harvesting, ship-board transport and holding of live Coral trout that alleviate stressors and improve survival.
5. To inform the industry and management of benchmark practices.
6. To assist with the implementation and to evaluate the implementation of benchmark practices in the live trout industry.

OUTCOMES ACHIEVED

This project has improved practices in the live Coral trout fishery resulting in increased survival of fish captured and improved profitability of enterprise.

It has also improved the ease with which fishers can move from the dead fish to the live fish industry by providing a training tool which helps remove the technology and knowledge barrier.

KEYWORDS: Coral trout, stress response, live fish trade, mortality

NON-TECHNICAL SUMMARY

The major stressors of Coral trout taken in the live trout fishing industry result from poor water quality in the dory tank. This is due to poor dory tank design or poor operation. These stressors result in significantly increased levels of cortisol and blood glucose which provide clear physiological indicators of Coral trout stress.

Key water quality parameters that appear to cause stress are ammonia greater than 0.5 milligrams per litre and dissolved oxygen saturation les than 70%. Further, these two factors may act in concert and so fishers should maintain water quality such that oxygen content is high and ammonia content is low.

A design for a cylindrical tank with the inlet at the top and the outlet at the bottom providing plug or laminar flow through the tank was developed. This tank, of 185 litre and sized to fit into a dory, maintains high flow velocities with limited mixing at an exchange rate of 6 exchanges per hour, a volume easily achieved with off-the-shelf 12 volt bilge pumps used in the industry.

The maximum stocking density for this type of tank was determined to be 20 fish per tank or approximately 100 kg per cubic metre, which is equivalent to the maximum average catch rate per session achieved in the earlier study of dories in the fishery.

Feeding Coral trout were found to have lower circulating cortisol than animals that were starved. Coral trout found an increase in temperature of 5 °C to result in significant stress but moving to water up to 10 °C cooler did not. Handling induced a stress response, but this did not differ between fish held out of water for up to 6 min. By 6 min however, the animals had lost their righting response indicating that they here severely hypoxic (had very low blood oxygen) and holding them out of the water for this period should be avoided. Tank colour (light vs dark) had no effect on the stress response of the fish.

Anaesthetic served to lessen the stress response of handling but the chemical used in this study is not registered for food use so this fact is of little use for fish destined for human consumption unless food grade anaesthetics can be demonstrated to be of similar value.

Stress indicators in Coral trout immediately post capture showed significant seasonal variation. Levels were highest in the spawning season between October and November indicating that at this time of year, Coral trout are more likely to be compromised by handling stressors and provides an explanation for the greater problems reported by fishers in maintaining fish alive during the spawning season.

Numerous parasites were found on Coral trout but the levels of parasitism were not related to overall levels of stress. Their presence during levels of stress when immune function is reduced by stress, may result in opportunistic infections. These may be controlled by low salinity (10 ppt or one part seawater: two parts fresh water) bathing of fish immediately upon capture to reduce the parasite load.

Other factors in the normal harvest cycle of Coral trout were also evaluated during this study. These were the effect of using a dehooker, swim bladder deflation and the effect of depth of capture. While these were found to be stressful, they were also considered to be unavoidable. The conclusion reached is that the animals should handled as gently and as rapidly as possible. Swim bladder deflation through the dorsal wall of the rectum was not related to infection and was the most easily achieved practice.

Use of a dehooker is also recommended. This allows rapid release of the fish into the dory tank and does not add to the stress response. Fish captured at depth (20 m) are more likely to suffer swim bladder extrusion from the mouth or other swim bladder problems. Fish taken from this depth either appear healthy or moribund when placed into a dory tank. Moribund fish should be killed immediately for ethical and food quality reasons and placed on ice since they will not recover. Fish that appear healthy upon release into the dory tank are likely to remain so if good water quality in the dory tank is maintained.

A number of recommendations were developed. These are:

- Water quality in all tanks used to hold fish throughout the harvest cycle should be below 0.5 $mg.L^{-1}$ ammonia and above 70% dissolved oxygen saturation.
- This can be achieved by ensuring good water exchange in the tank (at least 6 $exchanges.h^{-1}$ even in a well designed tank) and good design such that wastes are rapidly removed from the water.
- The maximum stocking density of Coral trout in a dory tank should be 20 $fish.tank^{-1}$ or approximately 100 $kg.m^{-3}$. This also serves as a useful indicator of maximum stocking densities in other tanks.
- Coral trout held in main tanks should be fed.
- When moved into a different tank, Coral trout should be placed in water at or below the temperature of the water from which they are being moved.
- When being moved, fish should be kept out of water for as short a time as possible and for not more than 5 min.
- Tank colour (light vs dark) is of no consequence but individual colours (eg red, blue etc) may have an effect. We found unstressed in tanks that were white, black or blue.
- If health regulations and product acceptability allow it, fish should be anaesthetised when handled.
- Particular attention must be paid to ensuring good water quality when fishing during the spawning season.
- Parasites may be controlled by low salinity (10 ppt) bathing of fish shortly after capture.
- Use of a dehooker is recommended to reduce the overall stress period when the animal is physically handled.
- Swim bladder deflation should be conducted quickly and with minimal physical force to restrain the fish. Deflation through the dorsal wall of the rectum with a 16 gauge needle provides an easy and efficient way to achieve this.
- Fish captured at depth (20 m) should be assessed to determine if they are moribund or healthy. Moribund fish should be removed from the live harvest process and killed immediately.

A video entitled “Live Trout, Not Dead” was produced encompassing these recommendations for use as a training tool by the industry. In addition, the recommendations were used to develop a revised version of the code of practice for live Coral trout fishing published by the Queensland Seafood Industry Association.

ACKNOWLEDGEMENTS

This project was funded by the Fisheries Research and Development Corporation (Project 97/341). Salaries for the Principle Investigator (Dr T. Anderson) and Dr P. Appleford were provided by James Cook University, salaries for Drs B. Mapstone and C. Davies were provided by the Cooperative Research Centre for the Ecologically Sustainable Development of the Great Barrier Reef, salaries for Drs M. Bennett, S Bennett and A. Bradley were provided by the University of Queensland and salary for Dr L. Evans was provided by Curtin University.

James Cook University and University of Queensland provided aquarium facilities.

James Cook University (School of Marine Biology and Aquaculture and The Office of the Pro-Vice-Chancellor, Research and International) provided administrative and operational support for the project.

The Queensland Fisheries Service mailed a copy of the video to each of 250 live trout fishers as part of the technology transfer program.

This project would not have been possible without the support and cooperation of many commercial line reef fishers who gave us time, access to fish and to their vessels for sampling, information and their encouragement. We are grateful to them for this and hope that the outcomes of this project provide some useful reward for their support.

BACKGROUND

THE LIVE REEF FISH FISHERY

The market size and value

The Queensland commercial line reef fishery has existed since the early 1940's (Mapstone *et al*, 2001). The fishery has traditionally targeted Coral trout (*Plectropomus leopardus*, *P. maculatus*, *P. laevis*), red-throat emperor (*Lethrinus miniatus*) and Spanish mackeral (*Scomberomorus commersons*), with Coral trout comprising 40 – 55% of landings (Mapstone *et al.*, 1996). Since 1993, an increasing number of fishers have retained their catch, particularly of Coral trout, alive to sell into the live reef fish trade centred in Hong Kong (Mapstone *et al*., 2001).

Official figures indicate that approximately 14,000 MT of live reef fish were imported into Hong Kong in 1999 (McGilvray and Chan, 2001). However, Chan (2000) suggested that, as a result of local boats not being required to report their catches, the total market for live reef fish in Hong Kong was more likely to be 30,000 to 35,000 MT. This market maintains high prices for live Coral trout, averaging HK$291.50 kg^{-1} (approx. AU$73 kg^{-1}) wholesale price (McGilvray and Chan, 2001) between November 1999 and January 2001 and providing a beach price (price paid to the fisher by the processor) of approximately AU$30 kg^{-1} (G. Muldoon, pers comm.). The beach price paid for live product is between 1.5 and 3 times the price paid for frozen fillet depending on the season (Mapstone *et al.*, 2001) and (assuming a 50% recovery) results in live fish being worth 3 to 6 times their value dead on a whole weight basis.

The greater value of live product over the same species dead has provided an incentive for fishers to change their practices and is perhaps the cause of the increased effort and capital investment reported in the industry (Mapstone *et al*., 2001).

It seems likely that the widespread live-fish trade and high-value markets throughout southeast Asia will continue to provide opportunities for Australian reef fishers to increase their margins by targeting live fish markets. In addition, competing countries supplying the 14000T live tropical fish market in Hong Kong are suffering from over-fishing (Johannes and Reipen, 1995) and demand for Australian product is expected to further increase as a result.

Limitations to the industry

A major limitation to the live Coral trout industry is the mortality in the fish due to injury and disease. Capture and holding of reef fish alive requires substantively different skills to fishing in the dead fish industry. The fisher must change from harvesting a product which is immediately killed and placed on ice to prevent spoilage to being able to take into account the physiological needs of the animal to allow the fish to remain alive and healthy. The value of the fish in the live fish industry is significantly compromised by poor condition related to infection and physical damage. Thus, while physiology of the animal harvested has some impact on the product quality in the dead fish industry, small changes in handling practice usually don't have the same impact on the saleability of the product as occurs in the live fish industry. In order to ensure that the value of live fish is maintained, fishers need to be aware of the appropriate handling practices for live product.

In 1997, most boats were restricted to a maximum of 5 to 6 days at sea as they are unable to hold the fish live for longer without great risk of disease and high costs of mortality. In addition, although mortality of live fish may be as low as 2% from processor to market, mortality between capture and transfer to processors may reach 50% at some times of the year. This leakage of

product from the high value live market to the fillet market resulted in significant loss of value in this fishery with loss of income to all sectors of the industry.

Meetings in 1995 between members of the CRC Reef "Effects of line fishing project" and commercial fishermen at a range of centres in the Great Barrier Reef region identified shorter fishing trips and greater risk (because of the risk of death or damage of fish before sale) as undesirable major changes in fishing practices, and losses of live fish in ship-board holding facilities prior to off-loading as a major cause of reduced profitability.

These concerns were reinforced in 1997 by the industry. Even though some fishers are content with the 5-6 day limit on trip length, others were either returning to the frozen fillet industry from the live industry or deciding to remain in the frozen fillet industry so they can continue to fish in more remote areas. Such decisions result in substantial loss of value for Coral trout captured on the Great Barrier Reef with impacts ranging from the individual to the national interest. Restricted length of trip is also apparently resulting in concentration of fishing effort close to major ports. A subsequent study of the reef line fish fishery (Mapstone *et al*., 2001) has indicated that fishers keeping their fish alive exhibit reductions in catch rates in both Coral trout and by-product species and more targeted harvest of their selected species compared with those killing their catch, supporting the contention that moving fishers into the live fish industry from the dead fish industry would be advantageous to the resource.

Practices related to the limitations

The processes involved in capture and transport to market of live Coral trout comprise a series of clearly defined activities, the conduct of which may result in stressors being placed on the fish. These stressors will cause changes in the animal's metabolism that result in changes in body composition, increased susceptibility to disease and compromised appearance and flesh quality (Pankhurst *et al.*, 1992). These are manifest in a variety of physiological and immunological responses and may be clearly reflected by the incidence of infection by opportunistic pathogens and parasites. As a result, survivorship is reduced and there are potential effects on product quality both in flavour and appearance.

In order to overcome the limitations imposed on the industry by handling practices, it is necessary to consider which practices and processes are likely to induce stress and thereby compromise the survival of the fish. By modifying those practices in ways that are acceptable to the fishers, it is possible to improve the retention of fish being sold as live product. This information can then be used to provide a sound basis for the development and improvement of Benchmark practices in the live fish industry.

STRESS IN FISH

INTRODUCTION

Recognition of the fact that stressors experienced by animals in aquatic environments will impact on their well-being has led to the development of an extensive body of literature describing the physiological stress response of fish. Much of this literature has addressed the effects of stressors on animals in aquaculture, with less relating to the impact of stressors in the fishing industry or in nature. Recently, however, some sectors of the fishing industry have moved into delivering product for live trade. With this shift in emphasis has come a shift from killing the product as quickly and humanely as possible, with emphasis on prevention of tissue degradation and spoilage, to maintaining the animal alive and limiting the subsequent susceptibility to disease and death. Many of the lessons learnt by the aquaculture industry are relevant to the conduct of best practice in the live fish industry. Consequently, understanding and minimising the impact of stressors during the harvest and processing cycle is extremely important in ensuring best value is achieved from the available resources.

THE GENERALISED STRESS RESPONSE

Although debate continues in the academic literature about the definition of the physiological stress response, a useful working definition is:

"THE PHYSIOLOGICAL RESPONSE OF AN ANIMAL TO DISTURBANCE OF ITS' HOMEOSTATIC BALANCE BY A STIMULUS, TERMED THE STRESSOR."

It is widely accepted that the stress response in fish generally conforms to the General Adaptation Syndrome (GAS) proposed by Seyle (1950). The GAS consists of three phases, the first being the initial alarm response to the stimulus. The second stage is that of resistance as the animal tries to compensate for the demands placed upon it and the third stage, exhaustion, is entered if the animal is unable to compensate sufficiently and homeostasis is lost. If the animal enters the third stage, it is physiologically compromised and is likely to be subject to infection and disease. The GAS assumes a general or non-specific nature to the response to a variety of stressors. A great deal of research since has questioned the notion that the stress response is non-specific but the three phases of the GAS theory are still generally used by physiologists in the discussion of stress.

Substantial information is available in the literature regarding the physiological processes that are invoked in fish in response to the presence of a stressor. The presence of a stress response in fish is part of an adaptive process whereby the animal is able to adjust its physiology and behaviour to deal with threats such as predators in the environment. However, if allowed to continue, the response becomes maladaptive and causes harm to the animal. The best described events are comprised of two separate cascades of endocrine and metabolic responses summarised in Figure 1. These cascades are termed the sympathetico-chromaffin (SC) axis and the hypothalamic-pituitary-interenal tissue (HPI) axis. The SC axis provides a very rapid response, which prepares the animal for escape or defence while the HPI axis provides a longer term maintenance of readiness to deal with the stressor.

A brief description of the SC and the HPI axes follows. A number of authors have provided detailed reviews of this information (Pickering, 1981; Adams, 1990; Barton and Iwama, 1991; Barton, 1997; Wendelaar Bonga 1997; Reid *et al.*, 1998) and the reader is referred to these for greater detail.

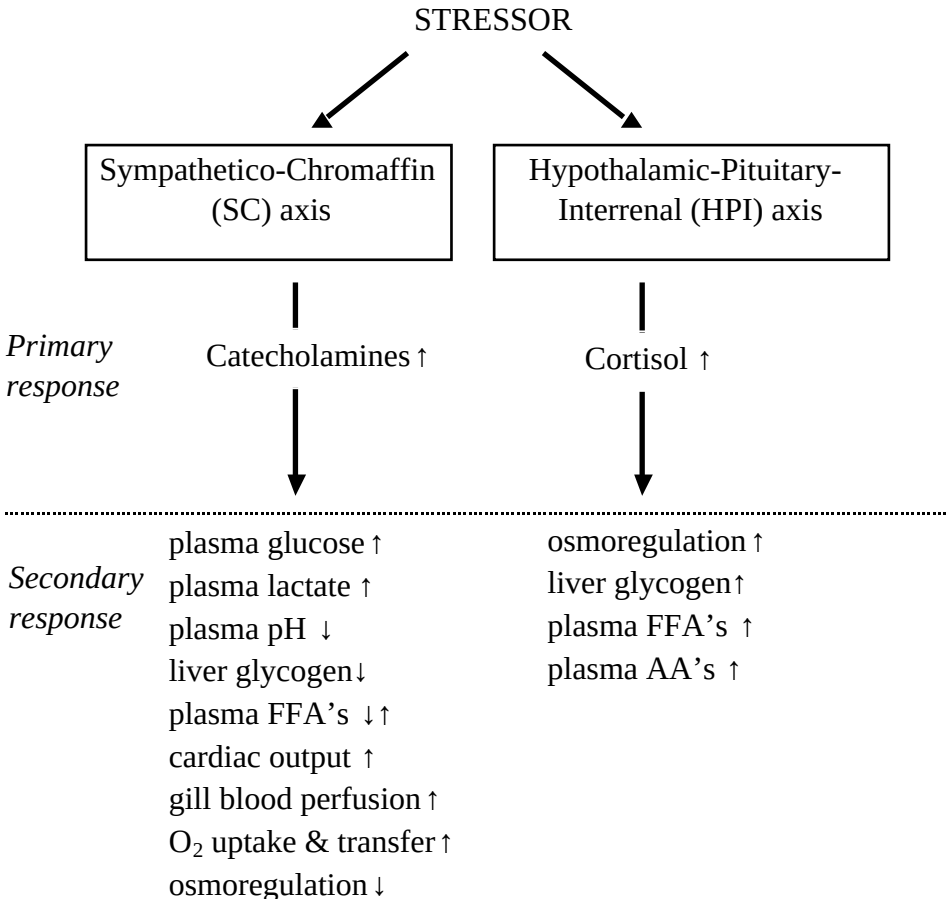

Figure 1. Diagrammatic representation of the cascade of events following imposition of a stressor on a fish. Arrows indicate the direction of change in the concentration of that hormone or metabolite.

Sympathetico-chromaffin axis

Stimulation of the SC axis results in the release of the catecholamines (dopamine, adrenaline and noradrenaline) from chromaffin cells in the kidney into the bloodstream (Reid *et al*., 1998). The stimulus originates in the hypothalamus in the brain and is transferred to the chromaffin cells via sympathetic nerve fibres. The major function of the SC axis is to modify the cardiovascular and respiratory systems to optimise oxygen delivery to the tissues and mobilisation of energy stores (Wendelaar Bonga, 1997).

Catecholamine levels rise within seconds from between 0.5 and 10 nmol/L in unstressed fish to levels of around 100nmol/L to 1000 nmol/L in severely stressed animals. Levels may remain elevated for days under conditions of chronic stress. A very short biological half-life of catecholamines means that levels will fall as quickly as they rose (Sumpter, 1997; Wendelaar Bonga, 1997). A number of other factors may stimulate or modify release including the hormones serotonin, angiotensin, natiuretic peptides, ACTH, cortisol, bioactive peptides, adenosine and catecholamine and non-humoral agents such as raised potassium levels, decreased blood oxygen content and acidosis (Reid *et al.*, 1998).

Catecholamines increase the number of circulating red blood cells by stimulating their release from the spleen. Catecholamines also induce red blood cell swelling, reflected by an increase in haematocrit, which increases the oxygen carrying capacity of the haemoglobin through the Bohr and Root effects. These effects increase the oxygen carrying capacity of the blood. Branchial

blood flow and oxygen diffusing capacity at the gills, enhanced by increased heart rate and stroke volume are stimulated by catecholamines (Wendelaar Bonga, 1997) and increase the rate of transfer rate of oxygen to the tissues. Together, these responses compensate for the higher metabolic oxygen demand associated with behavioural responses to stress (Randall and Perry, 1992).

Hyperglycemia (high blood sugar levels) appear rapidly after the onset of a stressor and are the result of stimulation of glycogenolytic enzymes that release sugar from stores in tissue glycogen (Wendelaar Bonga, 1997).

Hypothalamic Pituitary Interenal axis

Stimulation of the HPI axis results, initially, in the release of ACTH from the pituitary in response to an unknown corticotropic releasing factor produced by the hypothalamus. ACTH is produced by cleavage of the peptide, propiomelanocortin (POMC). Other products from different processing of POMC are melanophore stimulating hormone (MSH) which acts on the melanocytes in the skin causing changes in the animal's colour pattern and β-endorphin, the natural pain relieving hormone homologous to morphine (Fryer *et al*., 1983). ACTH enters the circulation and acts upon cells in the interenal tissue of the head kidney, stimulating them to produce and release corticosteroids (Fagerlund, 1970). The predominant corticosteroid in fish is cortisol (Gorbman *et al*., 1983).

As for catecholamines, there are a number of factors which modify the regulation of the release of cortisol by ACTH. These are less well understood but include arginine vasotocin (AVT), melanin concentrating hormone (MCH), atrial natriuretic peptide (ANP), urotensin I and II, MSH and β-endorphin (Sumpter, 1997). The resting levels of cortisol reported in the literature vary considerably. Animals that are well husbanded generally have cortisol levels below 10 ng/ml, although non-salmonid species may have levels up to 30-40 ng/ml (Barton and Iwama, 1991). Cortisol rises after the onset of stress and may reach levels of 100-200 ng/ml after an hour (Barton and Iwama, 1991). The maximum concentration of cortisol reached also varies widely between species.

Cortisol has both a mineralocorticoid (regulating tissue osmotic pressure) and glucocorticoid (regulating circulating sugar concentration) functions. Cortisol stimulates an increase in the number of chloride cells in the gills and an increase in branchial Na^+ and Cl^- excretion in saltwater adapted animals (McCormick, 1995).

Administration of cortisol generally invokes hyperglycaemia, increasing blood glucose concentration. The role of cortisol in short term hyperglycaemia is not clear, with greatest interest in the investigation of the effects of cortisol being in the long term. It is likely that the initial rise of plasma glucose concentration to an acute stressor is due to the glycogenolytic action of catecholamines (Frisch and Anderson, 2000). Leach and Taylor (1982) found that blocking the release of cortisol with metapyrone failed to prevent the immediate post-stress rise of glucose in mummichog but abolished the longer term hyperglycaemia. This suggests that the immediate rise in plasma glucose was not the result of cortisol action but that cortisol may play a role in the sustained hyperglycaemia.

Cortisol also stimulates lipid mobilisation and increases free fatty acids (Sheridan, 1987) and free amino acids (Van Der Boon *et al*., 1991) in the circulation, thereby further increasing available energy substrates in the circulation.

Since the metabolic actions of cortisol are not only related to stress, natural variations in the circulating cortisol levels can be expected. The most apparent of these, and that which is most likely to affect studies of the stress response, is the presence of diurnal rhythms. Some doubt exists regarding the presence of a diel rhythm in corticosteroids in teleosts. Boehkle *et al.* (1996) reported rhythmic variations in corticosterone and cortisone in channel catfish (*Ictalurus punctatus*), but Davis and Parker (1986) reported only slight diel changes in cortisol concentrations in that species. Nocturnal peaks in cortisol concentrations have been reported in brown trout (*Salmo trutta*) (Pickering and Pottinger, 1983) and Peter *et al.* (1978) reported a diel rhythm of cortisol in *Carassius auratus* that correlated with photoperiod. Other authors have correlated cortisol concentrations with time of feeding in *C. auratus* (Spieler and Noeske, 1984) and in brown trout (Pickering and Pottinger, 1983). In some salmonid species, no diel changes were found (Strange *et al.,* 1977; Barton *et al.,* 1980). Rhythms in circulating cortisol concentrations have also been reported in killifish (*Fundulus grandis*) (Garcia and Meier, 1973).

Other mechanisms

In addition to the HPI and SC axes, a range of other hormonal systems have been implicated in the stress response of fish. With the exception of shock protein production, the relationship between these alternative hormonal systems and stress is less clear and their measurement provides little assistance in determination of the stress status of a fish. These hormones include growth hormone (GH), insulin-like growth factor (IGF), prolactin (PRL) and somatolactin. The levels of somatolactin in the blood rises rapidly during stress but various studies have found contradictory effects of stress on PRL and GH with increases, decreases and no effect on concentrations all being reported. In addition, these factors are proteins and so their measurement is problematic since assays are not readily transferable between species.

STRESSORS IMPOSED BY THE ENVIRONMENT

A wide range of environmental stressors are known to impact fish and, in many instances, plasma cortisol and/or glucose concentrations reflect the degree of the stress (Davis *et al.,* 1984). Fish captured in wild-harvest fisheries and maintained alive are subject to a number of processes including capture, handling, transport, confinement and anaesthesia (Mazeaud *et al.,* 1977; Johnson and Metcalf, 1982; Maule *et al.,* 1993; Barnett and Pankhurst, 1998). During the harvest cycle a number of environmental changes occur. These act as stressors and include changes in water level (Einarsdottir and Nilseen, 1996), water temperature (Strange, 1980; Davis and Parker, 1986), nutrient levels (Carmichael *et al.,* 1984; Pickering and Pottinger, 1987a), salinity (Altimiras *et al.,* 1994), stocking density and oxygen availability (Matthews and Berg, 1997). In addition, the change in environment subjects the fish to changes in motion (Evans and Fewtrell, 1996) and pathogen encounter radius (Wedemeyer, 1997) that are different to those in its' natural environment.

The response of fish to common handling practices (Iwama *et al.,* 1997) and capture of wild stocks (Gustaveson *et al.,* 1991) has been investigated extensively. Apart from some disparity in results related to reproductive and seasonal changes, predictable changes in primary and secondary stress indices following capture and confinement occur. The time to the peak response, the magnitude of peak concentrations and the duration before pre-stressed levels are obtained differs significantly between species (Davis and Parker, 1986) and with severity of the capture event (Gustaveson *et al.,* 1991). This implies that improved outcomes can be achieved by improving handling techniques.

Stocking density

Holding and confinement of fish for culture or transportation results in stress associated with crowding. The degree of crowding, or stocking density, has been shown to have a marked effect on the stress response (Strange and Schreck, 1980; Pickering and Pottinger, 1987b). Barnett and Pankhurst (1998) reported significantly lower plasma cortisol levels in fish held at lower stocking densities (4.8 $kg.m^{-3}$) than those held at high densities (14.4 $kg.m^{-3}$). Similar trends have been reported for brown trout (Pickering and Pottinger, 1987b), red drum (Robertson *et al.*, 1987) and Chinook salmon (Mazur and Iwama, 1993). Pickering and Pottinger (1987b) showed that brown trout confined in high stocking densities can also suppress the short term activation of the HPI axis, however the effect was probably mediated by changes in water chemistry, rather than as a direct result of stocking density. Although Pickering and Stewart (1984) revealed that the effect of high stocking density was independent of water quality in brown trout, many studies do not account for changes in water quality when evaluating the response to stocking density.

The effect of water chemistry associated with stocking density has been used to formulate guidelines for densities (recommended values in $kg.m^{-3}$) and flow loading (recommended values in $kg.L^{-1}.min^{-1}$) of some salmonid species (Wedemeyer, 1997). These indices allow for an appropriate fish density to maintain sufficient water flow and minimise respiratory stress associated with reduced dissolved oxygen saturation and increasing nutrient concentrations (Banks *et al.*, 1979).

High stocking densities also promote activation of the HPI axis in fish through the formation of social hierarchies. Hierarchical dominance is often proportional to fish size. Elevated cortisol in subordinated coho salmon (Ejike and Schreck, 1980) and eels (Knights, 1987; Hyde and Perry, 1990) has been demonstrated. Additive effects of feeding suppression in subordinated fish ensure that dominant individuals usually remain larger. Aggressive behaviour associated with dominance can be alleviated to some degree by adjusting rearing densities. Wedemeyer (1997) reported that desirable schooling behaviours can be restored in tilapia and the decrease in dominance hierarchies in salmonids (Davis *et al.*, 1984), barramundi and Murray cod (Anderson, pers. observation) can be achieved by increasing stocking density.

Dissolved oxygen and carbon dioxide

Aside from the normal biological requirement of oxygen for respiration in fish, the increased respiratory and metabolic rate during stress lead to an increase in oxygen consumption and an increase in respired carbon dioxide. Wedemeyer (1997) suggested that the dynamic interaction between dissolved oxygen and carbon dioxide are two of the most important considerations in meeting the physiological demands associated with transport and holding of fish.

Aeration in holding systems serves to increase dissolved oxygen and facilitates exchange of unwanted gases such as carbon dioxide and nitrogen with the atmosphere. Failure to remove excess carbon dioxide from the water results in hypercapnia, acidosis, tissue hypoxia and carbon dioxide narcosis (Wedemeyer, 1997).

Supersaturation of holding tank water with oxygen can also be detrimental to fish. Wedemeyer (1997) suggests that oxygen concentrations of up to 18 $mg.L^{-1}$ can occur quickly in holding tanks supplied with oxygen diffusion. A consequence of oxygen supersaturation is the reduction of gill ventilation rate while metabolic rate, and thus carbon dioxide production, is maintained or increased. These changes result in an elevation of arterial carbon dioxide and a compensatory increase in blood bicarbonate concentration. When aeration is reduced to normal levels or, as is

often the case in transporting live fish, the fish are moved to less vigorously aerated holding tanks, blood pH rises quickly, leading to alkalosis and mortality.

Various studies have investigated oxygen depletion on the stress response of fish (Swift, 1981; Tomasso and Davis, 1981). Suppression of the HPI axis under conditions of very low oxygen saturation and high carbon dioxide levels is commonly observed. Pickering and Pottinger (1987a) reported that in brown trout, low oxygen levels alone lead to minor suppression of the stress response, but suppression was markedly greater in animals also exposed to high carbon dioxide and ammonia. Carmichael *et al.* (1984) reported similar results in largemouth bass and highlighted the soporific nature of exposure to low dissolved oxygen and high carbon dioxide levels. Behavioural shifts have been reported to accompany these changes in water chemistry, with fish usually sedate or appearing somnolent (Gebhards, 1965; Summerfelt *et al.*, 1967, Pickering and Pottinger, 1987a). Carmichael *et al*. (1984) also witnessed an extreme glucose response following 24 hours exposure to elevated carbon dioxide. The authors suggested this was due to metabolic demands associated with low oxygen utilization.

Excretory products

The degradation of protein to provide substrates for energy results in ammonia excretion (De Silva and Anderson, 1995). In an aquatic medium, direct excretion of ammonia is achieved across the gill surface by diffusion or exchange of ammonia ions for sodium ions (Evans and Cameron, 1986). Raised levels of unionised ammonia are typical of recirculation systems, and systems with little or no water exchange, such as during transportation.

Carmichael *et al.* (1984) found that plasma corticosteroids remained low, although plasma glucose remained elevated following exposure to ammonia and 24 h of recovery. This lead those authors to suggest a soporific effect of ammonia on the HPI axis, as observed in fish exposed to low oxygen/high carbon dioxide. In addition, an increase in plasma chloride ions 24 h after exposure suggested that ammonia had a suppressive effect on the HPI axis with a subsequent delayed stress response (Carmichael *et al.*, 1984).

Interactions between ammonia and dissolved oxygen may occur. Sousa and Meade (1977) reported that increases in ammonia concentration reduced the oxygen carrying capacity of the blood in coho salmon, although (Smart, 1978) suggested an overall increase in oxygen consumption. It has also been established that low levels of dissolved oxygen increase the toxicity of ammonia to rainbow trout (Lloyd, 1961).

Water temperature

Transport at a temperature a few degrees below the coldest extreme of those normally experienced by a species is considered to be a procedure which increases survival rate of the transported fish. Aside from the advantage of suppressing corticosteroids (see the discussion on seasonal effects below), reduced temperature also lowers the metabolic rate, reducing the consumption of oxygen and the production of ammonia. Conversely, the oxygen carrying capacity of water decreases at higher water temperatures and the associated metabolic demands on the fish increases. Thus, it has been suggested that respiratory failure is a likely cause of the mortality witnessed at high temperatures (Pickering, 1993).

Quite often a mismatch between the temperatures of the water from which fish are removed and that into which they are placed occurs during transportation of live fish (Wedemeyer, 1997). Stress associated with such thermal shock has been attributed to osmoregulatory dysfunction in *Fundulus heteroclitus* and often leads to high mortality (Carmichael *et al.*, 1988). Increases in plasma cortisol in response to thermal shock have been reported in salmonids (Strange *et al.*,

1977; Barton and Peter, 1982; Pickering, 1993) and a rapid decline in plasma chloride and osmolality can also be attributed to an associated osmotic shock (Carmichael *et al.,* 1984). Wedemeyer and Goodyear (1984) suggested the stress response resulting from thermal shock is sufficient to stimulate latent pathogen infections.

Salinity

The addition of mineral salt formulations to transport water mitigates stress during transportation of freshwater fish. Considerable work has been undertaken in freshwater salmonids transported in water with elevated NaC1 at concentrations of 0.5 – 1.0% (Mazik *et al.,* 1991; Wedemeyer, 1997). The main benefit afforded by this practice is the protection against plasma electrolyte losses resulting from increased cortisol release associated with transport stress (Carmichael *et al.,* 1984).

Few investigators have investigated the effects of salinity changes in saltwater species. Engel *et al.,* (1987) noted a fall in osmotic pressure in Atlantic menhaden (*Brevoortia tyrannus*) following a ten fold decrease in salinity. Kirschner (1995) suggested the metabolic cost associated with lowering salinity would increase metabolic rate. Contradictory to this, no changes in oxygen consumption were reported in the sea bream (*Sparus aurata*) (Altimiras *et al.,* 1994). The mineralocorticoid nature of cortisol was also reported by Altimiras *et al.,* (1994) with 8-fold increases in fish held in 3.7 $g.L^{-1}$ and 0.3 $g.L^{-1}$ salinity. Coral trout appear to be unaffected by short term exposure to salinities as low as 10 $g.L^{-1}$ (Frisch and Anderson, 2000).

In addition, rapid and relatively short term (0.5 – 24 h) salinity change is a simple, effective and widely used method of controlling external pathogens of fish (Rowland and Ingram, 1991). Thus modifying the salinity of the holding water has the potential benefit of reducing both the stress response and the pathogen load of the animals experiencing it.

Anaesthetic

Anaesthetics and hypnotic drugs added to transport water to reduce metabolic rate have been used in a variety of fish species. Lower oxygen consumption and excretory rates associated with such practices are particularly advantageous with high stocking densities (Ferreira *et al.,* 1984). Reduced activity levels also result in less injury result from the use of sedatives during transport (McFarland, 1959).

The effect of an anaesthetic depends on the type of anaesthetic, the fish species studied and the method of application. The local anaesthetic MS-222 prevented rises associated with handling of plasma cortisol in salmon (Strange and Schreck, 1978), plasma glucose in rainbow trout (Morales *et al*., 1990) and both cortisol and glucose in rainbow trout (Laidley and Leatherland, 1988). Etomidate suppressed the cortisol response in bass subjected to net confinement and a very high dose of MS-222 eliminated any change in plasma cortisol within five minutes of the commencement of sampling in that species (Davis *et al.,* 1982). Wedemeyer (1970) found no decrease in kidney ascorbate, used as an indicator of ACTH production and hence HPI axis stimulation, when anaesthetised with benzocaine. The general anaesthetic 2-phenoxyethanol was shown to induce a stress response in barramundi while 220 $mg.L^{-1}$ benzocaine did not (Percival, 1999). The anaesthetic Aqis-S$^{\hat{a}}$, a derivative of clove oil, was reported to reduce the cortisol in response to transport stress in rainbow trout (*Onchorynchus mykis*) and brown trout (*Salmo trutta*)(Auperin *et al*., 1998). Davidson *et al*. (2000), however, contradicted this finding that anaesthesia with Aqui-S$^{\hat{a}}$ resulted in a significant increase in plasma cortisol in rainbow trout.

Motion

A parameter afforded little attention in the research of transportation methods for live fish is the effect of water motion. Recently, it has been found that exposure to simulated unnatural water motion for 30 min resulted in a stress response in silver bream (*Rhabdosargus sarba*) (Evans and Fewtrell, 1998). A range of effects were observed including a significant elevation in cortisol, an increase in circulating thrombocytes and an increase in interenal cell size.

WITHIN AND BETWEEN ANIMAL VARIATION IN THE STRESS RESPONSE

Intraspecies variation

Differences in the physiological stress response of individuals within fish species have been identified. These have been related to both genetic variation and rearing conditions, although it is possible that the latter also result from genetic differences. Hatchery reared salmonids show more extreme changes in cortisol (Salonius and Iwama, 1993; Woodward and Strange 1987), glucose and chloride (Woodward and Strange, 1987) and depressed immune function (Salonius and Iwama, 1993) in comparison to wild animals. Strain differences in the stress response were apparent in rainbow trout (Fevolden and Roed, 1993; Pottinger and Moran, 1993; Pottinger *et al.*, 1994), lake trout (McDonald and Robinson, 1993) and Atlantic salmon (*Salmo salar*) (Fevolden *et al.*, 1993).

Dietary status

One of the usual consequences of stress in fish is depression of appetite. Food intake has been considered the main cause of reduced growth in many species, and reductions in feeding activity have been quantified following acute stress in brown trout (Pickering *et al.*, 1982) and eels (*Anguilla anguilla*) (Peters, 1982).

Nutritional stress has also been associated with dietary components and was recently reviewed by Fletcher (1997). The reduction in growth rate and tissue catabolism associated with insufficient dietary protein is well understood (De Silva and Anderson, 1995). Stress associated with lack of dietary protein results in immuno-suppression and increased severity of pathogen infection (Li and Woo, 1991). Insufficient dietary fatty acids, particularly low levels of the *n*-3 group of fatty acids, have been shown to be related to the induction of shock syndrome in rainbow trout following a handling stress (Castell, 1979). Lack of dietary vitamins has also been associated with stress. Vitamin C has been of great interest as it has been associated with promoting disease resistance (Fletcher, 1997). Kidney ascorbic acid has been shown to be significantly depleted following exercise (White *et al.*, 1993) and following ATCH injection (Wedemeyer, 1969).

Cessation of feeding prior to transport is a common practice and is used to reduce metabolic rate thereby reducing ammonia excretion and oxygen consumption. However, depriving an animal of nutrients in this way can interact with the nutrient mobilisation actions of catecholamines and impact on the stress response. Reubush and Heath (1997) found that fed hybrid striped bass showed a greater release of glucose following acute handling than starved animals.

Seasonal effects: Osmoregulatory ability

Seasonal changes in corticosteroids have been investigated in only a few fish species. Species that have distinct changes is osmoregulatory requirement, such as anadromous salmonids, have been studied because of the role cortisol is believed to play in osmoregulation. Hoar (1988)

showed that significant rises of cortisol in smolting steelhead trout (*Oncorhynchus mykiss*) reflect the changes in the capacity of these animals to osmoregulate in seawater. A strong correlation between plasma cortisol concentrations and plasma sodium concentrations observed by McLeese *et al.* (1994) also suggested that cortisol was involved in seawater adaptation in *O. mykiss*.

Seasonal effects: Water temperature

Environmental temperature has also been considered to effect the stress response in some fish species (Pickering, 1992). The rate of plasma cortisol elevation was affected by temperature in channel catfish (Davis *et al.,* 1984). Shorter term diel fluctuations in water temperature, however, did not effect cortisol dynamics in juvenile coho salmon (Thomas *et al.,* 1986) or cutthroat salmon (Strange *et al.,* 1977). Despite this, Barton and Schreck (1987) reported that stress induced mortality does increase with increasing water temperature. An inverse relationship between water temperature and plasma cortisol has been reported in channel catfish (Strange, 1980, Davis *et al.,* 1984). Davis *et al.* (1984) concluded that hormones are less effective at low water temperatures and consequently higher resting levels may exist to compensate. Therefore, the possible effect of temperature must be considered when interpreting seasonal differences in the stress response.

Seasonal effects: Sex and reproductive status

Much has been written on the effect of stress on reproduction in fish (Anderson, 2001), but little consideration of the reverse has been reported. Seasonal changes in reproductive status are also believed to modify the responsiveness of the HPI axis. Kubokawa *et al.* (1999), reported a distinct difference in stress response between female and male sockeye salmon (*Onchorynchus nerka*) during the breeding season. Although males showed a predictable corticosteroid response to capture, confinement and sampling, female fish showed no significant increase in cortisol concentration. Interestingly, female salmon had much higher basal cortisol levels (135.6 ± 13.8 ng/ml) than males (54.0 ± 22.5 ng/ml) at the beginning of the experiment, possibly associated with the physiological changes of reproduction. Androgen levels in both sexes were decreased after acute stress, with decreased 11-keto testosterone and testosterone in males and decreased testosterone in females (Kubokawa, 1999). The androgen response of the females led the authors to the conclude that females were not refractory to stress as has been reported in some bird species (Wingfield, *et al.*, 1992).

In contrast to the pattern reported for sockeye salmon, a reduction in the stress responsiveness of male rainbow trout and brown trout has been reported during sexual maturation (Sumpter *et al.*, 1987). Further investigation of this phenomenon by Pottinger *et al.* (1995) showed a correlation between corticosteroid dynamics and maturity in male rainbow trout of the same age and strain. Mature males showed a lesser cortisol response following a 24 h confinement than immature males. Furthermore, plasma ACTH levels were significantly lower in mature males, suggesting that the feedback equilibrium regulating the stress response had been modified to a lower “set point”. The authors suggested the physiological significance of the lower ‘set point’, may be to protect the fish from the deleterious effects of higher plasma cortisol on metabolism and reproduction.

STRESS AND DISEASE

Stress is known to significantly increase the disease susceptibility of animals both by compromising the metabolic processes of the animal and immuno-suppression (Barton and Iwama, 1991). The immune function of fish, as other animals, is comprised of specific (both

cellular and antibody) (Ellis, 1978; Manning and Tatner, 1985) and non-specific components. The latter comprises phagocytic, inflammatory and cellular responses that result in clotting (Alexander, 1985; Alexander and Ingram 1992; Sunyer and Tort, 1995). Whilst non-specific thrombocytes appear to be briefly elevated following a stressor (Cassillas and Smith, 1977; Frisch and Anderson, 2000), specific immunity is depressed by cortisol in killifish (Miller and Tripp, 1982), salmonids (Pickering and Pottinger, 1987b) and channel catfish (Ainsworth *et al.*, 1991).

STRESS PHYSIOLOGY OF CORAL TROUT

The stress response of Coral trout (*Plectropomus leopardus*) to wild capture or controlled shallow water stressors has been investigated (Frisch and Anderson, 2000). Those authors found that stress associated with capture from the wild, handling and transport and shallow water resulted in significant changes in circulating levels of cortisol, glucose, lactate, haemoglobin (Hb) and haematocrit (Hct) in Coral trout. The response of the SC axis was apparent by increases in plasma glucose, Hb and Hct over 30 to 60 minutes. These parameters then returned toward unstressed levels even if the stressor persisted. Similarly to other species, cortisol increased to a maximum level over 60 minutes following the onset of the stressor. Cortisol remained elevated over 4 hours in the case of a single short (30 minute) stressor or for 3 days in response to capture, handling and transport.

Frisch and Anderson (2000) also described the response of the immune cells to stress, reporting that the concentrations of circulating lymphocytes (responsible for specific immunity) were significantly reduced by shallow water stress, showing a negative correlation with cortisol (Anderson, 2001). They concluded that as lymphocytes are important immune components, reduced concentrations of these cells may explain the increases in disease susceptibility commonly observed in stressed fish during live transport. An increase in thrombocytes, responsible for non-specific immunity, was significant 150 min after stress (Frisch and Anderson, 2000) but returned to basal levels after 240 min. Cassillas and Smith (1977) have previously suggested thrombocytes are increased in response to catecholamine secretion to aid in blood clotting and the recovery of thrombocytes to normal levels whilst cortisol remains high in Coral trout supports this mechanism.

NEED

This research aimed to increase survivorship between capture and sale to processors, of Coral trout destined for the live fish market. In so doing, it attempted to overcome inefficiencies that resulted in significant devaluing of product in the live fish trade. This project directly addressed objective 3.3 of the Queensland Fisheries Research and Development Strategy (1995-2005) developed by the Queensland Fisheries Industry Research Advisory Committee by developing opportunities to add value to fisheries and fish products.

In 1997, the live Coral Trout fishery was conservatively valued at $3.5 million. Demand for live Coral Trout was not, and is not, being met and it is clear that the market could accept additional product. Anecdotal information also indicated that competing countries supplying the 14000T live tropical fish market in Hong Kong are suffering from over-fishing and demand is expected to further increase as a result.

A major limitation preventing the live Coral Trout industry expanding to fill the available market is the mortality due to injury and disease. Developments in technology and skill level were generally isolated to particular (eg very large) vessels. Most boats were restricted to a maximum of 5 to 6 days at sea as they were unable to hold the fish live for longer. In addition, although mortality of live fish may be as low as 2% from processor to market, mortality between capture and transfer to processors may reach 50% at some times of the year. Whilst current practice means that fish showing imminent sign of death are sacrificed to obtain fillet, this results in significant devaluing of the product. This leakage of product from the high value live market (@ $30+/kg) to the fillet market (+ $14+/kg) results in significant loss of value in this fishery with loss of income to all sectors of the industry.

The adoption of strategies to target the live trout, rather than the fresh frozen fillet, mark*et al*so results in reduced total catch per boat due to the significant price advantage and larger on-board facilities required to hold the product. By providing information that will allow a code of practice to achieve World Best Practice, information that was not available for Coral trout, this project that facilitated the movement of boats into the live fishery. It is likely that the total catch will be reduced and the long-term sustainability of the fishery will be enhanced.

OBJECTIVES

The objectives of this study were:

1. To increase fish survival in the live Coral trout fishery.

2. To identify practices in the harvest, ship-board transport and holding of live Coral trout that are the major stressors.

3. To identify the impact of these stressors on survival and disease resistance.

4. To develop benchmark practices for the harvesting, ship-board transport and holding of live Coral trout that alleviate stressors and improve survival.

5. To inform the industry and management of benchmark practices.

6. To assist with the implementation and to evaluate the implementation of benchmark practices in the live trout industry.

GENERAL METHODS

PROCUREMENT OF SPECIMENS

Coral trout (*Plectropomus leopardus*) were captured by commercial fishers or by project staff angling from vessels on the Great Barrier Reef (GBR). Fish were sampled from reefs at various locations on the GBR (Figure 2). All fish were measured to the nearest 100g immediately after sampling. All fish sampled were initially placed in a dory tank, followed by later transfer to a main vessel for transport to the James Cook University aquarium facility. Specific details on experiments and sample sizes are given in the relevant sections.

MEASUREMENT OF WATER FLOW AND WATER QUALITY (TEMPERATURE, SALINITY, PH, DISSOLVED OXYGEN, AMMONIA).

The capacity of all holding tanks from each of the commercial vessels was determined to the nearest 0.1 L by simple measurements. The flow rates of all inlet water was determined by the formula rate = time/volume. All flow rates were determined in the absence of fish and are therefore considered to be maximum rates. Water quality was measured at various intervals depending on the experiment. Percent saturation of dissolved oxygen (DO) and temperature were determined using a TPS Dissolved Oxygen – Temperature Meter (Model WP-82). Salinity and pH were determined using a TPS Conductivity – Salinity – pH – Temperature Meter (Model WP-81). Ammonia, nitrite and nitrate were determined using Dry Tab[â] Test Kits (Product number 61, 62 and 66 respectively : Aquarium Pharmaceuticals Inc., Chalfont).

AQUARIUM MAINTENANCE

Over the life of the project, approximately 240 *P. leopardus* were accommodated at the James Cook University Aquaculture Facility recirculation system. The fish were maintained in tanks of various size depending on the task. Tanks were either white plastic round 1000 L tanks (n = 30 per tank, 185 L rectangular plastic tanks (n = 6 per tank), 70 L rectangular tanks (n = 1 per tank) or 185 L round aluminium tanks (up to n = 30/tank). Unless other wise stated, the 1000 L tanks were supplied with water to allow 100% exchange per hour and the smaller (185 L or 70 L) tanks were supplied with water to allow 100% to 1200% exchange per hour from the main recirculating system. The salinity, temperature, and dissolved oxygen concentration of this system was maintained at 27-29 ppt, 26-27 °C, and 80-90% respectively. The specimens were fed daily on trash fish obtained from local fishermen. These were largely mackeral or trevalley fillets although pilchards were used on some occasions. These conditions were altered accordingly when the fish were subject to various experiments, as described in subsequent sections.

The experiments determining the effect of temperature change and handling were conducted at the University of Queensland. Animals were held in plastic tanks of approximately 1000 L or in glass aquaria of approximately 100 L serviced by a re-circulating sea water system.

BLOOD MEASUREMENTS AND ASSAYS

The base sampling unit used for all combinations of stressors and temporal response to stressors was 6 Coral trout. At each discrete sampling point (Table 1), the blood of 6 independent animals were sampled for various measurement of stress (cortisol, glucose, lactate, haemoglobin, haematocrit).

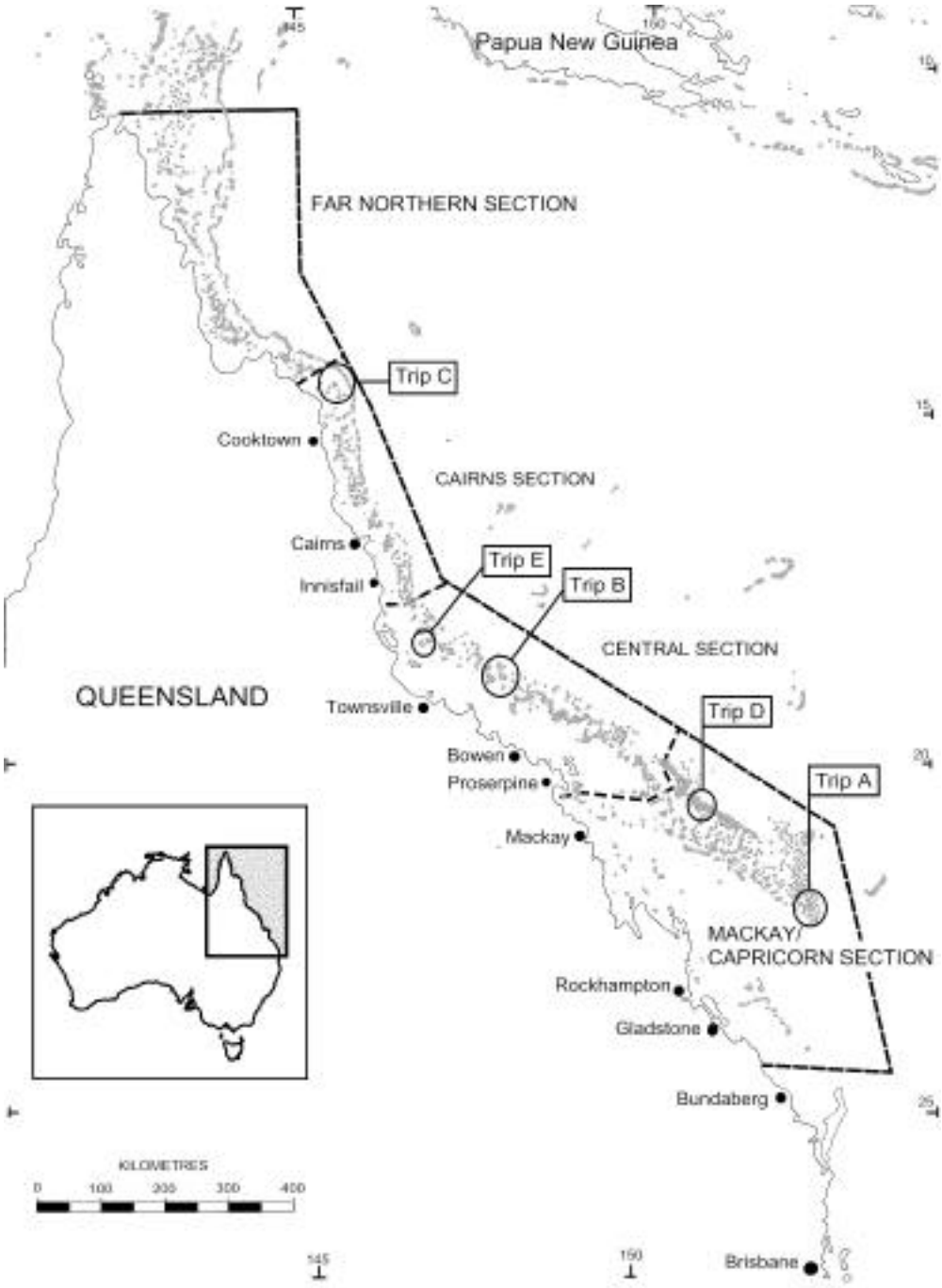

Figure 2. Map of the Queensland coast showing the areas where commercial fishing trips were undertaken for this study (Trips A to D) and where "ideal practices" were evaluated (Trip E).

After restraining the animals in a wet foam cradle, blood samples of 4 ml were taken from the caudal artery with an 16 gauge hypodermic needle on a 5 ml syringe. One ml of blood was transferred to a 1.5 ml eppendorf tube, and the remainder added to a 2 ml vial filled with fluoride heparin to prevent clotting. All blood samples were placed immediately on ice for no longer than 4 hours to await further processing. Haemocrit counts of the heparinised blood were undertaken the same day as the original sampling, and a 100 µl aliquot of whole blood was stored frozen at –20 °C for haemoglobin analysis. The remaining heparinised blood was centrifuged in an

Eppendorf bench centrifuge for 5 min at 10,000 g to separate the plasma, which was either assayed immediately, or stored at –20 °C. The non-heparinised blood was centrifuged in a similar manner, and the serum stored at –80 °C. Preliminary trials showed the measurement of haemoglobin and haematocrit to provide no additional data to glucose or lactate since all four parameters reflect the catecholamine response in Coral trout (Frisch and Anderson, 2000) and these parameters were not included in the experiments.

CORTISOL

A radioimmunoassay (RIA) technique were used to determine plasma cortisol concentrations. Plasma concentrations of cortisol were determined using Pantex radioimmunoassay (RIA) kits (Cat No. IM031, Immunodiagnostics, Brisbane, Australia). These kits contained a solution of radiolabelled (Iodine-125) hormone as tracer, first and second antisera, non-specific binder buffer solution (NSB) and a range of hormone standards. The principle of the assay is that tracer hormone competes with the hormone in either the standard or the sample, for binding sites on the first antiserum. The greater the hormone concentration in the standard/sample, the fewer the binding sites available to the radiolabelled hormone in the tracer.

Where necessary and with the exception of the second antiserum, all reagents were diluted 10-fold to increase the assay sensitivity and thus allow measurement of the hormone levels found in Coral trout. Hormone levels in plasma samples were each measured in triplicate. Inter assay variance in our laboratory is approximately10 % and intra assay variance is approximately 5%. The assay sensitivity is 1 $ng.mL^{-1}$ cortisol.

Details of the method are given in the instruction leaflet accompanying the kit. The radioactivity was measured using a gamma counter (Δ Packard, Canberra, ACT., Australia).

In order to determine the hormone concentration of the unknown samples, a standard curve was first generated from the standards. For each standard the fraction of radio-labelled hormone successfully bound to the first antiserum (R) was calculated as follows:

$R = (S – N)/(S^0 – N)$

where S = counts per minute (CPM) of the standard, N = mean CPM of the NSB standard and S^0 = mean CPM of the 0 standard

A standard curve of ln (R/1-R) vs $\log_{10}$[standard] was generated and the linear equation for this relationship was derived using Microsoft Excel. The concentration of hormone in each sample was determined by calculating R for each sample and back-calculating from the linear relationship of ln (R/1-R) vs $\log_{10}$[standard]. The mean hormone concentration of the replicates was used in data analysis.
The validity of this assay is demonstrated by the parallel relationship (H_0 $b_1 = b_2$, $t = 0.276$, df = 7, $p<0.05$) between the standards and serial dilutions of an unknown sample from Coral trout as shown in Figure 3.

GLUCOSE AND LACTATE

Plasma glucose and lactate were assayed using test-kits adapted for use with a Labsystems IEMS microplate reader. The glucose and lactate kits (No's. 716251 and 139084 respectively) were purchased from Boehringer-Mannheim (Castle Hill, Australia) and the haemoglobin kit (No. 525-A) was purchased from Sigma (Castle Hill, Australia).

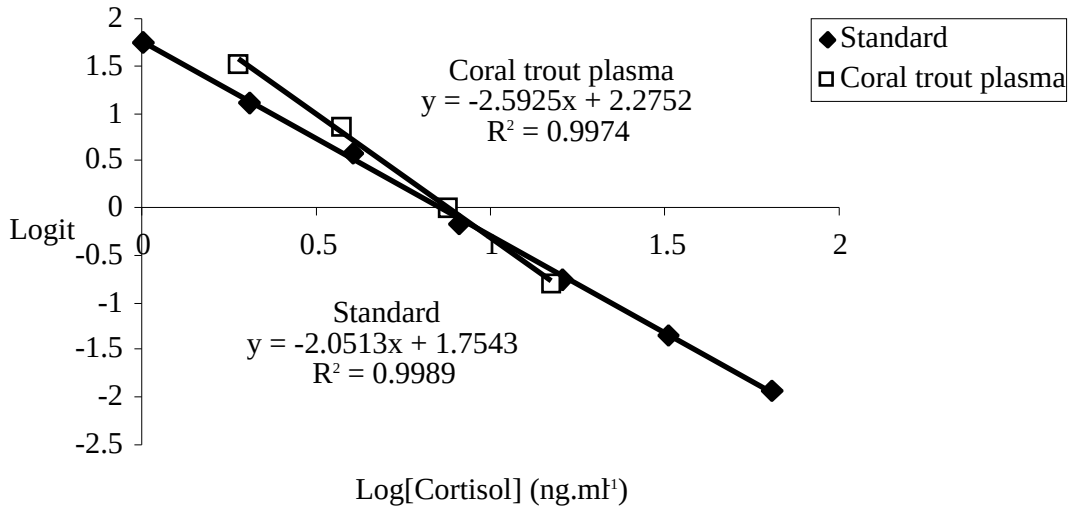

Figure 3. Log [Cortisol] vs logit plot of cortisol standards and serially diluted Coral trout plasma.

Glucose

The glucose assay followed the method described in the leaflet accompanying the kit except that it was modified by using 10% of the volumes recommended in the method to increase efficiency in the use of the reagents. Briefly, 20 µl of plasma were added to 80 µl of solution 1 (kit) and 122 µl of purified water in a flat-bottomed, plastic microplate well. The microplate was agitated for 30 secs in the microplate reader and absorbance at 340 nm measured after 3 min. Twenty µl of 1:10 diluted suspension 2 (kit) was then added to each well and after 15 min, the absorbance measured again. Standards (0.5, 2, 4, 6, 8 mM) and a blank (diluted suspension 2) were prepared and measured in the same plate as the samples. The first reading was subtracted from the second reading to correct for inter-well variation. Sample glucose concentrations were then determined from a regression equation derived from the standard curve using Microsoft Excel.

Lactate

The lactate assay also followed the method described in the leaflet accompanying the kit except that it too was modified by using 10% of the volumes recommended in the method to increase efficiency in the use of the reagents. Briefly, duplicate 100 µl aliquots of plasma were vortexed with an equal volume of ice-cold, 0.6 M perchloric acid. After 10 min on ice and 2 min centrifugation at 10,000 g in an Eppendorf bench centrifuge, 150 µl of supernatant was added to 6.4 µl of 3 M potassium hydroxide. This was vortexed, left for 15 min on ice, and centrifuged again. Twenty µl of sample and 162 µl of solution A (kit) were then added to each well of a microplate and the absorbance measured at 340 nm. Fifty µl of solution B (kit) was also added to each well, and after a further 30 min, the absorbance measured again. Standards (0.5, 1, 2, 3, 5, 7 mM) and a water blank were prepared and measured simultaneously with samples. As in the glucose assays, the first reading was subtracted from the second reading. Sample lactate concentrations were then determined from a regression equation derived from the standard curve using Microsoft Excel.

PART A. PRELIMINARY STUDIES OF COMMERCIAL FISHING PRACTICES AND CHARACTERISATION OF THE STRESS RESPONSE

INTRODUCTION

The capture of fish by angling subjects the fish to a number of potentially stressful events. The fish takes the hook in its mouth, is physically dragged to the surface, perhaps suffering barotrauma in the process and removed from the water. The hook is removed and the animal is placed into a dory tank. The physical and chemical parameters of the water into which the animal is placed and in which it remains for various amounts of time affect its stress levels. The dory moves from the point of capture to another fishing site or returns to the mother ship and during this time the water in the dory tank undergoes an unnatural movement within the tank. The fish is removed again from the dory tank and transferred to a tank in the mother vessel. Again, the physical and chemical parameters of this water affects the animal's stress. The mother vessel remains at sea for a period of time and then returns to port, again subjecting the fish to unnatural water movement. During the whole harvest process, the fish is handled a number of times and may also suffer physical damage thereby experiencing stress.

Since this process is very complicated involving a number of events, and the stress response of Coral trout is additive (Frisch and Anderson, 2000), it is likely that measurement of the stress response of fish at any one point in the harvest process will not reflect the experience immediately preceding that point but be confounded by the total experience of the animal. However, it is likely that the experience of an animal throughout the harvest cycle will include severe stressors and opportunities for recovery. An analysis of parameters describing the stress response of Coral trout throughout the harvest cycle may identify processes that induce stress and processes that allow recovery.

It was also likely that a variety of handling practices would be present in the industry, reflecting the different levels of prior experience, knowledge and commitment to minimizing fish stress of individual fishers. Frisch and Anderson (2000) undertook a study of the stress response of Coral trout in the capture and harvest cycle but applied experimental rather than ideal industry standards in their handling techniques. Thus, there was no description of the stress response of Coral trout using commercial best practice handling techniques by individuals well informed about the impacts of stressors on fish.

Therefore, the objective of this section of the project was to measure the stress response of Coral trout during capture and post-harvest holding using current commercial and an "ideal" practice.

MATERIALS AND METHODS

Identification of stressors

A project meeting held at James Cook University on 6th June 1997 identified a number of potential stressors along the capture and transport cycle. These are summarised in Table 1. The identification of these stressors also established the temporal sequence for sampling of stress measurements, also summarised in Table 1.

Table 1. Discrete sampling points along the capture and transport cycle.

Sampling Point	Potential Stressor	Time after stressor that fish were sampled.
1	Capture	30 minutes after capture
2	Holding onboard dory vessel	4 hours after capture
3	Transfer to primary vessel	30 minutes after transfer
4	Short term holding onboard primary vessel	24 hours after transfer to primary vessel
5	Long term holding onboard primary vessel	3-5 days after transfer to primary vessel, or immediately prior to steam to port (whichever is first)

Sampling

The stress response of Coral trout in the commercial fishery was determined by sampling fish captured by commercial fishers, angling on reef line fishing vessels on the Great Barrier Reef (GBR). Fish were sampled from a commercial line fishing vessel at reefs in four separate locations on the GBR (Trips A – D; Figure 2). Sampling was conducted between September 2 to 4, 1997 (Trip A), December 17 to 21, 1997 (Trip B), February 12 to 20, 1998 (Trip C) and March 25 to 31, 1998 (Trip D) and were of three, five, nine and seven days duration respectively.

All fish were initially placed in a dory tank, followed by later transfer to the main vessel. Where the fish showed loss of equilibrium, the swim bladder of the fish was deflated by inserting an 16 gauge needle into the swimmer bladder through the wall of the rectum Plate 1. Excess gas is allowed to escape until the animal regains its capacity to right itself.

Plate 1. Swim bladder deflation is most easily achieved by inserting an 16 gauge needle into the swim bladder through the wall of the rectum.

All fish were measured to the nearest 1 mm and 100g immediately after sampling. Due to the variation in the daily catch rate, it was necessary to sample for any one point over a number of

sessions and/or days. The stress response of Coral trout during an "ideal" harvest and handling cycle was determined by sampling of fish captured by members of the project team at Bramble Reef (Trip E; Figure 2).

Blood was sampled from the caudal sinus of the fish and cortisol, glucose and lactate assayed in the samples as described above in the General Materials and Methods.

Measurement of water flow and water quality (Temperature, Salinity, pH, Dissolved Oxygen, Ammonia).

The capacity of all holding tanks from each of the commercial vessels was determined to the nearest 0.1 L by simple measurements. The flow rates of all inlet water was determined by the formula
Rate = Time/Volume.
All flow rates were determined in the absence of fish and are therefore considered to be maximum rates. Water quality was measured at various intervals depending on the experiment. Water quality was monitored in dory tanks on each vessel on at least one occasion. Parameters were measured and were recorded every 30 min for 4 h in dory tanks and on a daily basis in day tanks and main tanks. Percent saturation of DO, temperature, ammonia, nitrite and nitrate were determined as described above in the General Materials and Methods.

RESULTS

Fish size and fishing dynamics

A total of 82 fish were sampled from commercial vessels and ranged in size from 375 mm to 571 mm and from 550 g to 2700 g. The relationship between weight and length in these animals is shown in Figure 4 and is described by the function
Length (mm) = $60.25\text{Weight}^{0.2853}$ ($F = 71706.0$, $df = 81,2$, $p<<0.001$, $r^2 = 0.953$).

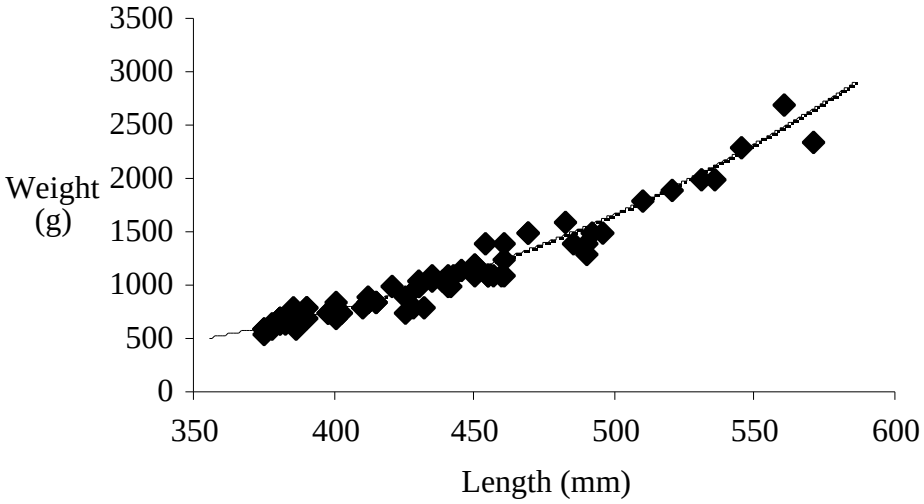

Figure 4. Weight (g) vs Length (mm) of Coral trout sampled from commercial fishing vessels.

Fishing sessions are usually conducted twice per day referred to in this document as AM and PM sessions. Sessions are of three to four hours, although they may be truncated if the fisher considers the dory tank to be overstocked. Each session consists of a number of hangs, the

periods of time when a fisher remains in one place with various distances travelled to and between hangs. Total catch per session in this study were significantly different between trips being highest in Trip A and Trip C and lowest during trips B and D. Total catch per session was significantly higher in AM than in PM sessions Table 2.

As a result of these varying catch rates, stocking densities also varied significantly between trips and between sessions (Figure 5). The mean stocking density observed in dory tanks in this study was 59 $g.L^{-1}$ and the highest stocking density recorded in a dory tank was 194 $g.L^{-1}$.

Table 2. Catch rate (kg) of live Coral trout per session (mean ± S.E.) during four commercial fishing trips.

Trip	AM session	PM session
A	20.133 ± 1.836	11.733 ± 1.362
B	7.4 ± 1.472	3.05 ± 1.092
C	17.756 ± 0.981	9.689 ± 0.728
D	5.357 ± 1.244	3.036 ± 0.923

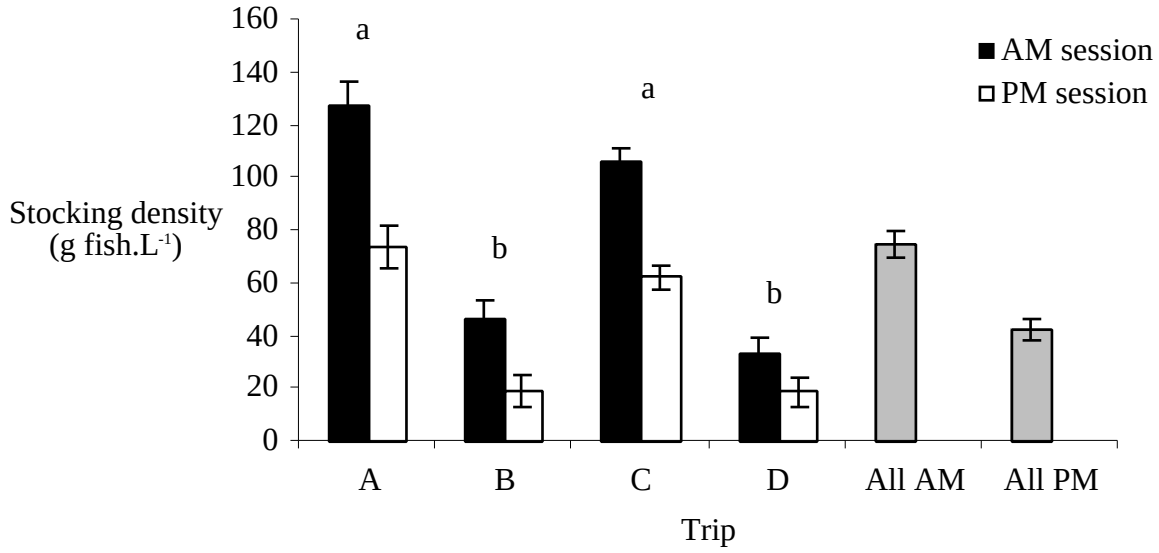

Figure 5. Stocking density of live Coral trout in dory tanks in the commercial fishery. Values are mean ± S.E., letters indicate homogenous subsets within session time.

Upon return to the main vessel, fish are transferred to day tanks. These tanks varied in volume in the vessels used in this study from 144 L to 380 L. Fish are held in these tanks for about 24 hours to ensure they are in a fit condition to survive until sale to the processor. Stocking densities in day tanks varied between trips in concert with catch rates and averaged 109 $g.L^{-1}$ (S.E.=7.32, range 17 to 289 $g.L^{-1}$, n = 59).

After approximately 24 h, fish were moved into the main tanks. These varied in the vessels used in this study from 1100 L to 20,000 L. Fish are held in these tanks until being off-loaded to the processor. Stocking densities increased with time over the course of the trips reaching maximum levels ranging from 22 to 148 $g.L^{-1}$ at the end of trip (mean 99 g L^{-1}, S.E. 22 $g.L^{-1}$, n = 6).

Observation summaries

Trip A

Main vessel supported four dories all of which appeared to have very poor water quality management practices. Generally fish handling was good, but flow rates in dories were poor (often none). This resulted in low dissolved oxygen and high ammonia concentrations. Fish from all four dories were placed in two day tanks for 24 h at very high stocking densities (approximately 80 – 100 kg.300 L^{-1}) also with qualitatively poor water flow and quality. The main tank has very high flow rates and apparently very good water quality maintained by a flow-through system. High mortalities were observed after 3 days and boat returned to port. Many fish (1/3 of catch) were rejected at point of sale.

Trip B

Main vessel supported four dories. Dory tanks were simple but flow rates and water quality parameters appeared good. Fish were held in day tanks at no more than 25 kg.150 L^{-1}. Flow rates in dory tanks were very good. Fish were transferred to one of three main tanks (500 – 700 L) also at low stocking densities. Water quality also good here. No fish were rejected on return to port, but as catch rates were low, stocking densities were low.

Trip C

This trip was conducted on the same vessel as Trip A but it had been highly modified. The main vessel supported four dories with excellent water quality management. There is one day tank (380 L) per fisher with maximum stocking densities of 30-50 kg.380 L^{-1}. Over 1200 kg were caught in the 9 days ands all fish were in very good condition at point of sale.

Trip D

The main vessel supported four very small dories with very small (70 L) and poorly designed dory tanks. Water flow was O.K. but the system so impractical that most of the fishers only fill tanks when required, that is when fish are floating around the tank. Day tanks were 160 L tanks with fair flow rates and mid range water quality. Main tanks are 1000 L cylindrical tanks, of which there are two. Water flow and quality are also apparently mid range. Again catch rates were very low, averaging 7 - 12 fish.day^{-1}.$dory^{-1}$. Consequently stocking densities at each stage was very low.

Water quality parameters

Dory tanks

Dissolved oxygen in dory tanks showed a rapid decline with the addition of fish to the dory tank (Figure 6) and at all stages was well below saturation. At the end of the fishing session, DO averaged 48% saturation and ranged between 15 and 70 % (Table 3).

Ammonia in the water of dory tanks showed a rapid increase with the addition of fish (Figure 7) and averaged 1.39 mg.L^{-1} by the end of the session. Values at the end of the session varied from 0.25 to 4.00 mg.L^{-1}.

Water pH (Figure 8), temperature and salinity remained relatively constant throughout the session, although they varied between trips according to conditions in the oceanic water being pumped into the tanks.

There were no significant correlations between fish number at the end of a session and any of the water quality parameters measured.

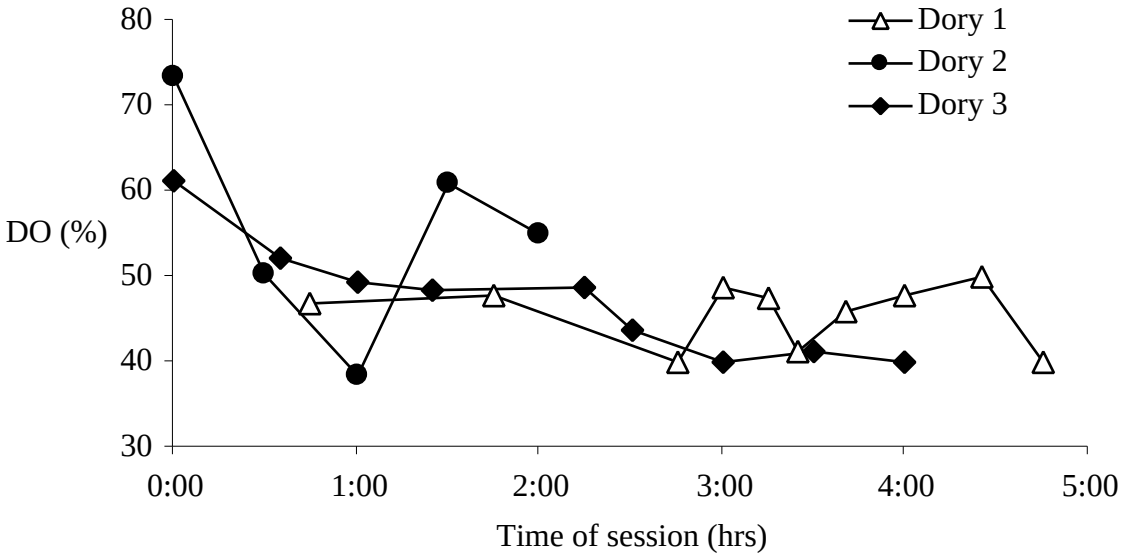

Figure 6 Dissolved oxygen vs time in the water in three dory tanks during commercial fishing trips.

Table 3 Water quality parameters (mean, range) in dory tanks at the completion of the fishing session.

	Average	**Range**
Number of Fish	17	7 - 25
DO	48%	15% - 70%
PH	8.01	6.99 - 8.17
Temperature	27.3 °C	24.7 - 29.5 °C
Salinity	32.4 $mg.L^{-1}$	30.0 - 33.3 $mg.L^{-1}$
Ammonia	1.39 $mg.L^{-1}$	0.25 – 4.00 $mg.L^{-1}$

Day tanks

Dissolved oxygen content in day tanks varied significantly between trips, being higher in trip C than in trips A and D (ANOVA $F = 4.352$, $df = 51,3$, $p < 0.01$; Figure 9). Dissolved oxygen in day tanks ranged between 19 and 82 %.

Ammonia concentration in day tanks did not vary significantly between trips (Kruskal-Wallis test, $\chi^2 = 7.7$, $df = 3$, $p > 0.05$), ranging from 0 to 1 mg L^{-1} (mean = 0.31, S.E. = 0.03, n = 55).

Water temperature in day tanks varied significantly between trips, being higher in trip C than in trips A and D (ANOVA $F = 1093$, $df = 51,3$, $p < 0.001$; Figure 10). Temperature in day tanks ranged between 24.8 and 29.5 °C.

Significant effects of trip on water pH and salinity were also observed but were considered biologically trivial. Water pH ranged from 8.09 to 8.26 (mean = 8.15, S.E. = 0.01, n = 55) and salinity ranged from 32.2 to 34.1 $mg.L^{-1}$ (mean = 33.2, S.E. = 0.04, n = 55).

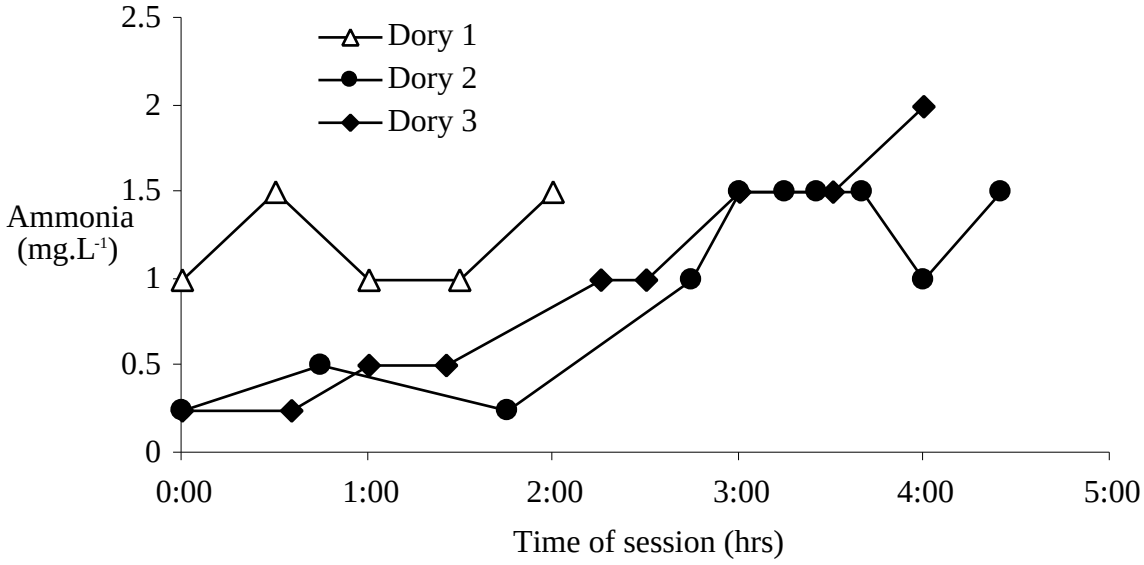

Figure 7 Ammonia vs time in the water in three dory tanks during commercial fishing trips.

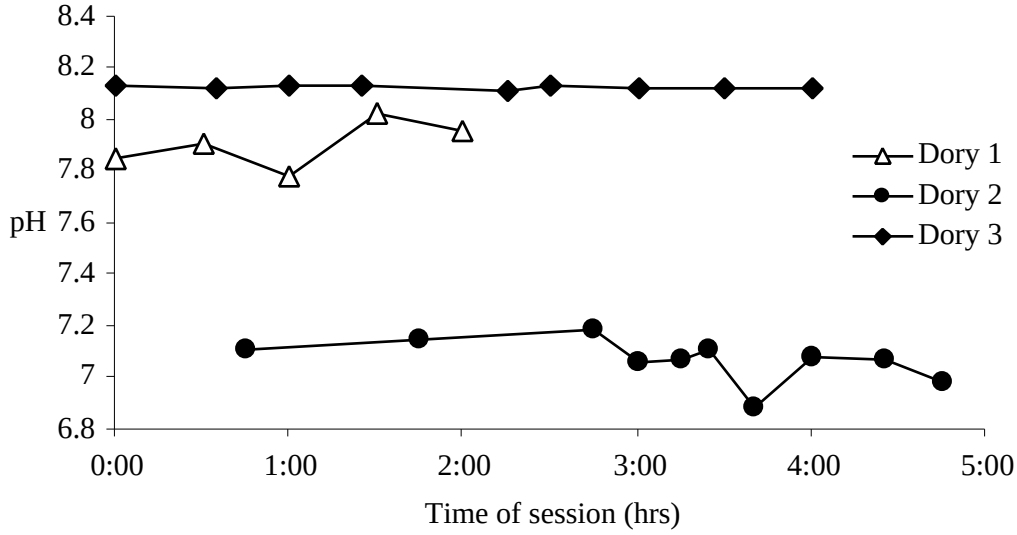

Figure 8 Water pH vs time in the water in three dory tanks during commercial fishing trips.

Main tanks

Dissolved oxygen decreased and ammonia and water temperature significantly increased with stocking density in the main tanks (Table 4). Dissolved oxygen also significantly varied between trips being highest in trips A and C (Figure 11), ranging between 41 and 95 % saturation.

Ammonia concentration was 0 mg L^{-1} in the main tanks during Trip A and not significantly different between the other three trips, ranging between 0.2 and 0.8 mg.L^{-1} (mean = 0.34 mg.L^{-1}, S.E. = 0.04, n = 28).

Water pH varied in the main tanks of vessels during trips A, B and D but the difference was not biologically significant. The range of pH in these tanks was 8.10 to 8.22. During trip C, pH

varied much more widely (Figure 12). The main tank of this vessel used a recirculating system and water quality management required management of the biofilter, whereas the vessels in other trips used flow through main tanks where water quality parameters more closely match the oceanic water being pumped through the tanks. During trip C, water pH varied from 7.10 to 8.08 (mean = 7.48, S.E. = 0.09, n=12).

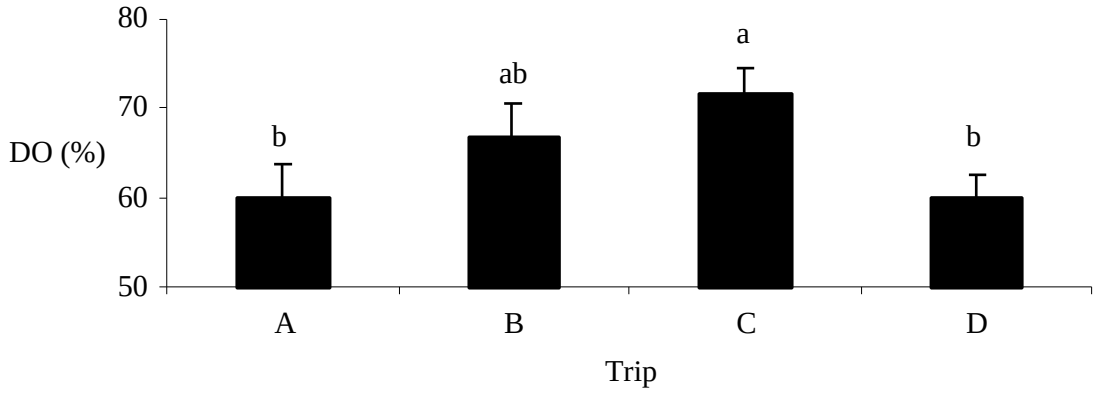

Figure 9. Dissolved oxygen concentration (% saturation) in day tanks during four commercial fishing trips. Values are mean ± S.E., letters indicate homogenous subsets.

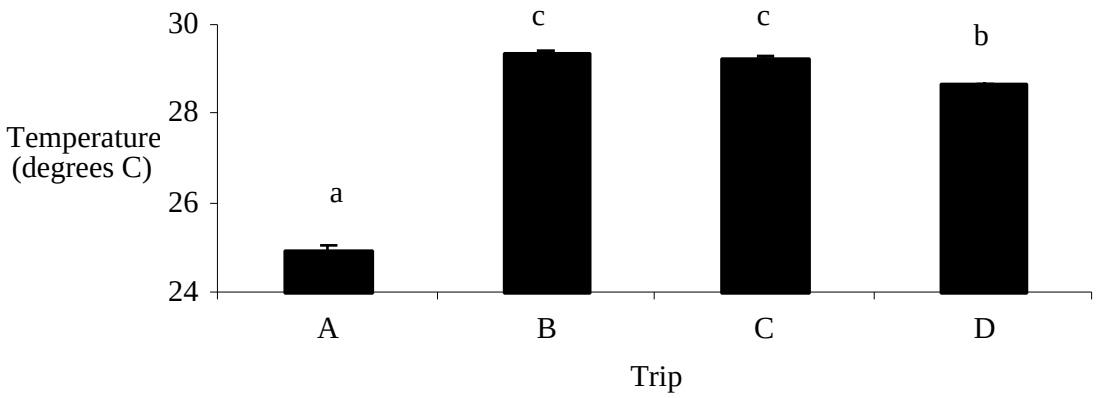

Figure 10. Water temperature (° C) vs trip in day tanks during four commercial fishing trips. Values are mean ± S.E., letters indicate homogenous subsets.

Temperature varied between trips being significantly lower during trip A and significantly higher during trip B than trips C and D (Figure 13). Water temperature variations as a result of day and resultant correlations with stocking density are considered to be an artefact of the different time periods of each trip and the different temperatures experienced during these trips and so are not considered biologically significant.

Salinity did not vary between days but was significantly different between trips. However, this difference was not considered to be biologically meaningful with salinity ranging between 30.7 and 33.9 $mg.L^{-1}$ (mean = 32.7, S.E. = 0.1, n = 35).

Table 4. Pearson's correlations between water quality parameters and stocking density in main tanks during four commercial fishing trips.

Parameter	vs Stocking density
Dissolved oxygen.	-0.721*
Ammonia	0.463*
pH	0.266
Salinity	-0.237
Water temperature	0.624*

* Pearson's correlation coefficient is significant at the 0.01 level (2-tailed).

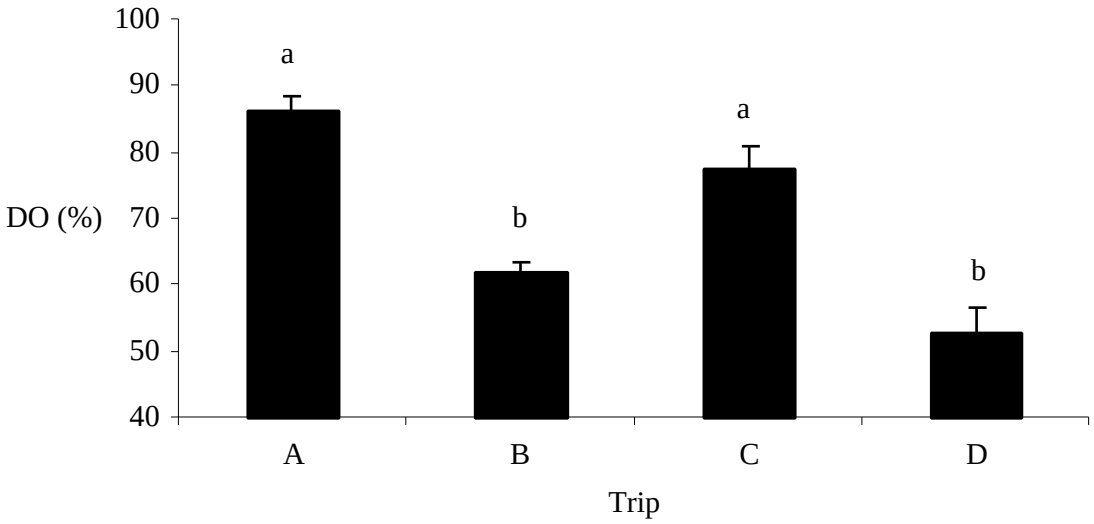

Figure 11. Dissolved oxygen concentration (% saturation) in main tanks during four commercial fishing trips. Values are mean ± S.E., letters indicate homogenous subsets.

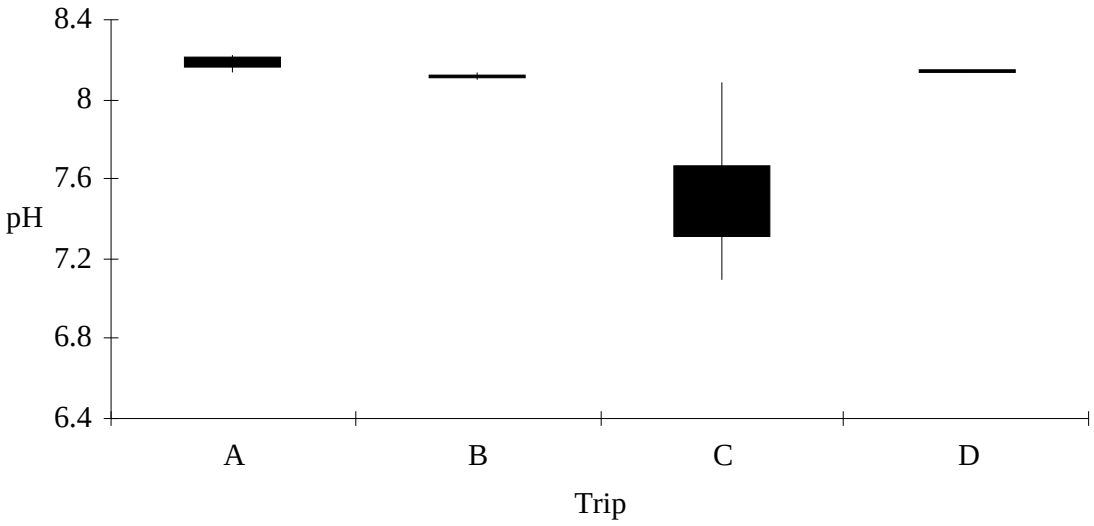

Figure 12. Box plot of water pH in main tanks during four commercial fishing trips. Boxes delineate upper and lower bounds of 95% confidence intervals and lines delineate maximum and minimum values.

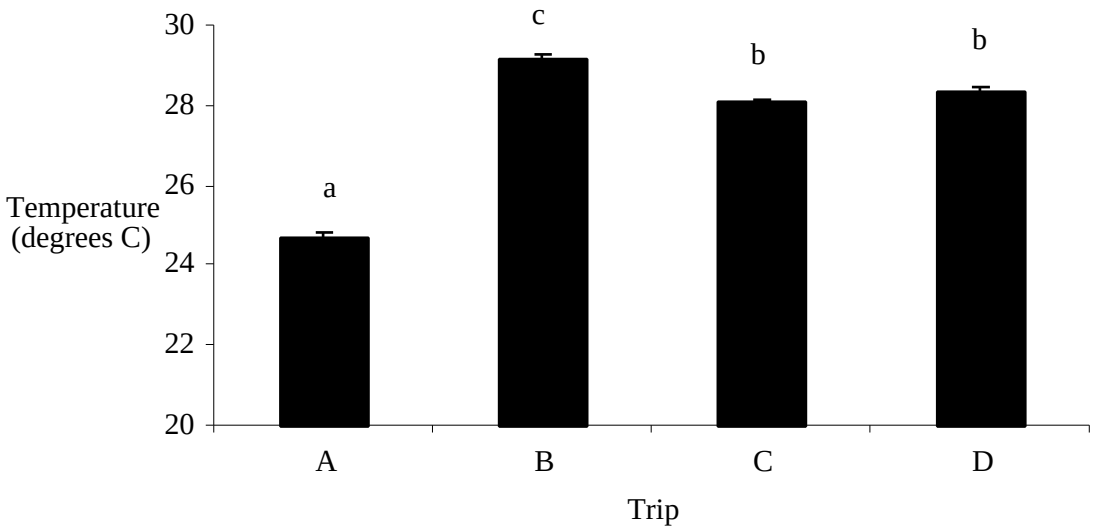

Figure 13. Water temperature (°C) in main tanks during four commercial fishing trips. Values are mean ± S.E., letters indicate homogenous subsets.

Table 5. Effect of trip and recovery time on stress response of Coral trout as measured by concentration of circulating hormones.

Cortisol*				
Source of Variation	*df*	*SS*	*H*	*P*
Trip	3	12846	25.02	<0.001
Recovery time	3	8062.1	15.7	<0.001
Trip *x* Recovery time	9	1295.7	2.52	ns
Error	63	513.5		
Glucose				
		MS	*F*	*P*
Trip	3	16.07	4.42	0.007
Recovery time	3	146.1	40.1	0.000
Trip *x* Recovery time	9	16.0	4.40	0.000
Error	70			
Lactate*				
		SS	*H*	*P*
Trip	3	7819	14.13	<0.001
Recovery time	3	27768	50.17	<0.001
Trip *x* Recovery time	9	3939	7.12	ns
Error	66	553.5		

***** Kruskall-Wallis non-parametric analysis. (see Zar, 1984)

Stress response of fish in the commercial fishery

Concentrations of cortisol, glucose and lactate were significantly different between Trips and among response times, but there were no interactions between time and trips for cortisol or lactate (Table 5). However, there was a significant interaction between Trip and Response time for glucose (Table 5).

Figure 14 shows that the mean cortisol concentration was highest on Trip A and Trip B, and lowest on Trip C, thus indicating variable practices between different boats. Trip A and Trip C were carried out on the same vessel which had considerably improved its practices in Trip C, compared to Trip A, and this was reflected in the average cortisol levels (Figure 14). The improved practices included improved dory water management by permanently mounted bilge pumps and lower stocking densities, and new filter equipment for the main tank recirculating system. Cortisol concentration was highest at 4 hours after capture, but at a similar level at 0.5, 4.5, and 24 hours after capture (Figure 15). This pattern was consistent across all trips, as indicated by the non-significant interaction of trip and recovery time (Table 5).

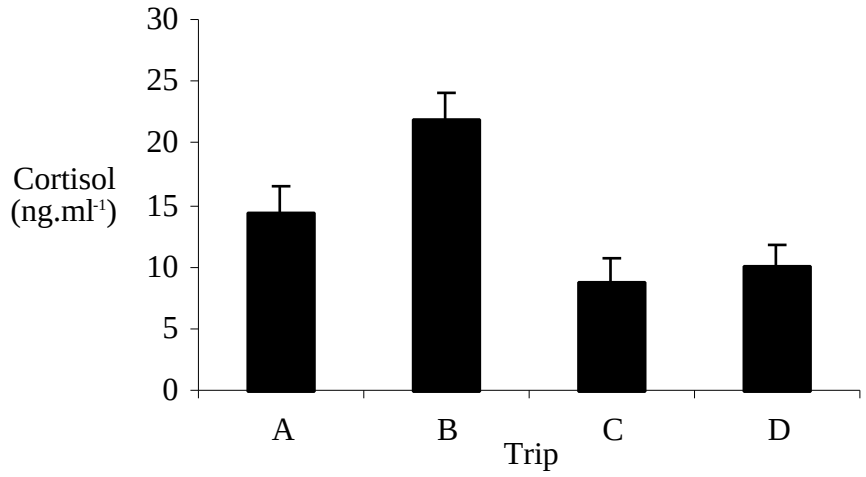

Figure 14. Mean cortisol concentration (ng.ml^{-1}) vs trip sampled in fish captured in the commercial fishery.

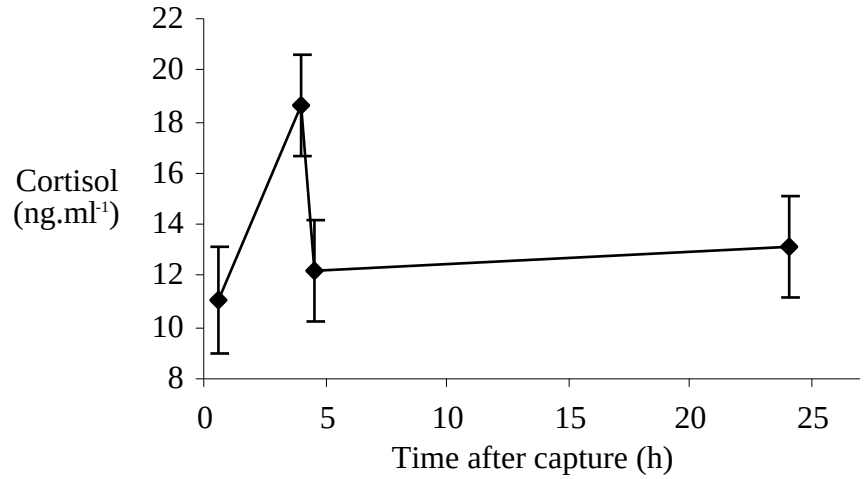

Figure 15. Mean cortisol concentration (ng.ml^{-1}) vs time sampled (hours) in fish captured in the commercial fishery.

To elucidate the nature of the interaction between trip and response time for glucose concentrations, two figures are presented. Trip B and C together (Figure 16), and Trip A and D together (Figure 17). Glucose in Trip B and Trip C dropped significantly between 0 and 4 hours,

the time spent in the dory tanks, indicating they were recovering from the capture-induced stress during this time (Figure 16). However, glucose then increased significantly in concentration between 4 and 4.5 hours after capture, the period in which Coral trout were transferred between the dory and main vessel, before dropping again after 24 hours. All times were significantly different in mean glucose concentration.

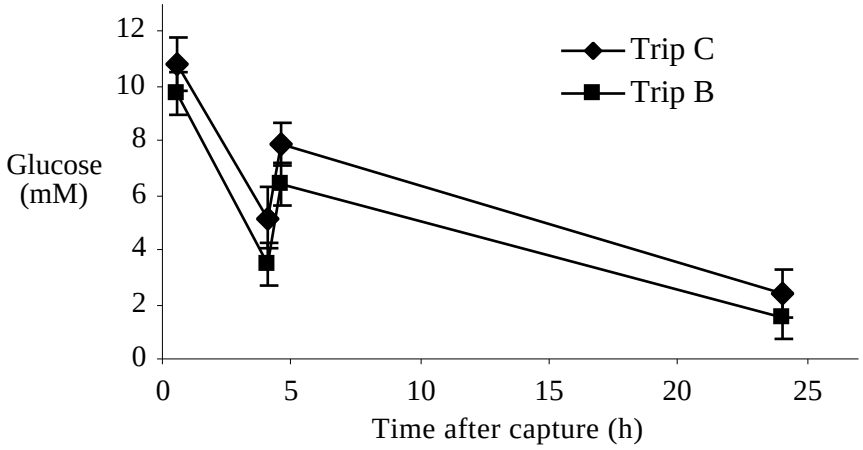

Figure 16. Mean glucose concentration (mM) vs time sampled (hours) in fish captured during Trip B and Trip C on commercial fishing vessels.

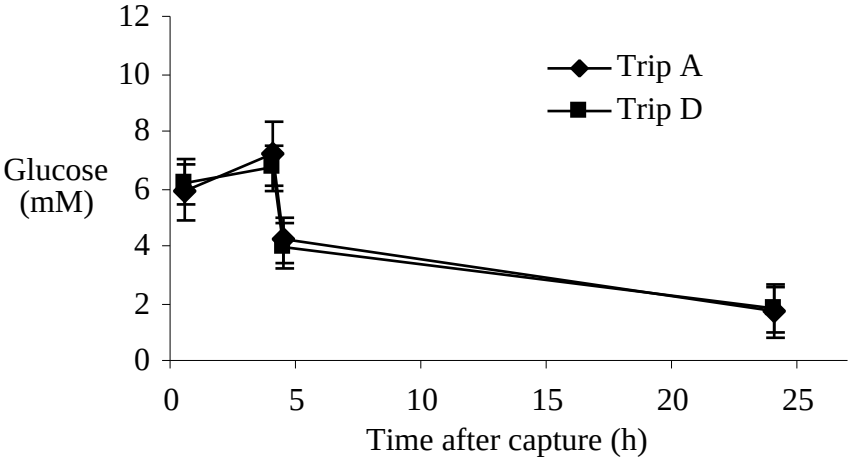

Figure 17. Mean glucose concentration (mM) vs time sampled (hours) in fish captured during Trip A and Trip D on commercial fishing vessels.

A rather different trend in glucose concentration was observed for Trips A and D (Figure 17). During the first four hours, the glucose levels were lower than observed in trips B and C (Figure 16) and did not change significantly, however glucose levels dropped significantly between 4 and 4.5 hours. This was in stark contrast to Trips B and C where it increased. This information suggests that there was a significant improvement in water quality between the dory tanks and the main vessel. Our observations that dory tank water flow was particularly low on the vessels used during Trips A and D correlated with the glucose results.

Lactate concentration was highest in animals caught in Trip B, and lowest in animals caught in Trip A, with no difference between Trips C and D (Figure 18). Lactate was highest in recently caught trout and decreased significantly over the first four hours, finally reducing to minimal concentrations at 24 hours (Figure 19). This pattern was consistent across all Trips.

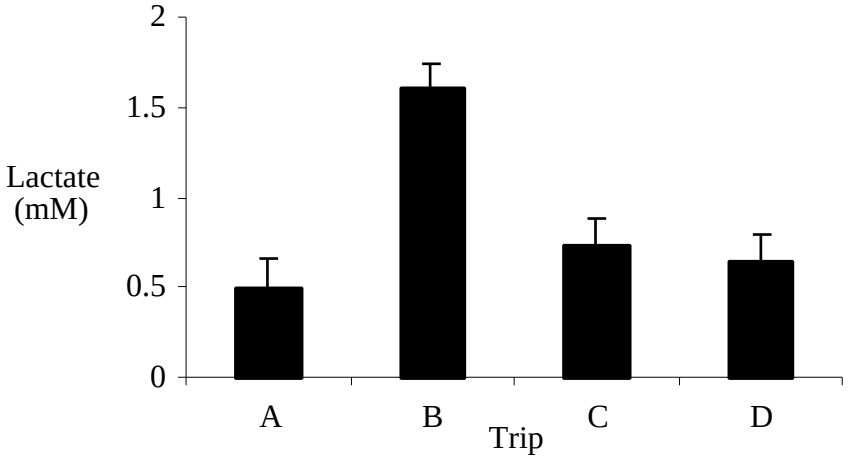

Figure 18. Mean lactate concentration (mM) vs trip sampled in fish captured in the commercial fishery.

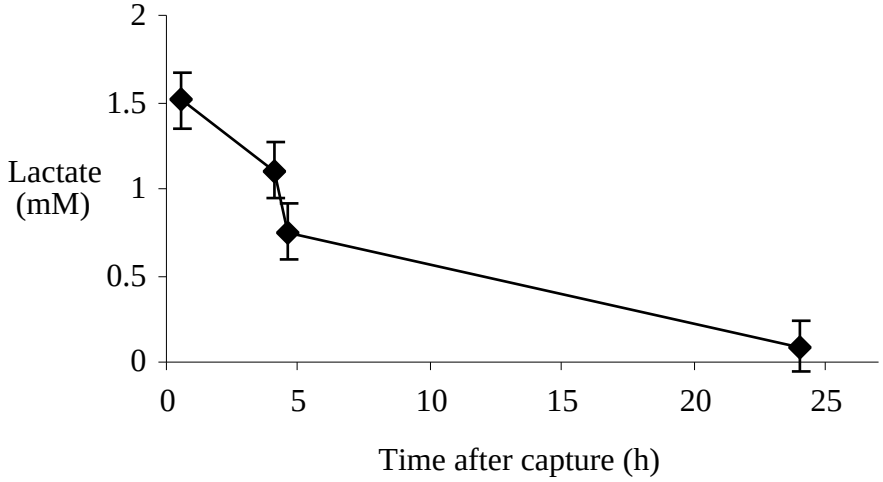

Figure 19. Mean lactate concentration (mM) vs time sampled (hours) in fish captured in the commercial fishery.

Stress incurred by Coral trout during an "ideal" capture and handling

The cortisol response to capture in this study was significant (Table 6), but highly variable and not well defined (Figure 20). Plasma cortisol levels at 120 and 240 min after capture were significantly different from basal concentrations, however at 30, 60, and 180 min after capture, they were not significantly different (Figure 20). Overall, the magnitude of the stress response is low and no pattern associated with any dory parameters is apparent.

Table 6. Characterisation of the response of circulating stress indicators over a 240 min time period.

Cortisol*				
Source of Variation	*df*	*MS*	*F*	*P*
Recovery time	5	0.383	7.213	0.000
Error	25	0.053		
Glucose				
Recovery time	5	13.14	13.72	0.000
Error	26	0.958		
Lactate#				
Recovery time	5	0.471	8.71	0.000
Error	23	0.054		

* ln transformed data
# square-root transformed data

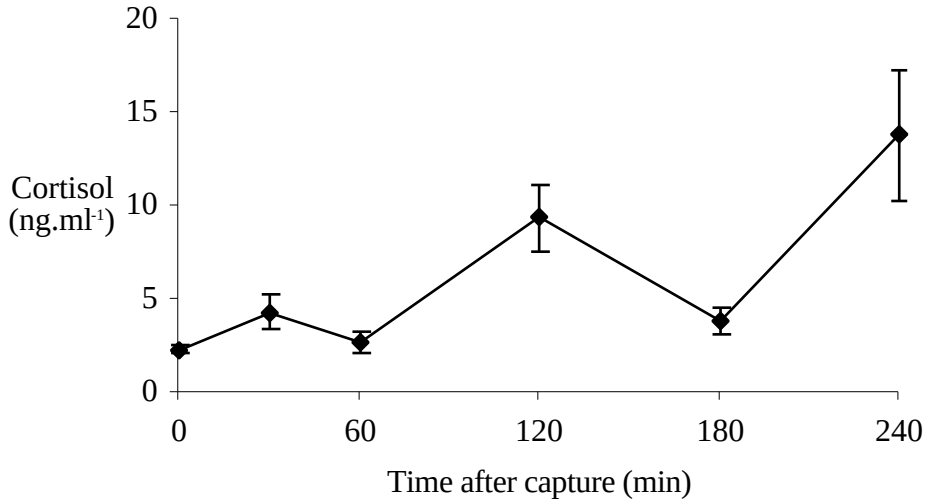

Figure 20. Mean cortisol concentration (ng.ml^{-1}) vs time sampled (min) in fish captured and subjected to "ideal" handling practices.

Plasma glucose provided a much more predictable and well defined response than plasma cortisol (Figure 21). Levels of plasma glucose increased significantly 30 min after capture and gradually decreased over the next 210 min (Table 6, Figure 21). After 240 min, plasma glucose levels were not significantly different from time zero levels (Figure 21).

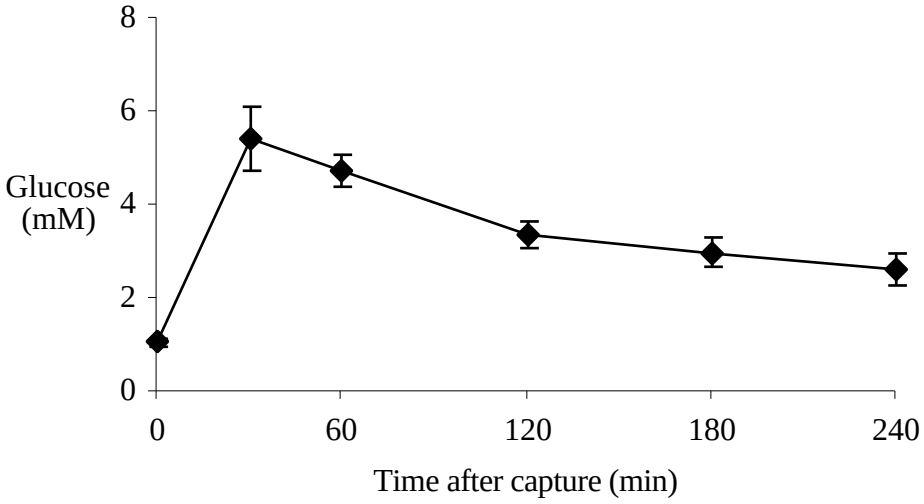

Figure 21. Mean glucose concentration (mM) vs time sampled (min) in fish captured and subjected to "ideal" handling practices.

Plasma lactate showed a pattern not unlike that of plasma glucose (Figure 22). Again levels had significantly increased by 30 min and had returned to basal levels by the end of the sampling period, i.e. 240 min (Table 6, Figure 22).

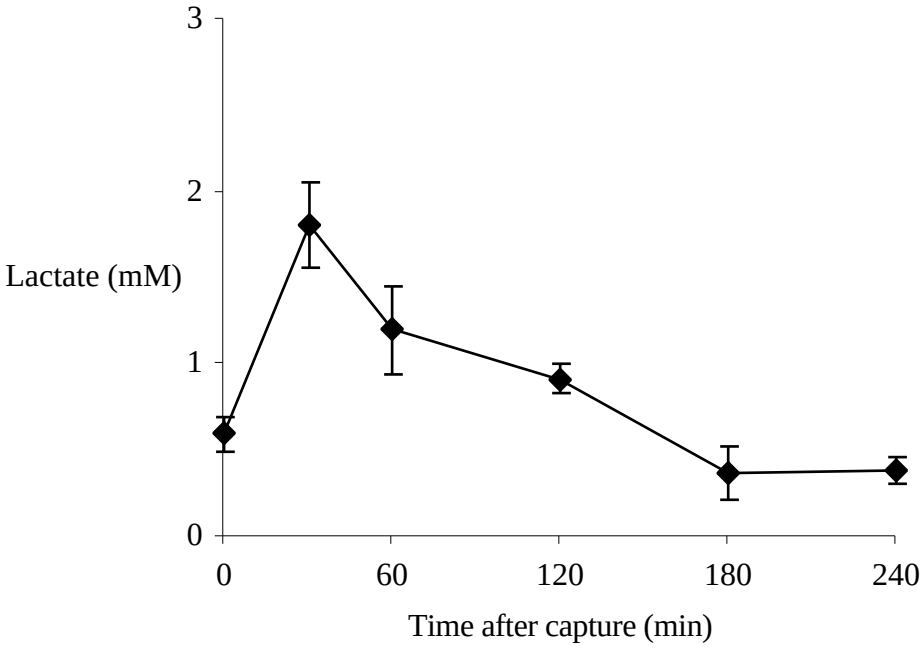

Figure 22. Mean lactate concentration (mM) vs time sampled (min) in fish captured and subjected to "ideal" handling practices.

DISCUSSION

Substantial variation in fishing practices in the commercial industry were identified that might lead to a compromised environment for the fish with resulting increases in the levels of fish stress. Catch rates varied between trips and were always higher in the morning than in the

afternoon. Similar variations in catch rates between AM and PM sessions were observed by Mapstone *et al*. (2001). As a result, stocking rates and water quality in dory tanks in the industry are highly variable. Poor water quality was particularly characterized by high ammonia and low dissolved oxygen. Dissolved oxygen in dory tanks began below saturation levels and declined thereafter throughout the session. In some cases, DO reached as low as 15% saturation in the dory tank. Conversely, ammonia often began at a low concentration but increased throughout the session reaching up to 4 $mg.L^{-1}$. Water quality in day tanks and main vessel tanks also varied between trips. In the main vessel tanks, stocking density was negatively correlated with dissolved oxygen and positively correlated with dissolved ammonia. A strong positive correlation between water temperature, which in most cases directly relates to environmental water temperature due to the flow through design of the water exchange systems used, and stocking density probably reflects the fact that fish are more active and so easier to catch when the water is warmer.

Three parameters were identified to provide indications of the response of the HPI and SC axes based on Frisch and Anderson (2000). These were cortisol as an indicator of the HPI axis response and glucose and lactate as indicators of the SC axis response. The response of the HPI and SC axes in Coral trout varied from one another and with trip and recovery time. The different response between cortisol and glucose is considered to reflect the different nature of the HPI and SC axis. As discussed above, the HPI axis is a relatively slow and longer acting response while the SC axis is rapid in both initiation and recovery (Anderson, 2001).

The cortisol levels generally increased from 30 min to 240 min after capture, recovering after the fish were moved to day tanks and remaining relatively consistent while fish were held in main vessel tanks. These data imply that a major driver of the cortisol levels in these Coral trout is the process of capture which induces a typical cortisol release and recovery pattern (Frisch and Anderson, 2000). Ammonia up to 0.8 $mg.L^{-1}$ and DO down to 50% saturation did not apparently cause these animals to increase plasma cortisol while in the main tanks. Average cortisol concentration varied between trips and showed a decrease between trips A and C that may be associated with improved practices on the vessel.

The change in plasma glucose concentrations with time was not consistent between trips. In all trips, circulating glucose declined after the fish were placed in the main vessel holding tanks indicating the SC axis was recovering. Plasma glucose was elevated at 30 min after capture reflecting the stress response of the initial capture. In Trips B and C, plasma glucose declined while the fish were in the dory tanks, indicating that these fish were recovering in these tanks. In Trips A and D, the glucose concentrations rose while in the dory tanks indicating that the fish in these tanks were continuing to suffer a stressor. This difference between trips is not correlated with stocking density since although Trip A showed the highest density in dory tanks, Trip D was the lowest observed. However, the difference does correlate with practices observed aboard the dories with poorer water quality observed in dory tanks from Trips A and D. Reduction in the stress response between Trips A and C was probably related to improved dory practices were observed.

Plasma lactate declined throughout the handling process being highest 30 min after capture. Lactate is produced by an animal both during severe exercise and stress as a result of anaerobic metabolism. The observed peak of lactate in the first time point after capture probably results from the effects of the struggle associated with capture and the anoxia resulting from the fish being held out of water whilst being dehooked and/or measured. The significantly higher lactate observed in fish from Trip B was not correlated with stocking density or DO in tanks and so is likely to have resulted from increased struggling during capture of these animals. This may have been associated with fish being taken from deeper water although this data was not recorded.

Length of time between hooking and landing the fish and depth of capture were not reported and so no definitive explanation of this difference is possible. It is interesting to note that on the vessel which improved practices from Trip A to Trip C, no differences in lactate response occurred (Figure 19), in contrast to cortisol (Figure 14) which decreased with improved practice.

The response of glucose and lactate observed in fish captured during the "ideal" sampling process showed a generally similar response to that described by Frisch and Anderson (2000). Both glucose and lactate concentrations increased and reached maximum levels 30 min after capture demonstrating the effect of the stimulation of the SC axis and anaerobic metabolism respectively. The cortisol response, however, was less clear with a general increase over time to a maximum at 240 min. This is to be expected, as the experiment was designed for optimal handling and holding conditions, and a chronic stress response (as measured by cortisol) was not produced. In contrast, a very clear cortisol stress response has been recorded for Coral trout by Frisch and Anderson (2000). In that study, cortisol was significantly elevated from basal levels 30 min after capture, climbing to a peak 4 h post-capture, and slowly decreasing after this. Recovery to time 0 time levels was not observed within the 72 h experimental time period (Frisch & Anderson, 2000).

Our preliminary observations on the interaction between the practices in the fishery and the stress response can be summarised thus:

1) there is considerable variability in holding and handling procedures between vessels;

2) glucose response appears to provide a more sensitive measure of differences in handling protocols, compared to cortisol and lactate. Cortisol appears to provide a more general measure of the stress experienced by the animal in its environment which is a complex combination of handling and water quality effects. This is a reflection of the fact that glucose is an indicator of acute stress response and cortisol is an indicator of the chronic response; and

3) potential exists to substantially improve dory handling and holding procedures to enable rapid recovery from stress by Coral trout.

These observations led us to focus our research effort on establishing and controlling the critical parameters of dory capture and holding procedure.

PART B. EFFECT OF CONTROLLED STRESSORS, DISSOLVED OXYGEN AND AMMONIA ON THE STRESS RESPONSE.

INTRODUCTION

In Part A of this study, it was apparent that there were significant differences in the stress response of Coral trout captured and held under different circumstances. These responses appear to be largely related to the experience of the fish in the dory tanks. In a previous study, Frisch and Anderson (2000) observed the stress response in Coral trout but there was no available calibration of the magnitude of the change in cortisol, glucose and lactate against a qualitative assessment of what would constitute a moderate or severe stressor.

Similarly in Part A of this study, it was apparent that the water quality parameters most likely to be related to the stress response of the fish in the dory tanks, and coincidentally the most likely to be able to be easily manipulated in the commercial fishery, were dissolved oxygen and ammonia.

Thus, the aims of Part B of the study were to identify the response of the Coral trout with regard to circulating cortisol, glucose and lactate to a moderate or severe stressor and to various concentrations of DO and ammonia.

MATERIALS AND METHODS

Controlled stressor

Animal maintenance

Fish were obtained from commercial fishermen and maintained in twelve 200L rectangular white plastic tanks stocked at 6 fish per tank. They were fed daily on trash fish. Water was supplied from a recirculating system with water temperature maintained between 26.1 and 27.5 °C.

Stressor treatments

Fish were subjected to one of three stressors termed Control, Moderate and Severe. Control fish were removed from their tank and immediately sampled at time 0, 30, 60 and 240 min (one tank per time point, each fish sampled once only). Fish subjected to the Moderate stressor were removed to a 70L plastic tank containing a small amount of water (insufficient for the fish to do other than lie on their side) for 1 min and returned to the 200 L tank. Fish subjected to the Severe stressor were removed to a 70L plastic tank containing a small amount of water which was rocked side-to-side every 20 secs for 10 min before being returned to the 200L tank. Moderate and Severe fish were sampled at 30, 60, 120 and 240 min after the completion of the stressor. The experiment was repeated once to determine if there was an effect of acclimation time to the stress response.

Dissolved oxygen and ammonia

Animals

The experiment was conducted on board a commercial fishing vessel. Twenty four fish caught the previous day were starved overnight in 380L circular plastic tanks with constant flow through of fresh oceanic water. At the beginning of the experiment, fish were either sampled or

divided between each of three systems. Each system comprised a 185 L tank with a closed lid and fitted with a standpipe overflowing into a 70 L sump tank. Water in each system was recirculated at a rate of 1080 $L.h^{-1}$ (six $exchanges.h^{-1}$) by means of a 12 V pump.

Fish were subjected to either the ammonia or DO treatment for 30 min on the first day. The experiment was then repeated for 240 min using a second group of animals captured from the same reef on the subsequent day.

Water treatment

For the ammonia experiment, ammonia solution was added to the water of each system to achieve 0.5, 1.0 or 1.5 $mg.L^{-1}$ ammonia. For the dissolved oxygen experiment, 0.07 $g.L^{-1}$ sodium sulphite (Na_2SO_4) was added to deoxygenate the water. Oceanic water was then added to the system to achieve a DO of 10%, 40% or 70% saturation. Fish were placed in each system once the desired conditions had been achieved.

Water analysis

Ammonia was measured in samples taken from the outlet water running into the sump using the cyanurate method described above. DO and pH were measured by means of probes located in the experimental tank.

Sampling

Animals were anaesthetised in 0.08 $mg.L^{-1}$ benzocaine until ventilation ceased. Blood was drawn from the caudal sinus, treated and assayed for cortisol, glucose and lactate as described previously.

RESULTS

Controlled stressor

In the first experiment, mean cortisol levels for the Control (~31 $ng.ml^{-1}$) and Moderate (~35 $ng.ml^{-1}$) stressor are significantly higher than those in fish subjected to the same treatments

Table 7. Effect of experiment (One, Two), stressor (Control, Moderate, Severe) and recovery time (30, 60, 120 and 240 min) on cortisol stress response of Coral trout.

Cortisol*				
Source of variation	*df*	*MS*	*F*	*P*
Experiment	1	24.9	67.7	0.00
Stressor	2	0.48	1.36	0.26
Recovery time	3	0.196	0.55	0.65
Experiment *x* Stressor	2	3.86	10.7	0.00
Experiment *x* Recovery time	3	0.16	0.44	0.73
Stressor *x* Recovery time	6	0.31	0.87	0.52
Experiment *x* Stresor *x* Recovery time	6	0.44	1.24	0.29
Error	88	0.36		

***** Log-transformed data

in the second experiment (~7 and ~7 ng.ml^{-1} respectively; Table 7, Figure 23). However, there is no difference between the two experiments when the Severe controlled stressor was applied to the trout (Figure 23). This result indicated that the trout were already stressed prior to the experimental treatments in the first experiment. Therefore, the results from the second experiment, following a period of acclimation to captivity, are deemed more representative of actual cortisol stress response by Coral trout to induced stressors.

The cortisol response of Coral trout in the second experiment was significantly greater in the Severe stress treatment at 30 min and 60 min but there was no difference between treatments by 120 min (Figure 24).

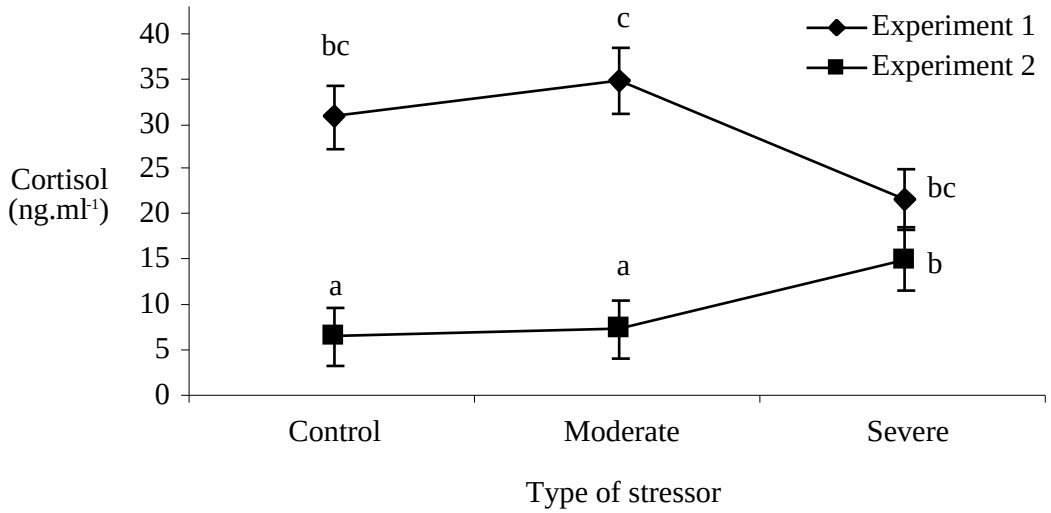

Figure 23. Circulating cortisol concentrations (ng.ml^{-1}) in Coral trout subject to no (Control), Moderate or Severe stressors in two experiments. Values are mean ± S.E., letters indicate homogenous subsets.

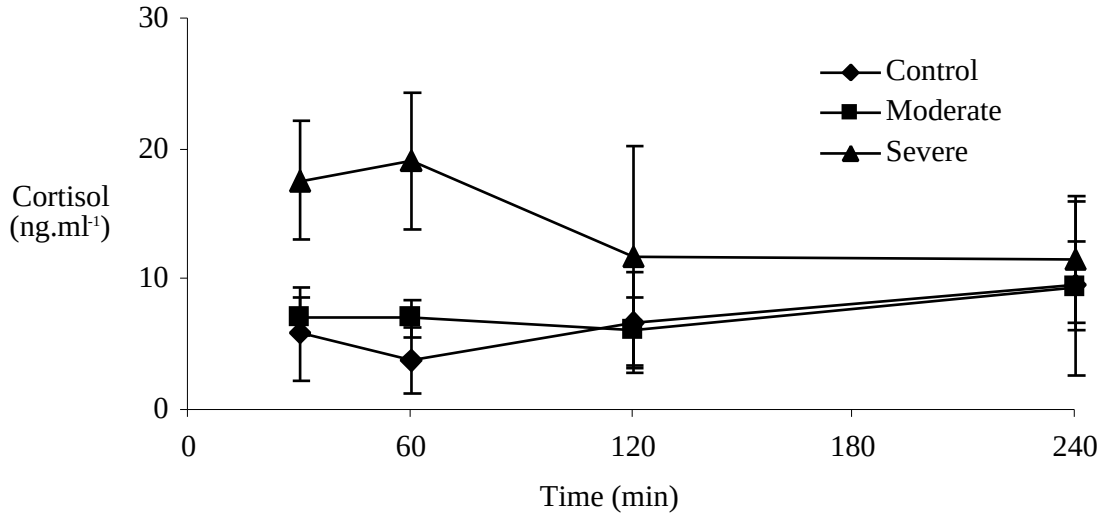

Figure 24. Mean cortisol concentration (ng.ml^{-1}) vs time sampled (min) in fish subjected to various levels of stress in experiment 2.

The glucose stress response of Coral trout to simulated stressors was significantly different between stressors, and response time (Table 8). There was also a significant interaction between

stressor and response time (Figure 25). The only relatively clear pattern is that glucose concentrations in Coral trout subjected to the Severe controlled stressor were significantly higher at 30 min after capture compared to fish from the Control or Moderate stress treatment (Figure 25). However, by 60 min there was no difference in glucose response between the different experimental treatments.

Table 8. Effect of stressor (Control, Moderate, and Severe) and recovery time on stress response of Coral trout as measured by concentration of circulating hormones or metabolites.

Cortisol				
Source of Variation	*df*	*MS*	*F*	*P*
Stressor	2	772.7	1.87	0.167
Recovery time	3	228.4	0.55	0.649
Stressor *x* Recovery time	6	196.6	0.48	0.476
Error	42	412.9		
Glucose				
Stressor	2	0.626	3.04	0.056
Recovery time	3	1.098	5.34	0.003
Stressor *x* Recovery time	6	1.401	6.82	0.00
Error	56			
Lactate*				
Stressor	2	0.034	2.0	0.145
Recovery time	3	0.182	10.6	0.000
Stressor *x* Recovery time	6	0.016	0.925	0.485
Error	54			

***** square root transformed data

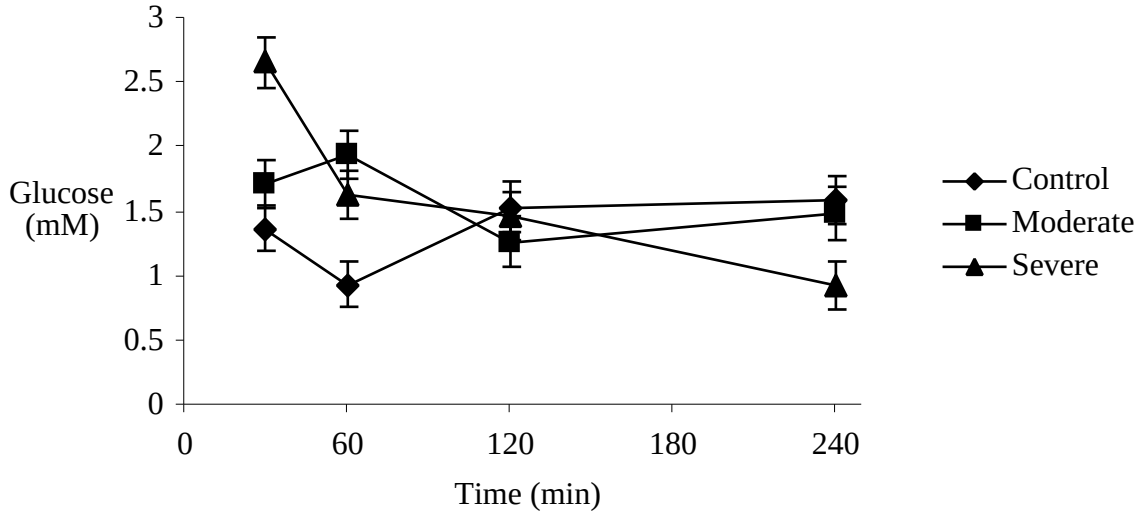

Figure 25. Mean glucose concentration (mM) vs time sampled (min) in fish subjected to various levels of stress.

No difference in lactate stress response of Coral trout was observed between any of the stress treatments, nor was there any interaction between stress treatment and recovery time (Table 8).

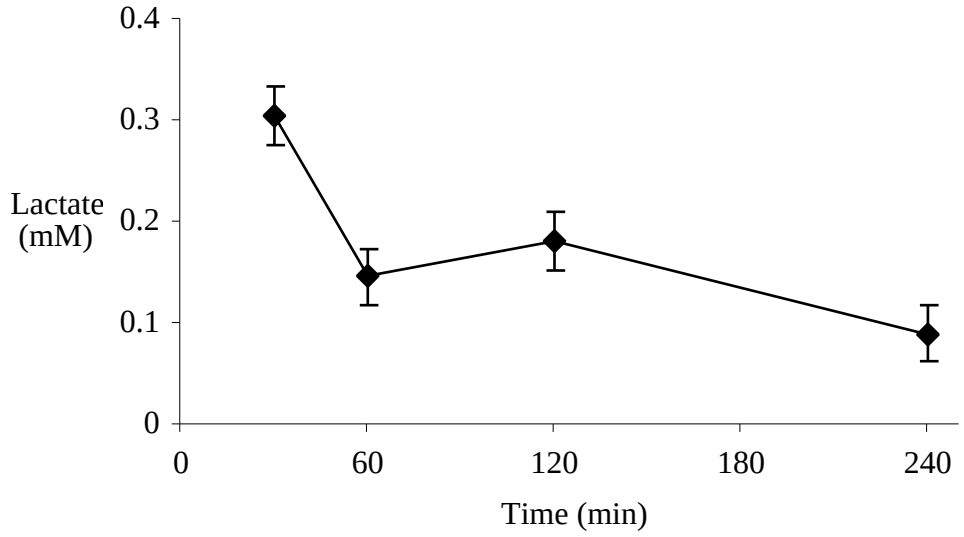

Figure 26. Pooled mean lactate concentration (mM) vs time sampled (min) in fish subjected to various levels of stress.

Table 9. Effect of dissolved oxygen and time of exposure (30, 240 min) on the stress response of Coral trout as measured by concentration of circulating hormones and metabolites.

Cortisol#				
Source of Variation	*df*	*SS*	*H*	*P*
Dissolved Oxygen	3	117.8	0.63	> 0.05
Time of exposure	1	40.8	0.22	> 0.05
DO *x* Time of exposure	3	1457.2	7.75	<0.1
Error	39	7035		
Glucose#				
Dissolved Oxygen	3	0.025	0.5	> 0.05
Time of exposure	1	0.025	0.5	> 0.05
DO *x* Time of exposure	3	0.152	3.1	> 0.05
Error	39	0.049		
Lactate#				
DO and Time of exposure	4		9.87	< 0.05

***** log-log transformed data
# Analysis by non-parametric ANOVA

The only significant effect was recovery time, indicating that the lactate stress response and associated recovery was consistent across all experimental treatments. Figure 26 shows the lactate response, summed across all experimental treatments. Lactate concentration was highest at (~0.3 ng/ml) after 30 min. There was no significant difference between lactate concentrations at 60, 120, or 240 minutes after cessation of the experimental stressors.

Dissolved oxygen and ammonia

Dissolved Oxygen

There was no significant effect of DO or time of exposure on concentration of cortisol in the blood of Coral trout (Table 9). Patterns of change in circulating cortisol over time are apparent (Figure 27). However, the interaction between DO and response time was significant at $\alpha = 0.1$ but not at $\alpha = 0.05$ (Table 9). Cortisol increased between the time 0 and 30 min value at 10% saturation of oxygen. At 30 min, fish held at 30% and 70% saturation of oxygen appeared to have the same level of circulating cortisol as fish at time 0 (Figure 27). At 240 min, however, the fish held at 10% and 70% saturation had a circulating cortisol concentration similar to that of fish at time 0 while those held at 30% saturation of oxygen appeared to have a higher level of circulating cortisol. The overall mean cortisol level for the experiment was 20.6 ± 2.7 SE $ng.ml^{-1}$ (Figure 27).

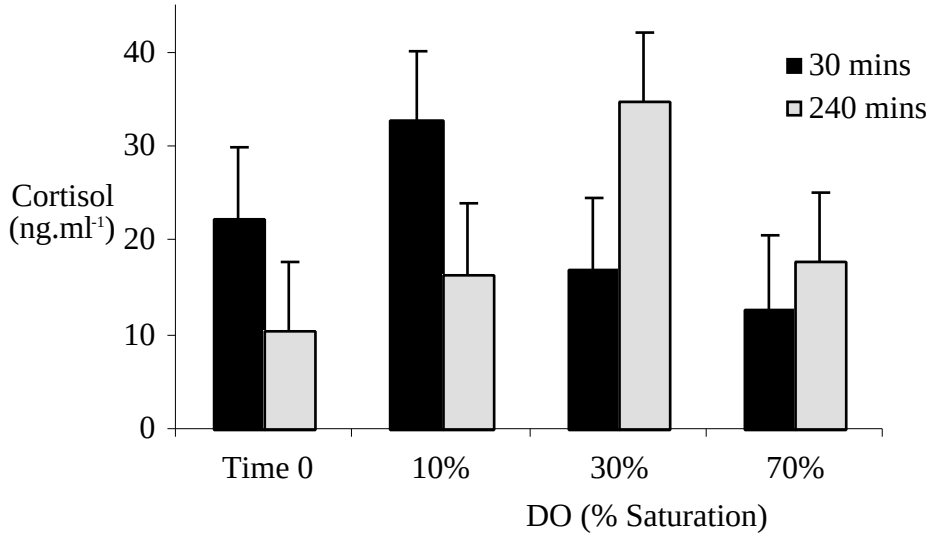

Figure 27. Circulating cortisol concentrations ($ng.ml^{-1}$) in Coral trout subjected to different levels of dissolved oxygen for 30 min or 240 min. Time 0 values are those determined in fish for each experiment (on consecutive days). Values are mean ± S.E.

There was no effect of DO levels or exposure time on circulating glucose, nor was there an interaction between these two factors (Table 9). Overall mean glucose level for this experiment was 2.51 mM ± 0.11 SE.

Lactate response to varying levels of dissolved oxygen was significant (Table 9). Concentration of lactate in unstressed trout (Time 0) was below the sensitivity of the assay at both 30 and 240 min (Figure 28). Blood from Coral trout exposed to 10% oxygen saturation for 240 min was significantly higher in lactate than blood from trout exposed to all other treatments, which were not significantly different from each other (Figure 28).

Ammonia

There was a significant difference in cortisol levels amongst Coral trout subject to different concentrations of ammonia (Table 10). Basal levels of cortisol were lowest, and significantly different from cortisol in fish exposed to 1 and 1.5 $mg.ml^{-1}$ (Figure 29). Cortisol was highest in fish exposed to 1.5 $mg.ml^{-1}$, and this value was significantly different to fish subject to 0.0 or 0.5

mg.ml^{-1} of ammonia (Figure 29). The cortisol response to ammonia was not dependent on recovery time, and there was no interaction between ammonia concentration or recovery time (Table 9). This result suggests a clear influence of ammonia on long term stress response as measured by cortisol.

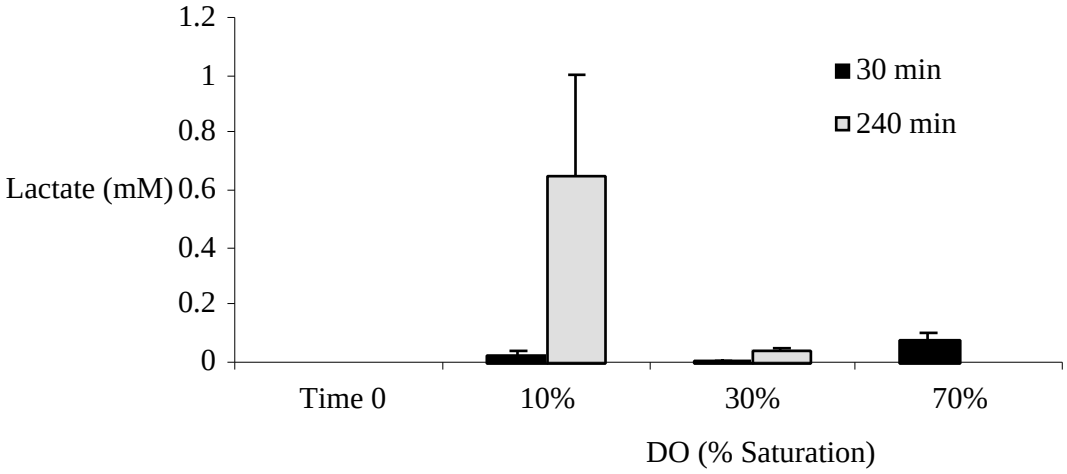

Figure 28. Circulating lactate concentrations (mM) in Coral trout subjected to different levels of dissolved oxygen for 30 min or 240 min. Time 0 values are those determined in fish for each experiment (on consecutive days). Values are mean ± S.E.

Table 10. Effect of ammonia and time of exposure (30, 240 min) on stress response of Coral trout as measured by concentration of circulating hormones and metabolites.

Cortisol*				
Source of variation	*df*	*MS*	*F*	*P*
NH_4 concentration	3	0.56	8.83	0.00
Time of exposure	1	0.11	1.68	0.20
NH_4 concentration *x* Time of exposure	3	0.12	1.89	0.15
Error	37	0.06		
Glucose#				
		SS	*H*	*P*
NH_4 concentration	3	2141	10.9	<0.02
Time of exposure	1	4219	21.5	<0.001
NH_4 concentration *x* Time of exposure	3	434	2.12	> 0.05
Error	40	196		
Lactate#				
			H	*P*
NH_4 concentration and Time of exposure	4		5.72	> 0.05

***** log-log transformed data
# Analysis by non-parametric ANOVA

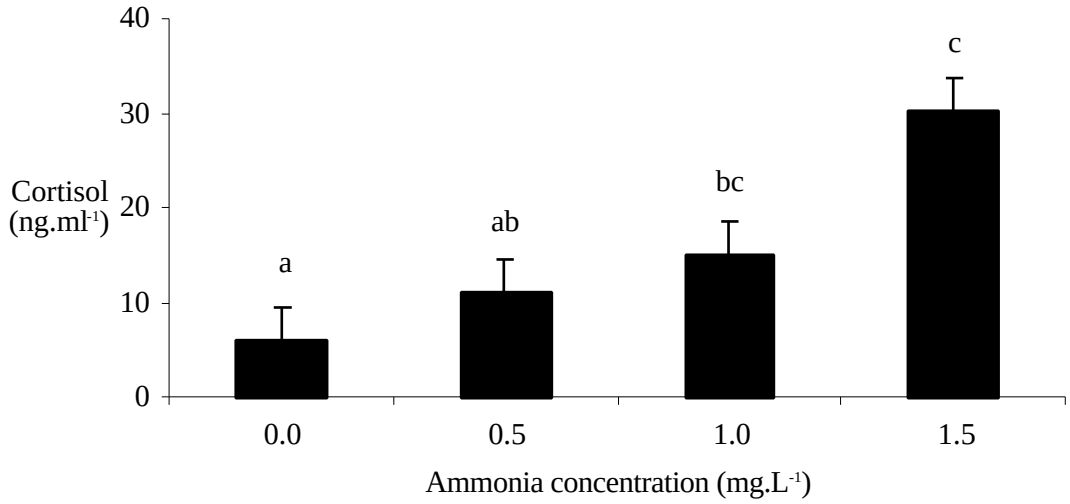

Figure 29. Circulating cortisol concentrations (ng.ml^{-1}) in Coral trout subjected to different levels of ammonia. Values are mean ± S.E., letters indicate homogenous subsets.

Glucose concentrations were significantly different between different ammonia treatments and exposure times, but there was no interaction between these factors (Table 9). After 30 min of exposure to any level of ammonia there was no increase in blood glucose. After 240 min exposure, there was no difference between basal levels and trout exposed to 0.5 mg.ml^{-1}, and blood glucose in fish experiencing these levels of exposure was significantly lower than glucose at 1.0 and 1.5 mg.ml^{-1} (Figure 30). In fish experiencing 1.5 mg.ml^{-1} ammonia, blood glucose reached extreme concentrations with a mean value of 14.2 mM.

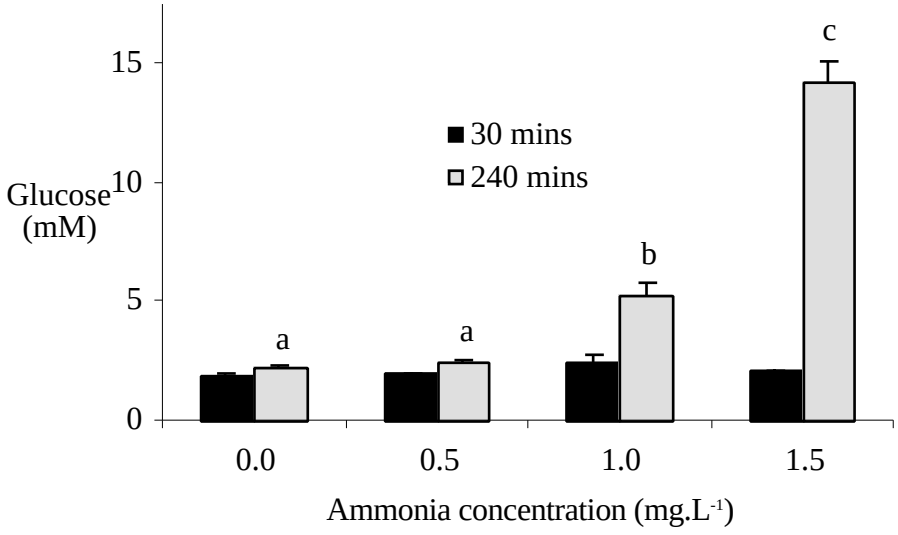

Figure 30. Mean glucose concentration (mM) in Coral trout vs ammonia concentration (mg.L^{-1}) in the water. Values are mean ± S.E., letters indicate homogenous subsets within the 240 min exposure treatments.

No discernible changes in lactate concentrations were observed as a result of the experimental treatments (Table 9). There was no measurable lactate in the blood of trout at 30 min. The mean concentration found in the remaining treatments was 0.07 ng.ml^{-1} ± 0.01.

DISCUSSION

Controlled stressor

Although it is of considerable value to be able to measure a single or few parameters to be able to apply a semi-quantitative value to the level of stress experienced by an individual, determination of relative responses in stress parameters are rare. This may be due to the difficulties in determining the effects of many influences including prior history, genetic effects, diet, holding conditions prior to stress and so on. However, in the present study where education and modification of the behaviour of fishers was desired, it was considered to be useful to be able to place the measured parameters (cortisol, glucose, lactate) in a framework of the level of stress experienced by the fish. Thus, we undertook to determine the effect of stressors that were subjectively ascribed as Control (none), Moderate and Severe on the circulating levels of cortisol, glucose and lactate.

This was undertaken in two experiments and showed a differential response in the cortisol concentrations depending upon whether the animal was already stressed or not. In the first experiment, cortisol concentration in the control was found to average ~31 $ng.ml^{-1}$, a value clearly indicative of a stressed animal (Frisch and Anderson, 2000). In this case, there was no significant effect of a moderate stressor (1 min air exposure) but in fish subjected to the chronic treatment (10 min air exposure with intermittent rocking), cortisol levels were lower. A number of factors influence the amount of cortisol secreted by an animal under any given circumstances. The latent period prior to release of cortisol (Lowe and Wells, 1996; Frisch and Anderson, 2000) indicates that cortisol is synthesised in response to a stressor rather than being stored in tissue. It is possible that the substrates for synthesis are exhausted in chronically stressed Coral trout that are then subjected to the Severe stressor. Frisch and Anderson (2000) found the cortisol response to be sustained for more than 72 h but it is not known how long the fish in the first controlled stressor experiment had been experiencing the stressor. Thus, it can not be determined how these fish relate to that observation and whether or not substrate exhaustion was possible. Alternatively, it may be that the Severe stressor served to invoke the feedback mechanism on ACTH release that is known to regulate maximum levels of cortisol (Barton and Iwama, 1991).

Control fish in the second controlled stressor experiment had low levels of circulating cortisol (~7 $ng.ml^{-1}$) indicative of unstressed animals and responded more predictably. There was no effect of the Moderate treatment on circulating cortisol, but the Severe treatment invoked a significantly higher cortisol response that followed the previously described release and recovery cycle (Figure 24; Frisch and Anderson, 2000). These animals were taken to provide a better indication of the relationship between circulating hormones and metabolites in Coral trout and the degree of stress experienced shortly after the stressor. On this basis, the level of cortisol measured in fish captured in the commercial line fishery indicates that they are experiencing stressors that invoke a greater response than the response to air exposure with intermittent rocking for 10 min.

Although not statistically significant ($P = 0.056$), the Severe stressor resulted in higher blood glucose than the Control and Moderate treatments at 30 min but this was no longer apparent by 60 min (Figure 25). Even so, the glucose levels determined in fish immediately after capture or when held in dories with poor water quality ($2 – 4x$) were much greater than those measured in this experiment, indicating the relative severity of the stressor experienced by fish during capture and holding in conditions of poor water quality.

Lactate measured in this experiment did not vary with treatment and showed rapid recovery within 60 min. Again, measured values were much lower than those in fish immediately after capture providing support for the severity of the stress imposed by the capture process.

Dissolved oxygen and ammonia

Only the interaction between DO and Exposure time was statistically significant in affecting circulating cortisol in Coral trout (Table 9) and this interaction is clear in Figure 27. This experiment was conducted using relatively newly captured fish aboard a vessel and concentration of cortisol was high. However, further increases in cortisol were apparent. Subjecting Coral trout to DO of 10% saturation induced a small rise in cortisol at 30 min but levels had returned to control values by 240 min. Suppression of the HPI axis under conditions of very low oxygen saturation and high carbon dioxide levels has been previously observed in brown trout (Pickering and Pottinger, 1987a) and largemouth bass (Carmichael *et al.*, 1984). There was no apparent response in Coral trout cortisol to 30% DO at 30 min but by 240 min, cortisol appeared to have increased. At 70% DO, no response was apparent at either 30 or 240 min.

Only subjecting Coral trout to DO of 10% saturation for 240 min elicited an increase in plasma lactate. An increase in plasma lactate is the result of increased reliance on anaerobic metabolism and is common to studies of the effects of hypoxia. The critical point at which anaerobic metabolism is activated between 10 and 30% for Coral trout appearing only after 240 min is similar to ranges from 13 to 20% in goldfish, tilapia and carp (van Ginneken *et al*., 1998), 11 to 15% in barramundi (Percival, 1999) and 25% in rainbow trout (van Raaij *et al*., 1997). These data indicates that deleterious effects of hypoxia in Coral trout will only be observed at very low DO.

No effect of low DO on plasma glucose was observed in this experiment. Other species have demonstrated varied responses with both increased blood glucose concentrations (Morata *et al*., 1982; van Raaij *et al*., 1997) and unchanged concentrations (Dunn and Hochachka, 1986) being reported. The absence of hyperglycaemia may indicate that the catecholamine release invoked by low blood oxygen concentration (Wright *et al*. 1989) was not initiated at 10% saturation in Coral trout.

While surprisingly few effects on glucose metabolism and the SC axis were seen in response to low dissolved oxygen, the data indicate that DO should be maintained at or above 70% saturation in holding tanks for Coral trout to avoid a cortisol response.

Excretory products

In contrast to DO, a clear and consistent dose response to ammonia concentration was observed in both plasma cortisol and glucose. Cortisol was not affected by exposure time and was significantly elevated over control values by 1.0 and 1.5 $mg.L^{-1}$ ammonia. Glucose concentrations were increased at 240 min relative to those at 30 min and were also significantly elevated in response to 1.0 and 1.5 $mg.L^{-1}$ ammonia.

There is limited information about the effect of ammonia on the stress response of fish. Carmichael *et al.*, (1984) found that plasma corticosteroids remained low, although plasma glucose remained elevated following exposure to ammonia and 24 h of recovery in largemouth bass. An increase in plasma chloride ions 24 h after exposure suggested that ammonia has a suppressive effect on the HPI axis a with a subsequent delayed stress response (Carmichael *et al.*, 1984). However, the HPI response in Coral trout is apparent within 30 min, although it may be suppressed by 24 h as in bass.

Clearly, ammonia must be maintained below 0.5 $mg.L^{-1}$ in holding tanks for Coral trout to avoid a stress response. Further, interactions between ammonia and dissolved oxygen may occur further exacerbating the effects of poor water quality. Sousa and Meade (1977) reported that increases in ammonia concentration reduced the oxygen carrying capacity of the blood in coho salmon, although (Smart, 1978) suggested an overall increase in oxygen consumption. It has also been established that low levels of dissolved oxygen increase the toxicity of ammonia to rainbow trout (Lloyd, 1961).

PART C. EFFECT OF WATER FLOW, TANK DESIGN AND STOCKING DENSITY ON STRESS RESPONSE

INTRODUCTION

In Part A of this study, the significant differences in the stress response of Coral trout taken by fishers appeared to be largely related to the experience of the fish in the dory tanks. This was associated with measurements of poor water quality (high ammonia, low DO) in many of the dory tanks. In Part B it was demonstrated that high ammonia and low DO caused the fish to become stressed. A number of practices by dory fishers that might contribute to poor dory tank water quality were observed including failure to ensure a flow of clean water through the tanks at all times. Whilst these practices might be easily overcome by attention to detail by the fisher, other aspects such as tank design and flow require modifications to existing equipment to achieve best practice.

In view of this, the aims of Part C of the study were to design a dory tank that would facilitate water exchange and ensure a clean environment for the fish at all times and be suitable for location and operation in a dory whilst at sea. This part of the study also aimed to identify the necessary rates of exchange of water and maximum stocking densities of fish in the tanks to ensure minimal stress on the animals.

MATERIALS AND METHODS

Tank design

Two tanks were compared in this study. The first was a commercial rectangular plastic tank obtained from Nally Industries (Sydney) configured to reflect standard practice on board commercial dories (Figure 31). These tanks were of 185 L capacity and were 450 mm high and 400 mm *x* 750 mm at the base and 600 mm *x* 900 mm at the top.

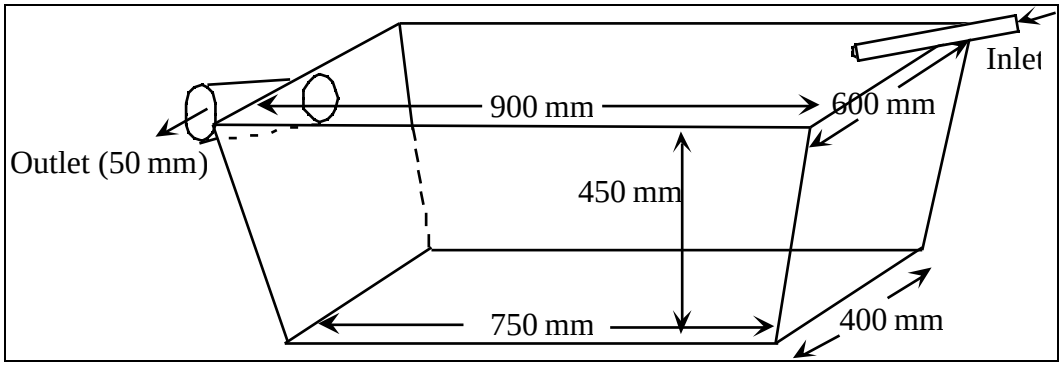

Figure 31. Diagram of the rectangular plastic tank.

The second tank was designed according to standard principles of aquaculture tank operation (Figure 32). The tank is cylindrical with a central drain in the floor which is sloped at approximately 10° from the outside to the drain. The drain is protected from blockage by two U-shaped bars forming a cross above the drain opening and constructed from 10 mm aluminium rod welded to the bottom of the tank. Immediately below the floor of the tank, the 50 mm aluminium pipe carrying the effluent bends at a right angle and carries to a point outside the body of the tank. The body of the tank is comprised of a cylinder measuring 400 mm high *x* 750 mm in diameter and with the sloping floor. A second cylinder measuring 200 mm high *x* 460

mm in diameter is mounted at the top and is sealed with a hinged lid secured by a clasp. This provides a baffle to prevent excessive water movement. The inlet to the tank comprises a 20mm aluminium pipe set at approximately 30° to the perpendicular to provide for circular water movement within the tank. The inlet is mounted 100 mm from the base of the upper cylinder. The outlet of the tank is fitted with plumbing fittings such that there is an external stand-pipe which is led over the side of the dory and a valve mounted at the base of the stand-pipe which allows the fisher to easily and quickly flush bait disgorged by the fish.

The tanks were arranged in the recirculating seawater system at James Cook University as described in the General Materials and Methods.

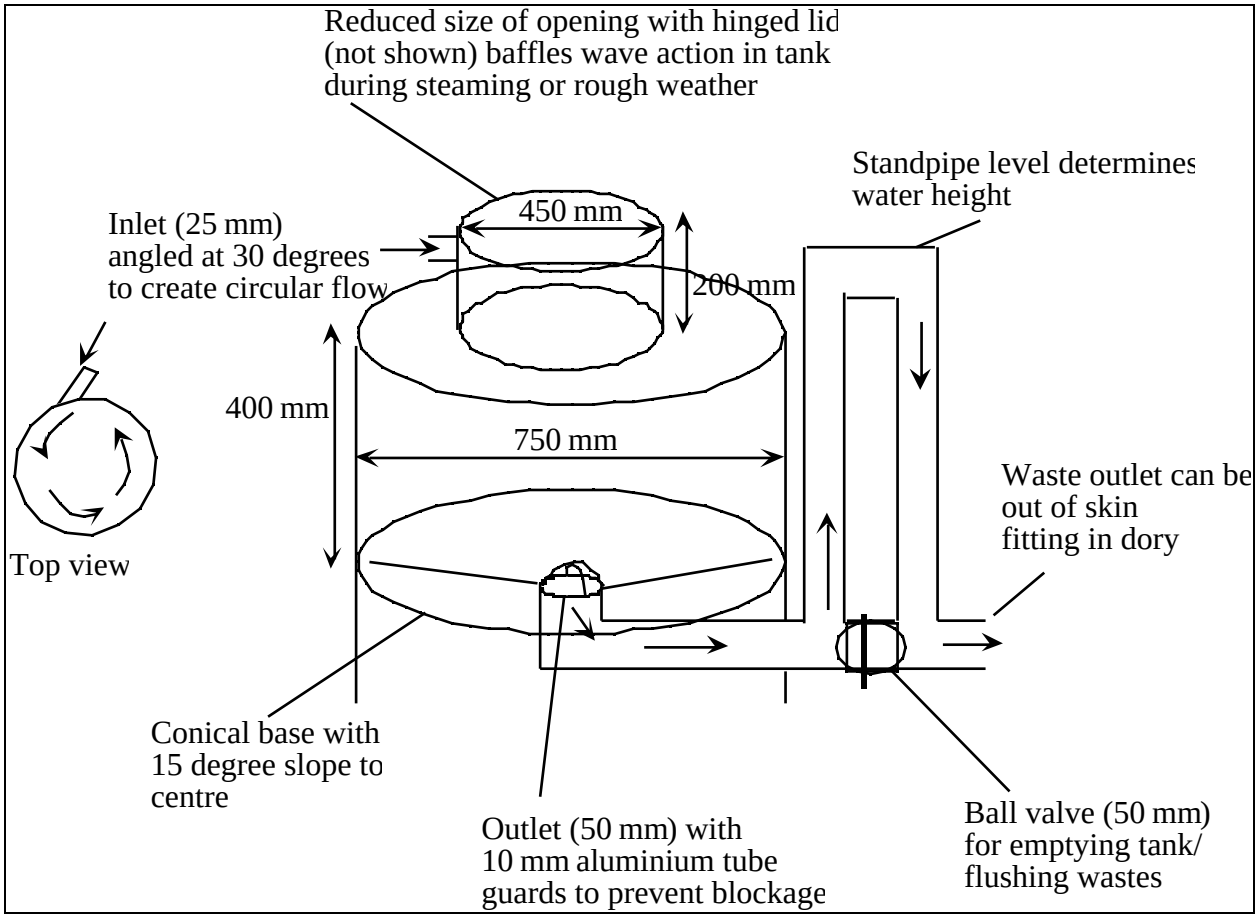

Figure 32. Diagram of the cylindrical dory tank design.

Determination of flow rates

Flow rates were determined in tanks without fish by use of a Flomate 2000 flow meter (Marsh McBirney, Fredericksberg Maryland, USA). Thirty replicate measurements of flow were taken in each position in each of four directions and averaged to give the final value.

Exchange rate

The effect of exchange rate on flow rates, water quality and the stress response was determined at 2, 6, or 10 tank volumes per hour. Prior to the experiment, 60 fish were held in two 1000 L tanks and fed daily to satiety on trash fish. Six fish were anaesthetised to obtain basal, or resting levels of blood parameters and, following sampling, were replaced in the original tank. Eighteen other fish were divided equally into each of three round dory tanks with flow rates of 2, 6, or 10

tank volumes per hour. Specimens within the dory tanks were sampled for stress response after 30 min, and DO, pH and ammonia levels were recorded. The procedure was repeated with another 24 fish the next day, which were left in the dory tanks for 240 min. After two weeks recovery, the experiment was repeated using the square dory tanks.

Stocking density

One hundred fish were held in 3 *x* 1000 L circular plastic tanks and fed daily to satiety. Basal levels of stress were established by sampling 10 fish from one tank prior to any disturbance. Sixty fish from the other two tanks were netted and placed in a 185 L tub with approximately 30 L seawater to simulate initial catching and holding stressors at sea. After approximately 3 min, fish were re-netted and placed into 3 round dory tanks described above to produce stocking densities of 10, 20, 30 fish/tank. At 30 min, fish were removed and sampled. The experiment was repeated following a rest period of two weeks and stress response measured at 240 min. During the experiment, water quality parameters were monitored and maintained at consistent levels to avoid confounding effects.

Ammonia was measured in samples taken from the outlet water using the cyanurate method described above. DO and pH were measured by means of probes located in the experimental tank.

Sampling

Animals were anaesthetised in 0.08 $mg.L^{-1}$ benzocaine until ventilation ceased. Blood was drawn from the caudal sinus, treated and assayed for cortisol, glucose and lactate as described previously.

RESULTS

Flow characteristics of tanks

Round Tank

Flow characteristics of the round tank at different exchange rates are seen in Figures 33 to 35. Flow throughout the tank is consistently in one direction. At 2 tank volumes an hour, flow rates vary from 2 $cm.sec^{-1}$ at the centre of the tank near the inlet, to 7 $cm.sec^{-1}$ near the tank walls (Figure 33). With increasing flow, speeds increase near the tank walls and the bottom, rising to 17 $cm.sec^{-1}$ with 6 tank volumes an hour (Figure 34). At 10 tank volumes an hour, water flow varies from 18 – 24 $cm.sec^{-1}$ within the tank (Figure 35).

Square Tank

Flow characteristics for the square tank are quite directionally variable and average flow speeds markedly lower. At 10 tank volumes per hour, flow speeds within the square tank vary from 0 – 7 $cm.sec^{-1}$ (Figure 36), which is substantially lower than speeds observed within the round tank for the equivalent exchange rate.

Effect of tank design, water flow, and recovery time on the stress response.

Blood parameters

There was no significant effect of tank design or water flow on cortisol stress response by Coral trout (Table 11). In fact, the only factor that had a significant influence on cortisol levels was

recovery time (Table 11). Mean cortisol concentration at 30 min (11.4 ng.ml^{-1} ± 0.7 SE) was significantly higher compared to 240 min (7.0 ng.ml^{-1} ± 0.7 SE).

The glucose response of Coral trout was significantly different between tank design, and water flow (Table 11). Figure 37 shows that glucose levels in round tanks were significantly higher than in square tanks. However, there was also a significant interaction between water flow and recovery time (Table 11). Figure 38 shows that glucose response at 30 min was significantly lower at basal water flows, compared to the exchange rates of 2, 6, and 10 tank volumes per hour. At 30 min, there was no difference in glucose levels from flow rates of 2, 6, or 10 tank volumes per hour (Figure 38). This result is to be expected as the fish are still within the time at which they exhibit their peak response, so differences in water flow are not important in the early stages of recovery. At 240 min after capture the situation is different. Glucose concentrations in fish at 6 and 10 tank volumes per hour had returned to basal levels and were significantly lower than fish subject to exchange of only 2 tank volumes per hour (Figure 38).

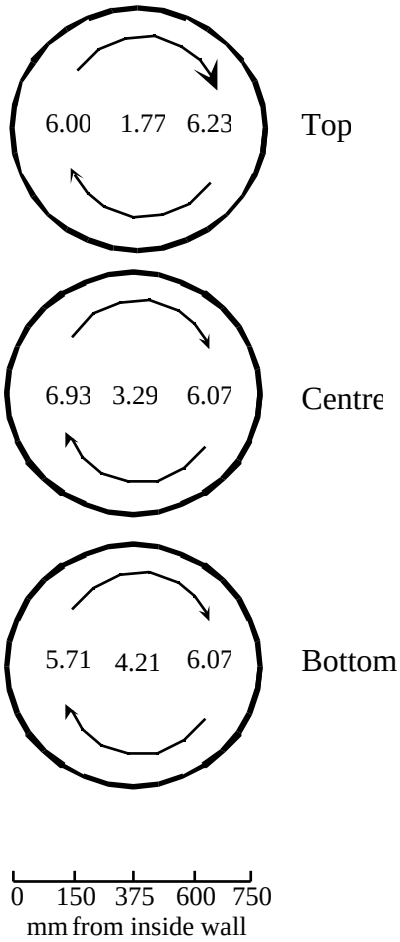

Figure 33. Flow velocities in the cylindrical tank at 2 tank volumes exchanged.h^{-1}. Values are cm.sec^{-1}. Arrows indicate the direction of water flow.

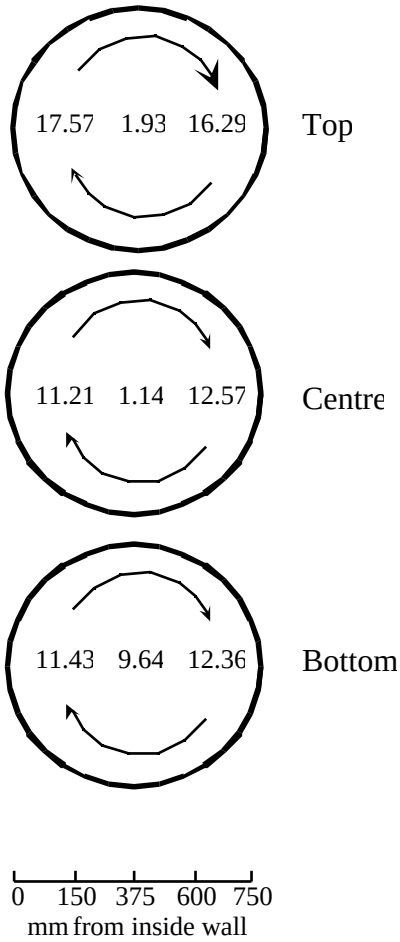

Figure 34. Flow velocities in the cylindrical tank at 6 tank volumes exchanged.h^{-1}. Values are cm.sec^{-1}. Arrows indicate the direction of water flow.

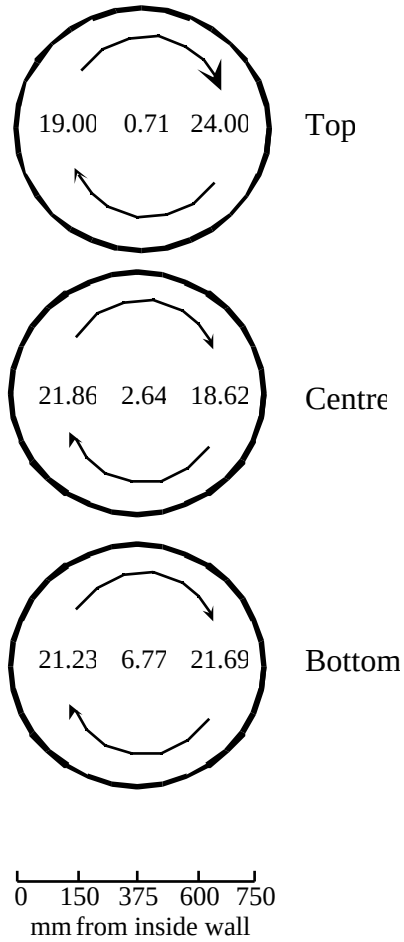

Figure 35. Flow velocities in the cylindrical tank at 10 tank volumes exchanged.h^{-1}. Values are cm.sec^{-1}. Arrows indicate the direction of water flow.

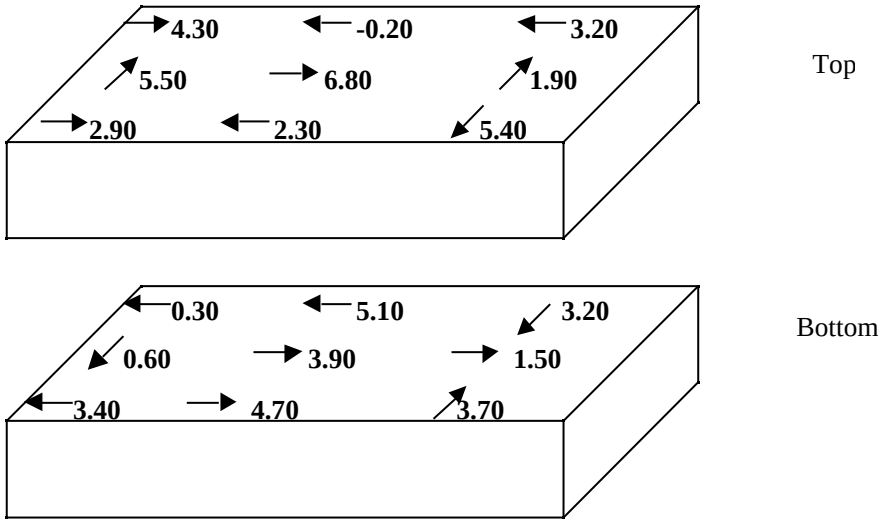

Figure 36. Flow velocities in the rectangular tank at 10 tank volumes exchanged.h^{-1}. Values are cm.sec^{-1}. Arrows indicate the direction of water flow.

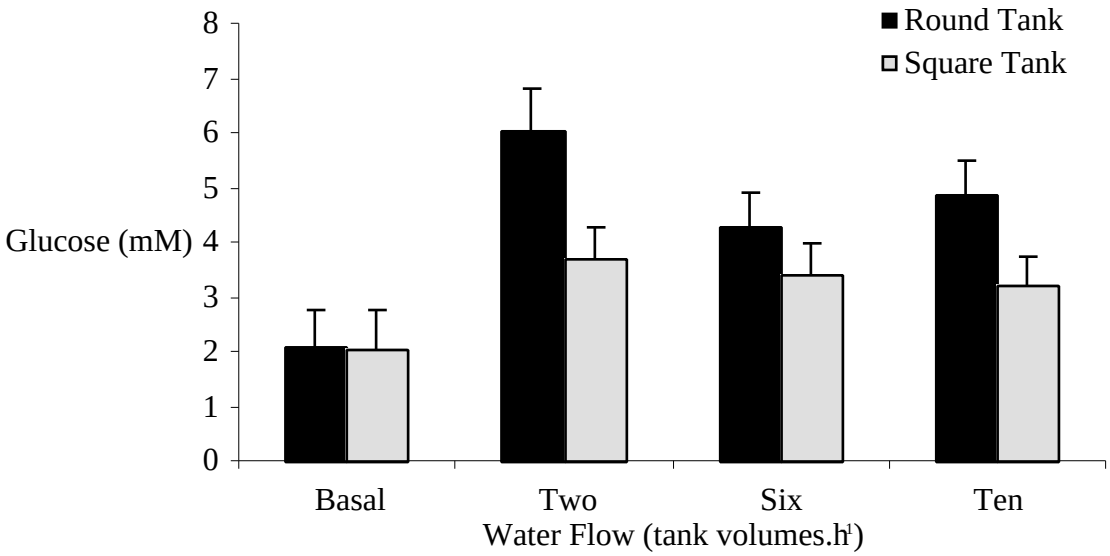

Figure 37. Mean glucose concentration (mM) in Coral trout vs flow rate (tank volumes.h^{-1}) in Square or Round tanks. Values are mean ± S.E.

The lactate stress response was quite clear (Table 11; Figure 39). There was a significant effect of water flow, but no difference between tank design, and no interaction between water flow or tank design. Figure 39 shows the lactate response at 240 min to different water exchanges. After 240 min, lactate was significantly higher at basal levels of flow, compared to 2, 6, and 10 exchanges per hour.

Water quality

Water quality varied between treatments. Tanks with an exchange of two tank volumes.h^{-1} showed significantly poorer water quality parameters than tanks exchanging 6 or 10 tank volumes.h^{-1} (Table 12). This was the case for all of DO, ammonia and pH.

Table 11. Effect of tank design (Round, Square), water flow (0, 2, 6, 10 tank volumes.h^{-1}), and recovery time (30, 240 min) on stress response of Coral trout as measured by concentration of circulating hormones and metabolites.

Cortisol*				
Source of Variation	*df*	*MS*	*F*	*P*
Tank design	1	0.11	0.62	0.43
Water flow	3	0.25	1.46	0.23
Recovery time	1	5.05	29.3	0.00
Tank design *x* Water flow	3	0.36	2.06	0.11
Tank design *x* Recovery time	1	0.16	0.93	0.34
Water flow *x* Recovery time	3	0.17	0.98	0.41
Tank design *x* Water flow *x* Recovery time	3	0.12	0.74	0.53
Error	86	0.17		
Glucose#				
		SS	*H*	*P*
Tank design	1	4540	5.50	<0.05
Water flow	3	17556	21.28	<0.05
Recovery time	1	940	1.14	NS
Tank design *x* Water flow	3	1914	2.32	NS
Tank design *x* Recovery time	1	460	0.56	NS
Water flow *x* Recovery time	3	12964	15.71	<0.05
Tank design *x* Water flow *x* Recovery time	3	991	1.20	NS
Error (MS)	83	825		
Lactate				
		MS	*F*	*P*
Tank design	1	0.001	0.01	0.91
Water flow	3	0.511	6.36	0.00
Tank design *x* Water flow	3	0.024	0.29	0.83
Error	45	0.080		

***** log-transformed data
# non-parametric analysis

Table 12. Water quality parameters in dory tanks containing 6 Coral trout and receiving water exchange at 2, 6 or 10 tank volumes.h^{-1}. Superscripts indicate homogenous subsets (Tukey's test, p<0.05).

Exchange rate	**2 tank volumes.h^{-1}**	**6 tank volumes.h^{-1}**	**10 tank volumes.h^{-1}**
DO	61.44 ± 0.83^{a}	70.34 ± 0.33^{b}	72.35 ± 1.01^{b}
NH_3	0.12 ± 0.01^{a}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}
pH change	-0.11 ± 0.03^{a}	-0.04 ± 0.01^{b}	-0.04 ± 0.01^{b}

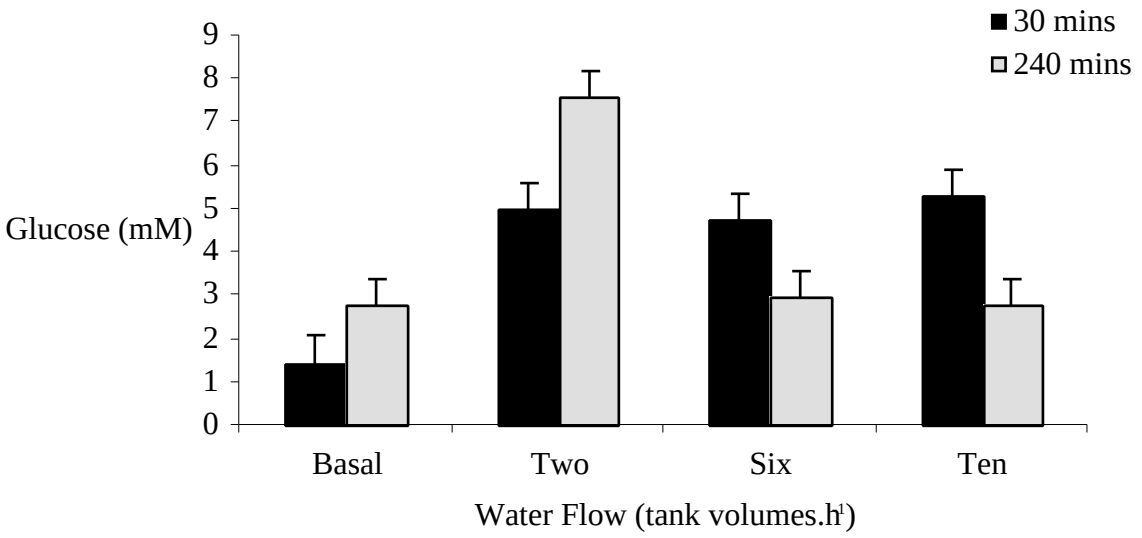

Figure 38. Mean glucose concentration (mM) in Coral trout vs flow rate (tank volumes.h^{-1}) at 30 min or 240 min Recovery time. Values are mean ± S.E.

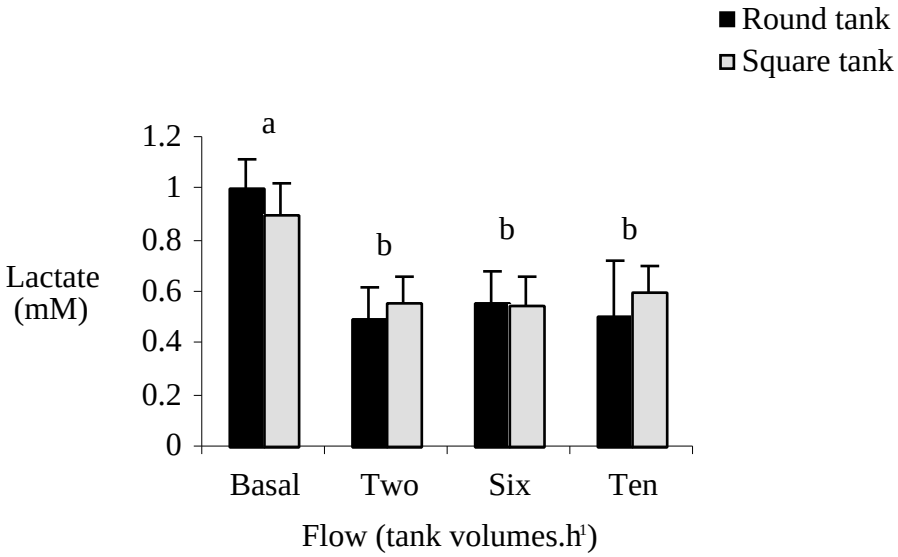

Figure 39. Mean lactate concentration (mM) ± S.E. in Coral trout vs tank volumes exchanged in round or square tanks.

Dissolved oxygen in the tanks declined quickly compared to system water in the 2 and 6 tank volumes.h^{-1} but reached a plateau by 30 min (Figure 40). DO in the 10 tank volumes.h^{-1} did not reach the plateau until between 30 min and 60 min.

Similarly, ammonia concentrations rose rapidly in all treatments immediately fish were placed in the tank, reaching maximum values between 30 min and 60 min after confinement. The greater concentration of ammonia in the tank receiving 2 tank volumes.h^{-1} was readily apparent (Figure 41).

Rapid onset of changed pH was also clearest in the tank receiving the lowest exchange rate, although in all treatments, pH returned to that of system water by 120 min (Figure 42).

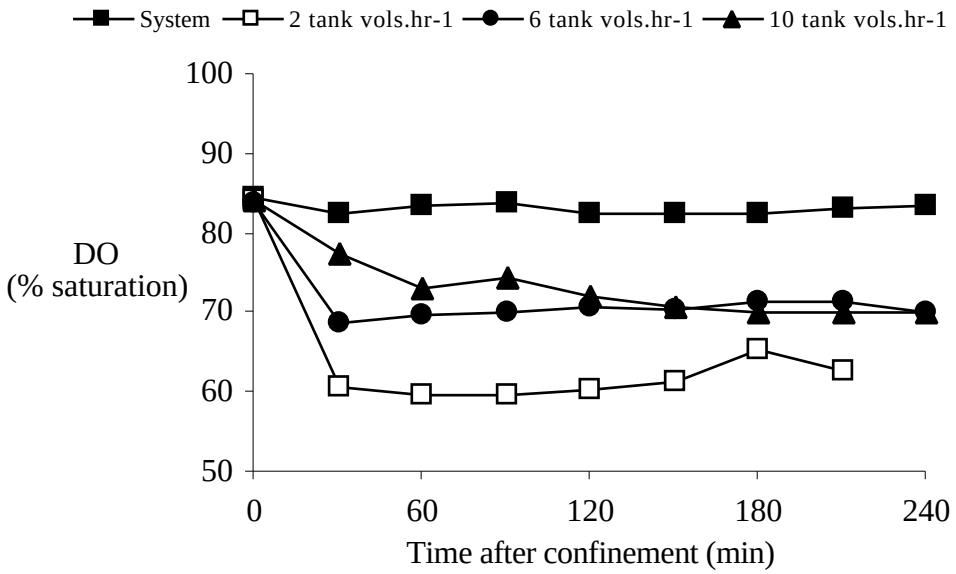

Figure 40. Dissolved oxygen (DO % saturation) vs time after confinement (min) in the source system water and at exchange rates of 2, 6 and 10 tank volumes.h^{-1} in round dory tanks.

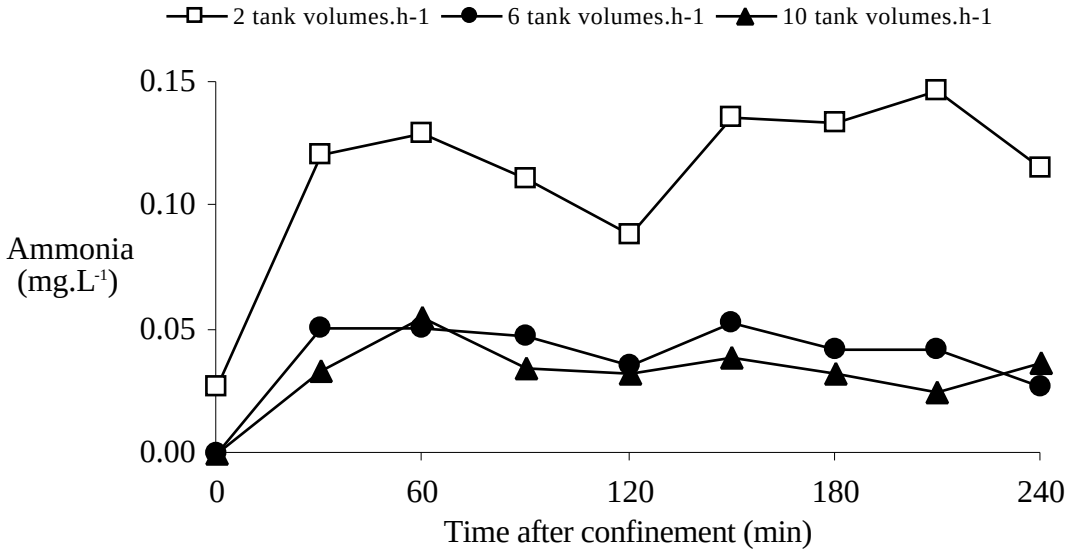

Figure 41. Dissolved ammonia (mg.L^{-1}) vs time after confinement (min) at exchange rates of 2, 6 and 10 tank volumes.h^{-1} in round dory tanks.

Effect of stocking density on the stress response

There was a significant effect of stocking density and recovery time on the cortisol response of Coral trout placed into dory tanks (Table 13). This effect was largely due to a rapid and large increase in the circulating cortisol concentration in fish stocked at the highest density of 30 fish.185 L^{-1} at 30 min after stocking (Figure 43). At 240 min after stocking into the tank, cortisol levels had returned to values that were not different to those of fish prior to stocking (Basal) (Figure 43).

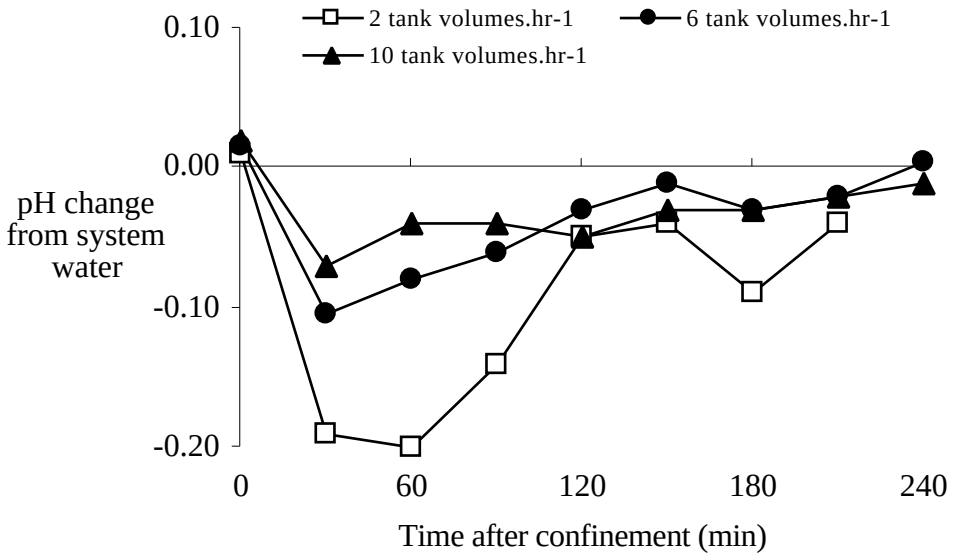

Figure 42. Change in pH from the source system water vs time after confinement (min) at exchange rates of 2, 6 and 10 tank volumes.h^{-1} in round dory tanks.

Table 13. Effect of Stocking density (10, 20 or 30 fish.185 L^{-1}) and Recovery time (30, 240 min) on stress response of Coral trout as measured by cortisol and glucose.

Cortisol*				
Source of Variation	*df*	*MS*	*F*	*P*
Stocking density	3	2.57	4.15	0.012
Recovery time	1	3.86	6.24	0.017
Stocking density *x* Water flow	3	0.58	0.93	0.435
Error	39	0.62		
Glucose#				
		SS	*H*	*P*
Stocking density	3	10583	108	<0.001
Recovery time	1	2368	24.12	<0.001
Stocking density *x* Water flow	3	6256	63.84	<0.001
Error	7	15929		

***** log-transformed data

non-parametric analysis

Plasma glucose levels also showed a significant response to stocking density and recovery time and there was a significant interaction between these parameters (Table 13). Glucose concentration was significantly higher at 30 min in fish stocked at 30 fish.185L^{-1} than in fish stocked at other densities (Figure 44). As with cortisol, by 240 min plasma glucose had returned to basal levels (Figure 44).

DISCUSSION

Tank design and water flow

Tank design had a significant effect on water flow characteristics. The rectangular tanks similar to those normally used in the industry were characterized by a relatively high degree of turbulence and mixing with flow in adjacent segments and levels of the tank often in opposite directions. Even though measurements were conducted at 6 tank volumes.h^{-1}, the maximum flow velocity was substantially lower than that achieved at this exchange rate in cylindrical tanks.

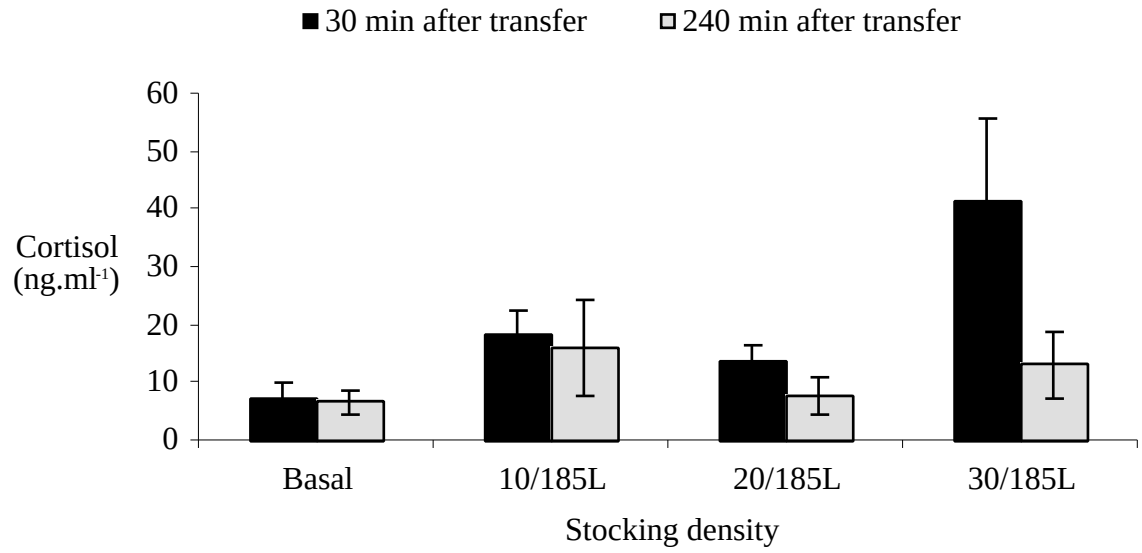

Figure 43. Mean Cortisol concentration (ng.ml^{-1}) in Coral trout vs Stocking density (No. of fish.185 L tank^{-1}) at 30 min or 240 min recovery time. Values are mean ± S.E.

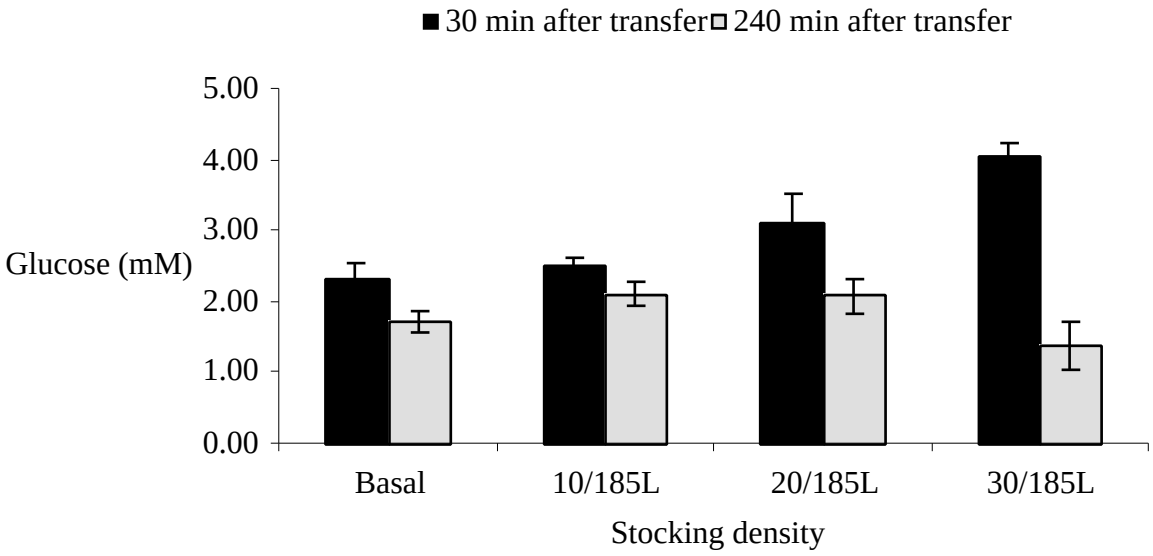

Figure 44. Mean Glucose concentration (mM) in Coral trout vs Stocking density (No. of fish.185 L tank^{-1}) at 30 min or 240 min recovery time. Values are mean ± S.E.

Conversely, the cylindrical tanks had a regular laminar flow in a single circular direction. Maximum velocities of water were achieved at the periphery of the tank with very low velocities in the centre of the tank. The centre drain in the base of the tank resulted in a relatively unmixed plug flow effect where water flowing out the drain was replaced by clean water coming in at the top of the tank. This would result in much better removal of metabolic waste and delivery of fresh oxygenated water to the fish than is possible in rectangular tanks configured as in this experiment.

It should be recognized that the presence of fish in either tank will lead to enhanced mixing and, in the case of the cylindrical tank, perturbation to some extent of the plug flow characteristic. Notwithstanding this, however, it is clear that the flow characteristics of the cylindrical tank are preferable and tank design should seek to achieve this.

Tank design, water flow, recovery time and the stress response

Despite the substantial differences in characteristics and flow velocities observed, tank design and water flow did not have an effect on plasma cortisol. Recovery time did have a significant effect with cortisol elevated at 30 min with return to basal levels by 240 min reflecting a typical response to a handling stressor. This is different to the response of fish to dory tanks observed in the fishery where cortisol rose during the period in the dory tank. The difference is likely to be due to the greater attention to ensuring water flow during the 240 min of the present experiment compared to the variable water exchange in dory tanks observed in the fishery. It further indicates that flow velocities achieved in rectangular tanks or with relatively limited exchange (2 tank volumes.h^{-1}) are sufficient to allow recovery of the cortisol stress response of the fish.

Plasma glucose appeared to be more sensitive to design and flow rate, being highest at an exchange rate of 2 tank volumes.h^{-1} at 240 min after transfer. At all exchange rates, there was an increase in glucose at 30 min but at 6 and 10 tank volumes.h^{-1}, this had declined by 240 min. At 2 tank volumes.h^{-1}, plasma glucose continued to increase and appeared higher at 240 than 30 min indicating that the animals continued to be stressed and recovery was not achieved at the lower flow rate.

Plasma lactate was relatively high compared to other samples in fish sampled for basal levels and was significantly lower in the treatment animals. The reason for the high basal level was not apparent but the fish were clearly able to recover during the period they were in the experimental dory tanks.

The tanks with the exchange rate of 2 tank volumes.h^{-1} had lower DO and higher ammonia than was observed in the tanks exchanging at 6 and 10 tank volumes.h^{-1}. Even though DO was lowest in the tank with the lowest exchange rate, it remained above 60% saturation throughout. We have previously shown a relatively limited effect of low DO, even at much lower concentrations than measured in the experimental dory tanks, and so the stress response at 2 tank volumes.h^{-1} is unlikely to be due to low DO. We had, however, earlier shown a dose dependent stress response to ammonia in Coral trout. It seems more likely therefore that the stress response at the lower exchange rate is attributable to the increase in ammonia.

The increase in ammonia in the experimental dory tanks was less than that which previously invoked the response in the controlled experiment. Interactions between high and ammonia and low DO may have occured. Sousa and Meade (1977) reported that increases in ammonia concentration reduced the oxygen carrying capacity of the blood in coho salmon and low levels of dissolved oxygen increase the toxicity of ammonia to rainbow trout (Lloyd, 1961).

Stocking density and the stress response

A high stocking of 30 Coral trout.185 L^{-1} (approximately 160 $kg.m^{-3}$) was associated with significantly increased circulating cortisol at 30 min after stocking but this had recovered to basal levels by 240 min. Similarly, there was an increase in plasma glucose at 30 min at the highest stocking density which was no longer apparent at 240 min.

A number of studies have investigated the effects of stocking density on the stress response in fish with the degree of crowding known to have an effect on the stress response (Schreck 1981, Pickering and Pottinger, 1987a). Significantly lower plasma cortisol levels are found in flounder held at lower stocking densities (4.8 $kg.m^{-3}$) than those held at high densities (14.4 $kg.m^{-3}$) (Barnett and Pankhurst, 1998) and similar data have been reported for other species (Pickering and Pottinger, 1987a; Robertson *et al.*, 1988; Mazur and Iwama, 1993). The stocking densities investigated in other studies are generally well below those used here where even the lowest density was approximately 54 $kg.m^{-3}$.

Pickering and Stewart (1984) revealed that the effect of high stocking density was independent of water quality in brown trout, many studies do not account for changes in water quality when evaluating the response to stocking density as was the case here. The previous experiment investigating the effect of exchange rate was undertaken at a density of approximately 100 $kg.m^{-3}$. Water quality parameters (DO and ammonia) in that experiment was found to remain relatively good (DO averaged ~70% saturation; ammonia increased by an average of 0.04 $m.L^{-1}$ in the 6 tank volumes.h^{-1} treatment). It is likely that water quality at 160 $kg.m^{-3}$ would be poorer than that determined at 100 $kg.m^{-3}$ and so it is not possible to remove the effect of water quality from the present analysis. It is also not possible to remove the effect of hierarchies which are known to occur in groups of fish and which effect the stress response (Ejike and Schreck, 1980; Knights, 1987; Hyde and Perry, 1990). These are less likely to be in effect in higher stocking densities (Davis *et al.*, 1984; Wedemeyer, 1997) if in fact they are able to develop within the short period of time the fish were held in the experimental dory tanks.

Irrespective of interactions between water quality and stocking density, it is apparent that a stress response is invoked at 160 $kg.m^{-3}$ and not at 100 $kg.m^{-3}$ and stocking densities in the industry should be maintained at a maximum of 100 $kg.m^{-3}$ in tanks.

PART D. EFFECT OF FEEDING, TANK COLOUR, ANAESTHESIA, TEMPERATURE CHANGE AND HANDLING ON THE STRESS RESPONSE OF CORAL TROUT.

INTRODUCTION

In Parts A to C of this study, we examined the effects of water quality and it's management on the stress response of Coral trout taken by fishers. Although water quality appeared to be a major effector of the experience of the fish in the dory tanks, a number of other parameters which varied in the way different fishers husbanded their animals are worthy of investigation. It is known that temperature, nutritional status and handling affect the stress response of fish (see literature review) and it was considered by some fishers that tank colour may also have an effect.

In view of this, the aims of Part D of the study were to evaluate the effects of tank colour, feeding, handling and temperature change on the stress response of Coral trout as determined by circulating plasma glucose and cortisol.

MATERIALS AND METHODS

The effects of tank colour and feeding were determined by studies at James Cook University while the effects of temperature change and handling were examined at the University of Queensland.

Feeding

Animal maintenance

Fish were obtained from commercial fishermen and maintained in two groups of 30 per 1000L round plastic tanks. One group were fed daily to satiety on trash fish while the other group was not fed for 5 days prior to the experiment. Water was supplied from a recirculating system with water temperature maintained at 26.0 ± 0.5 °C.

Stressor treatments

On the morning of the sixth day, 6 fish were netted from their tank and immediately sampled. These fish were then placed in a third tank. The remaining fish in the two experimental tanks were chased with a net for five min. At each of 30 and 240 min after completion of the initial stressor, a further 6 fish were removed and sampled.

Samples were analysed for cortisol, glucose and lactate as described previously.

Tank colour and anaesthesia

Animal maintenance

Fish were obtained from commercial fishermen and maintained in 9 groups of 6 fish per 185L rectangular plastic tanks. All fish were fed daily to satiety on trash fish. Water was supplied from a recirculating system with water temperature maintained at 26.0 ± 0.5 °C.

Stressor treatments

Tanks were assigned to one of three treatments. Treatment 1 fish were held in black tanks and sampled after anaesthesia. Treatment 2 fish were held in white tanks and sampled after

anaesthesia. Treatment 3 fish were held in white tanks and sampled without anaesthesia. Where used, anaesthesia was achieved by addition of benzocaine at 0.08 $mg.L^{-1}$ to the experimental tank.

All fish from one tank from each treatment were sampled at time 0. Fish in the remaining tanks were chased with a net for 5 min and subsequently removed from the tanks and sampled at 30 min and 240 min after the cessation of the stressor.

Samples were analysed for cortisol, glucose and lactate as described previously.

Handling

Animals

Fish obtained from a processor in Cairns and were transported to Brisbane by air freight. They were held for a minimum of 10 d in recirculating seawater systems of approximately 1,500 L. Fish were fed with W.A. pilchards.

Stressor

Individual fish were rapidly and carefully netted from their holding tank and transferred to another holding tank with the same water conditions. Transfer was either Direct, with an air-exposure time of less than five seconds, or incorporated a period of air-exposure of 2, 4, or 6 min. During this time fish were placed on a wetted smooth polystyrene surface to simulate the measuring process on board ship. Fish were then returned to a holding tank. The nets used were industry standard, made of soft, knot-less nylon.

Two hours post-handling/transfer, the fish were rapidly re-netted and sampled within 45 seconds.

Samples were analysed for cortisol, glucose and lactate as described previously.

Temperature

Animals

Coral trout were obtained from a processor in Cairns and transported to Brisbane by air-freight. Fish were released directly into glass aquarium tanks (one $fish.tank^{-1}$) that were part of a recirculating seawater system containing a biological filter. Each tank was aerated, and was covered so that the fish were not disturbed by visual stimuli. Fish were allowed to recover for 48 h prior to the experiment.

Stressor

Fish were subjected to temperature change from either 25 °C to 15 °C over 15 min (Cooling by 10 °C; n=15) or from 25 °C to 30 °C over a period of 15 min (Heating by 5 °C; n=8). The only change to the system was the water temperature; pH, dissolved oxygen and salinity were unaffected by the water cooling. Fish were sampled 120 min after the start of the temperature change. Each fish was then removed from the tank by net and sampled within 45 seconds.

Samples were analysed for cortisol, glucose and lactate as described previously.

RESULTS

Feeding

There was a significant effect of feeding on both cortisol and glucose response (Table 14). Cortisol levels were significantly higher in unfed fish including during basal measurements (Figure 45). There was a significant effect of recovery time, with cortisol declining throughout the experiment. Cortisol values determined in basal samples were higher than would be expected in unstressed fish (Figure 45) at mean values of 30 – 60 $ng.ml^{-1}$.

Glucose was also significantly different between fed and non-fed treatments, between recovery times and there was a significant interaction. This effect is largely due to the significant elevation in plasma glucose concentration at 30 min post stressor in the fed fish. Generally, glucose was lower in non-fed Coral trout, and higher in fed Coral trout (Figure 46). No difference existed at prior to the stressor or at 240 min post stressor (Figure 46).

The lactate response to feeding regime was not significant (Table 14). There was a significant difference between recovery times, with basal levels of lactate being significantly lower than levels at 30 min post stress (Figure 47). This is effect is greater in the fed than the unfed fish.

Tank colour

There was no effect of tank colour on cortisol, glucose, or lactate concentrations in the blood of Coral trout (Table 15). Means for cortisol, glucose, and lactate levels were 16.6 ± 2.0 ($ng.ml^{-1}$), 1.5 ± 0.2 (mM), and 0.72 ± 0.07 (mM) respectively.

Table 14. Effect of feeding and recovery time (Basal, 30, 240 min) on the stress response of Coral trout as measured by concentration of circulating hormones and metabolites.

Cortisol*				
Source of Variation	*df*	*MS*	*F*	*P*
Feeding	1	5.38	15.23	0.00
Recovery time	2	1.25	3.52	0.04
Feeding *x* Recovery time	2	0.13	0.35	0.70
Error	27	0.35		
Glucose				
Feeding	1	5.04	13.97	0.00
Recovery time	2	2.44	6.76	0.00
Feeding *x* Recovery time	2	1.76	4.88	0.02
Error	28	0.36		
Lactate*				
Feeding	1	0.08	3.36	0.08
Recovery time	2	0.15	6.48	0.01
Feeding *x* Recovery time	2	0.05	2.21	0.13
Error	27	0.02		

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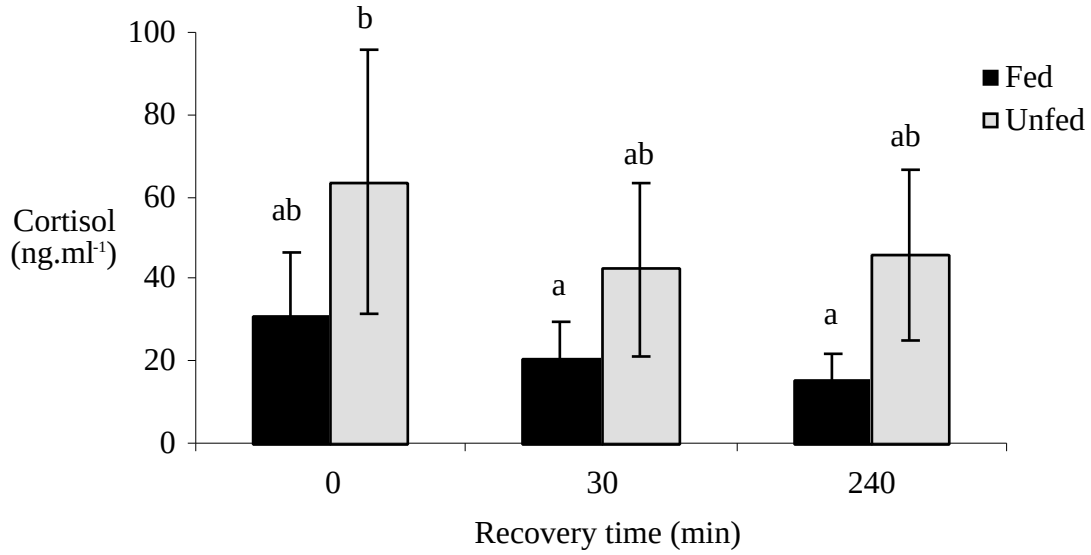

Figure 45. Effect of feeding and recovery time (Basal (0), 30, 240 min) on plasma cortisol. Values are mean ± S.E., letters indicate homogenous subsets.

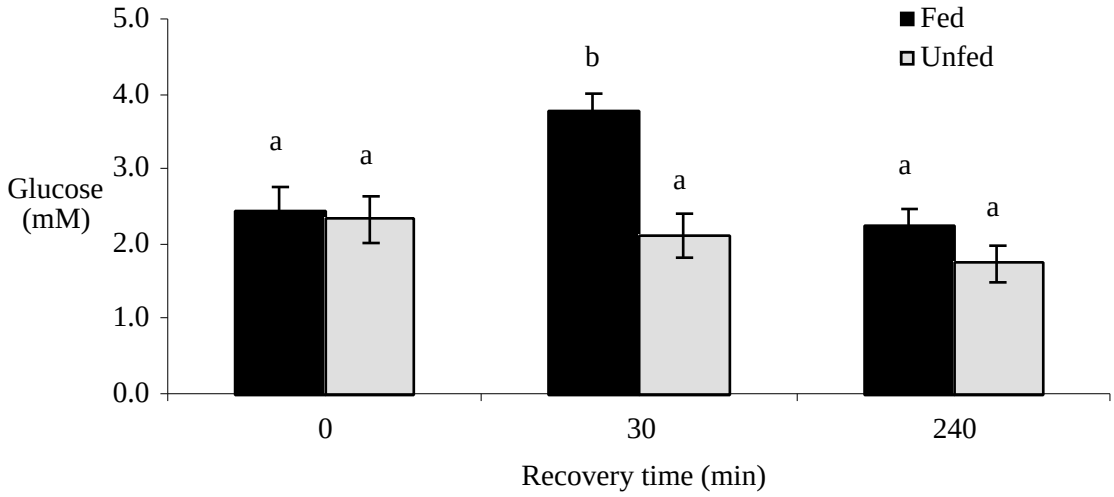

Figure 46. Effect of feeding and recovery time (Basal (0), 30, 240 min) on plasma glucose. Values are mean ± S.E., letters indicate homogenous subsets.

Anaesthetic

There was no effect of anaesthetic or recovery time on plasma cortisol (Table 16) which had a mean value (± S.E.) of 12.8 ± 1.3 ng.ml^{-1}. There was no effect of anaesthetic on plasma glucose and all animals showed the standard glucose response to stress with elevated levels at 30 min which had returned to basal levels by 240 min (Table 16, Figure 48). Plasma lactate concentrations did not vary with recovery time, but did increase significantly with anaesthesia. This increase was relatively small in absolute terms (Table 16, Figure 49).

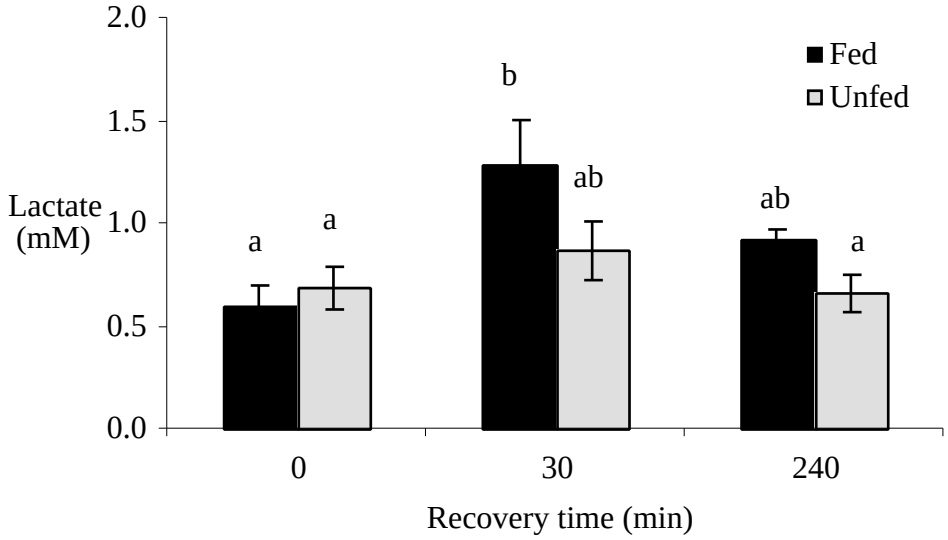

Figure 47. Effect of feeding and recovery time (Basal (0), 30, 240 min) on plasma lactate. Values are mean ± S.E., letters indicate homogenous subsets.

The lactate response to feeding regime was not significant (Table 14). There was a significant difference between recovery times, with basal levels of lactate being significantly lower than levels at 30 min post stress (Figure 47). This is effect is greater in the fed than the unfed fish.

Table 15. Effect of tank colour and recovery time (Basal, 30, 240 min) on stress response of Coral trout as measured by concentration of circulating hormones and metabolites.

Cortisol				
Source of variation	*df*	*MS*	*F*	*P*
Tank Colour	1	60.24	0.60	0.45
Recovery time	2	2.61	0.03	0.97
Tank Colour *x* Recovery time	2	11.37	0.11	0.89
Error	21	100.4		
Glucose				
Tank Colour	1	0.88	1.08	0.31
Recovery time	2	0.88	1.08	0.35
Tank Colour *x* Recovery time	2	0.37	0.46	0.94
Error	24	0.81		
Lactate				
Tank Colour	1	0.07	0.46	0.50
Recovery time	2	0.14	0.97	0.39
Tank Colour *x* Recovery time	2	0.12	0.83	0.45
Error	24	0.14		

***** log transformed data

Table 16. Effect of anaesthetic and recovery time (Basal, 30, 240 min) on stress response of Coral trout as measured by concentration of circulating hormones and metabolites.

Cortisol				
Source of variation	*df*	*MS*	*F*	*P*
Anaesthetic	1	78.8	1.65	0.21
Recovery time	2	46.2	0.97	0.39
Anaesthetic *x* Recovery time	2	26.1	0.55	0.59
Error	23	47.7		
Glucose				
Anaesthetic	1	0.00	0.00	0.99
Recovery time	2	1.74	7.24	0.00
Anaesthetic *x* Recovery time	2	0.63	2.64	0.09
Error	25	0.24		
Lactate*				
Anaesthetic	1	0.096	7.80	0.01
Recovery time	2	0.007	0.58	0.57
Anaesthetic *x* Recovery time	2	0.083	6.93	0.00
Error	24	0.093	7.80	

***** log log transformed data

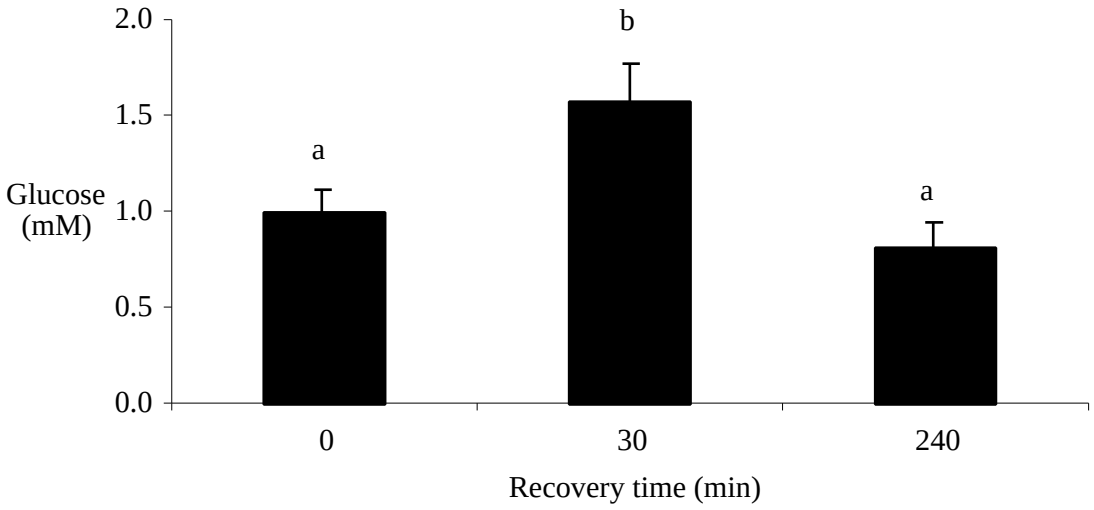

Figure 48. Effect of recovery time (Basal (0), 30, 240 min) in anaesthetized and unanaesthetised Coral trout on plasma glucose. Values are mean ± S.E., letters indicate homogenous subsets.

Handling

There was no effect of handling and air exposure on plasma cortisol, glucose, or lactate concentrations in the blood of Coral trout sampled 2 h after the stressor. Means for cortisol, glucose, and lactate levels were 5.78 ± 1.28 ($ng.ml^{-1}$), 4.37 ± 0.89 (mM), and 1.06 ± 0.30 (mM) respectively. Fish given a six minute air-exposure had lost their righting reflex on returning them to the tank which is indicative of a significant hypoxia. None of the experimental fish died.

Temperature change

A rapid drop of temperature from 25 °C to 15 °C did not elicit any significant change in measured blood parameters. However, a rapid increase in temperature by 5 °C was associated with a significant increase in plasma cortisol 2 h after the stressor (Figure 50).

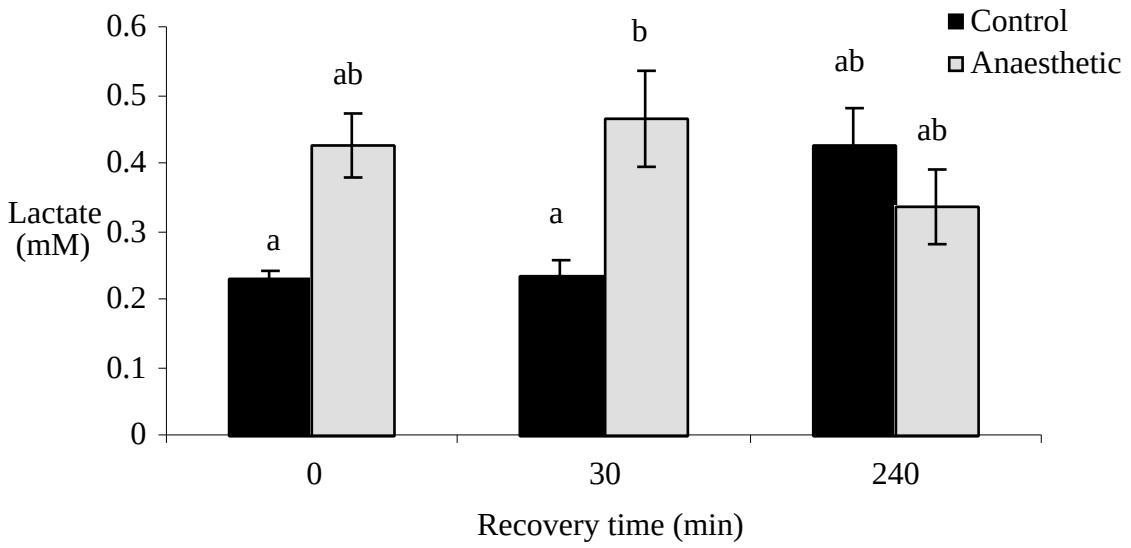

Figure 49. Effect of anaesthetic and recovery time (Basal (0), 30, 240 min) on plasma lactate. Values are mean ± S.E., letters indicate homogenous subsets.

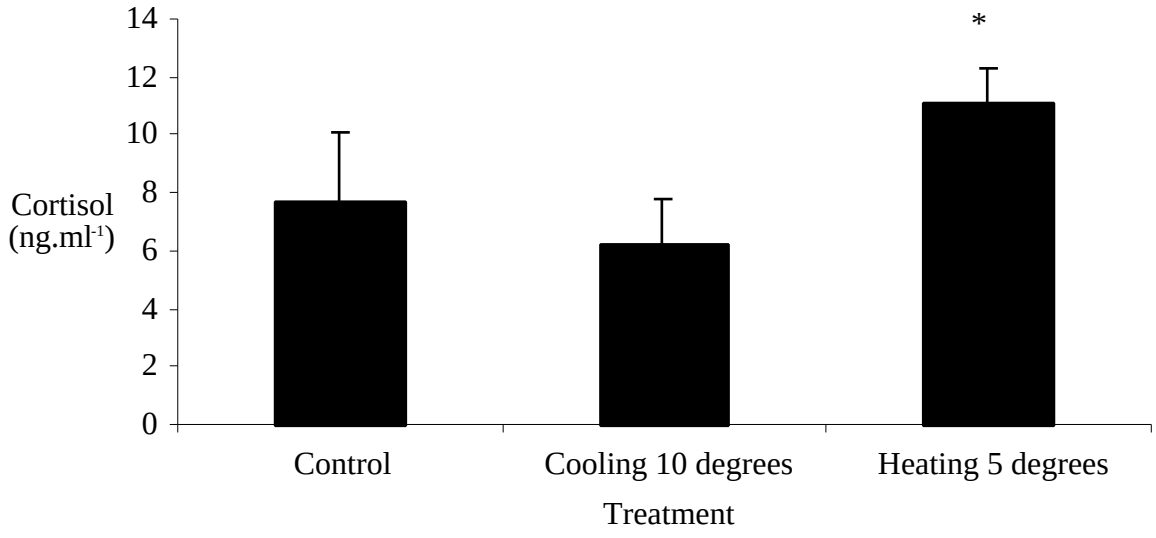

Figure 50. Effect of temperature change on plasma cortisol in Coral trout. Values are mean ± S.E., * indicates a significant difference to the Control.

DISCUSSION

Feeding

Considerable discussion exists amongst fishers about the value of feeding fish on vessels. Providing feed increases costs and may cause water quality problems associated with the presence of faeces, uneaten food and metabolic waste in the tanks. These can be overcome with routine husbandry practices and good tank and flow design and should not be seen as a constraint if there is a benefit to the health and well-being of the animal from feeding.

Fed Coral trout showed a significantly lower cortisol response throughout the experiment than starved Coral trout. Surprisingly, in all animals, the cortisol level appeared to decline during the experimental period and did not show the rise at 30 min in circulating cortisol normally observed. Although the analysis of variance found a significant effect of recovery time, this was not evident from a *post hoc* Tukey's test of each treatment group. The absence of the typical response may have been due to the high levels of cortisol in basal samples which indicated that these animals may have been chronically stressed. In any case, it is clear from this experiment that starved fish show a higher cortisol response and are therefore more likely to suffer the detrimental effects of high cortisol than fed fish.

Cessation of feeding prior to and during transport is a common practice and is used to reduce metabolic rate thereby reducing ammonia excretion and oxygen consumption. However, depriving an animal of nutrients in this way can interact with the nutrient mobilisation actions of catecholamines and impact on the stress response. The glucose response to stress in Coral trout was sensitive to feeding. The response in fed fish reflected the normal stress response with increased plasma glucose at 30 min in fed animals which had declined by 240 min. In contrast, there was no change in blood glucose with chasing in starved fish. The changes in the levels of blood lactate in response to the stressor emulated that of glucose. Similar data was obtained in striped bass (Reubush and Heath, 1997) and may indicate that tissue glycogen stores are greater or more readily mobilised in fed than starved fish.

Tank colour

There was no difference in response of Coral trout between black and white tanks in this experiment. Whilst we cannot dismiss possible effects of particular colours, the use of dark or light tanks in the live-fish industry should have no effect on fish stress and survival.

Anaesthesia

The use of anaesthetics results in reduced injury to both fish and handler, and a reduced stress response (Strange and Schreck, 1978; Davis *et al*, 1982; Laidley and Leatherland, 1988; Morales *et al.*, 1990). Such an effect is dependent upon the type and method of delivery of the anaesthetic. The general anaesthetic 2-phenoxyethanol was shown to induce a stress response in barramundi (Percival, 1999).

The change in all parameters in response to anaesthesia in Coral trout was very small. Significant increases were observed in glucose at 30 min compared with 0 and 240 min and in lactate in anaesthetised fish compared with unanaesthetised fish. Changes in glucose concentration reflected a mild stress response probably associated with transfer of fish to the anaesthetic bath while the increase in lactate concentration probably reflects a slight hypoxia associated with the cessation of gill irrigation and the associated reduction in oxygen transport.

Since the Coral trout are being taken for sale as human food, any anaesthetic use must take into account the requirements of drug registration and withholding times. The drug used here, benzocaine, whilst widely used in fish hatcheries throughout the world is not currently registered for use with fish for sale. The anaesthetic Aquis-S^{â}, which is a derivative of clove oil and is registered for use with food fish, was reported to reduce the cortisol in response to transport stress in rainbow trout (*Onchorynchus mykis*) and brown trout (*Salmo trutta*)(Auperin *et al*., 1998). Davidson *et al*. (2000), however, contradicted this finding that anaesthesia with Aqui-S^{â} resulted in a significant increase in plasma cortisol in rainbow trout. Whilst clearly of benefit, identification and registration of suitable anaesthetics will require further work. In the interim, the use of benzocaine in the handling of fish that may be required for broodstock in the hatchery industry is clearly beneficial.

Handling

Rather surprisingly there was no effect of the length of air exposure on the stress response at 120 min. Data for 30 min were not collected in this experiment so it is not possible to determine the short term effects but it is clear that the animals were able to recover from their handling experience. This indicates that necessary on-board management processes such as measuring fish to demonstrate that they meet legal size limits, and rapid transfer out of water between dory and main vessel and main vessel and processor, if conducted quickly should not unnecessarily compromise the fish. In spite of this, such air exposure should be kept to a minimum as the additive effects of serial stressors on Coral trout have been previously described (Frisch and Anderson, 2000). Since tasks such as measuring and transfer to main vessel occur in a sequence of stressful events including capture, swim bladder deflation and holding in a dory tank potentially with poor water quality, the additional stress provided by air exposure may have a more deleterious effect than when it occurs alone. This conclusion is supported by data from fish sampled on vessels which appear to have recovered to some extent in the dory tanks. When they are transferred to the main vessel, an increase in blood glucose was observed (Figure 16).

Temperature

Holding fish at lower temperatures has been proposed by members of the industry to provide benefits by reducing the stress of the transported fish. In addition, there is often a mismatch between the temperatures of the water from which fish are removed and that into which they are placed during transportation (Wedemeyer, 1997). Coral trout were stressed by an increase of 5 °C, showing a significant increase in plasma cortisol. Stress associated with such thermal shock has been attributed to osmoregulatory dysfunction and often leads to high mortality (Carmichael *et al.,* 1988). Increases in plasma cortisol in response to thermal shock have been reported in salmonids (Strange *et al.,* 1977; Barton and Peter, 1982; Pickering, 1993). Wedemeyer and Goodyear (1984) suggested the stress response resulting from thermal shock is sufficient to stimulate latent pathogen infections. In contrast, rapidly cooling Coral trout by 10 °C did not result in a stress response. Thus, when Coral trout are moved from one tank to another, it is necessary to ensure that the temperature of the receiving water is at or below the original temperature, and definitely not above it. This will be important throughout the harvest process, when fish are captured and placed in the dory tank (perhaps the most likely place for increased water temperature to occur), or when transferred into day tanks, low salinity baths, or main vessel tanks.

PART E. SEASONAL VARIATION IN THE STRESS RESPONSE OF CORAL TROUT

INTRODUCTION

During the course of industry consultation for this study, it was identified that there were differences in the ability of fishers to maintain Coral trout alive during some parts of the year, particularly the spawning season (September to November). In view of this, it was hypothesized that there is some physiological changes associated with reproductive development that occur in Coral trout that affect the response of the animals to stress.

Thus, the aims of Part E of the study were to evaluate the effects of seasonality on the stress response of Coral trout and to link these with the reproductive status of the fish.

MATERIALS AND METHODS

Fish were captured by hook and line at Bramble Reef (Figure 2) during the week immediately before, or immediately after the New Moon phase, of September, October and November, 1998, and May, June, September, October, November and December, 1999. The months sampled were those during which weather conditions permitted sampling. In each month, between 9 and 20 Coral trout were captured. Immediately upon capture, fish were blood sampled and placed in a 70 L bin containing aerated water. Fish were re-sampled 30 min after capture.

Fish were then sacrificed and dissected. Gonads from each fish were fixed for in FACC (formaldehyde 100 mL L^{-1}, acetic acid 50 mL L^{-1}, 0.02 M $CaCl_2$), dehydrated and embedded in paraffin wax. Five µm sections were stained with haematoxylin and eosin and examined using light microscopy to determine the sex of each fish.

Samples were assayed for cortisol and glucose to determine stress levels, and testosterone as an indication of reproductive status.

RESULTS

There was a significant effect of season on cortisol stress response (Table 17). Highest concentrations were found in October 1998, followed by October and December 1999 (Figure 51). Lowest levels of cortisol were found in trout sampled during September and November, 1999. In both 1998 and 1999 there was a significant increase in cortisol from September to October, followed again by a significant decline in November (Figure 51). Cortisol was also significantly lower at 0 min (17.7 $ng.ml^{-1} \pm 1.2$), compared to 30 min (21.6 $ng.ml^{-1} \pm 1.4$) for data pooled over season.

Glucose in Coral trout was not significantly different between seasons (Table 17). However, there was a difference between response times. Glucose was significantly higher at 30 min (8.73 mM ± 0.35), compared to 0 min (0.66 mM ± 0.04). This result is consistent with the glucose response to stress observed in other experiments.

Table 17. Effect of season and recovery time (0, 30 min) on stress response of Coral trout as measured by concentration of circulating hormones.

Cortisol*				
Source of Variation	*df*	*SS*	*H*	*P*
Season	8	7.2×10^5	99	<0.05
Recovery time	1	2.7×10^4	5.14	<0.05
Season *x* Recovery time	8	2.8×10^4	5.36	NS
Error	240	5229		
Glucose*				
Season	8	2.6×10^4	3.84	NS
Recovery time	1	9.6×10^4	15.5	<0.05
Season *x* Recovery time	8	4.1×10^4	15.5	NS
Error	232	5355		
Testosterone*				
Season	8	4.1×10^5	78	<0.05
Recovery time	1	1.1×10^6	205	<0.05
Season *x* Recovery time	8	1.1×10^6	212	<0.05
Error	232	3.7×10^5		

* Analysis by non-parametric ANOVA

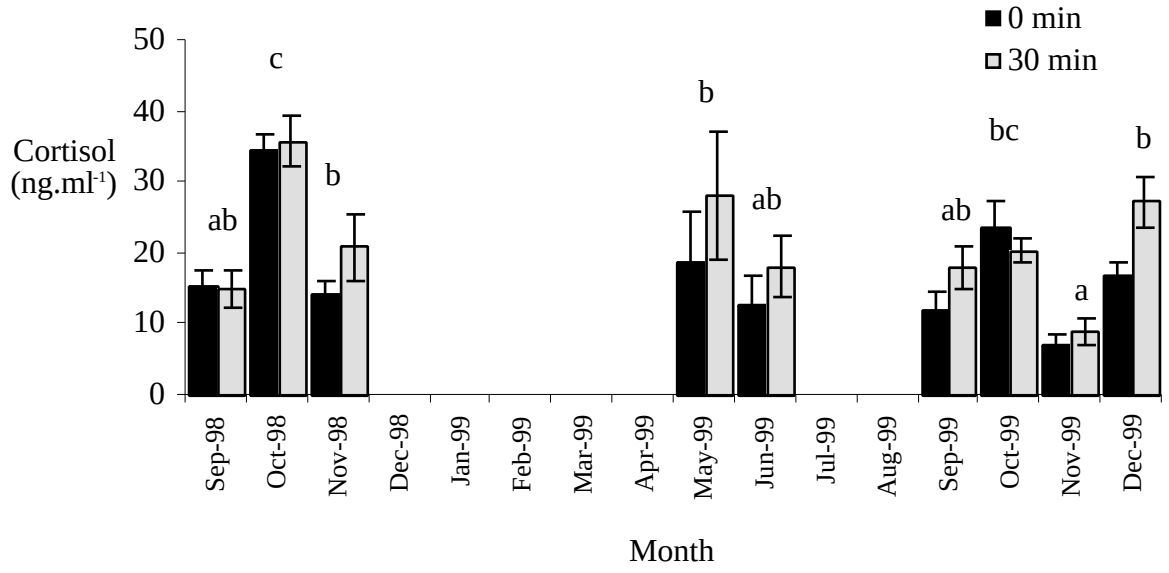

Figure 51. Effect of season and recovery time (Basal (0), 30 min) on plasma cortisol. Values are mean ± S.E., letters indicate homogenous subsets for data pooled over recovery time.

There was a significant effect of season on plasma testosterone (Table 17). Highest concentrations were found in October 1998 and October and November, 1999 (Figure 52). Lowest levels of testosterone were found in trout sampled during May and June, 1998. In 1998 there was an increase in testosterone from September to October, followed by a significant decline in November (Figure 52). However, in 1999, the plasma testosterone reached a maximum in November. Testosterone was significantly lower at 30 min (1.54 ± 0.20 ng.ml^{-1}), compared to 0 min (3.87 ± 0.46 ng.ml^{-1}) for data pooled over season (Table 17).

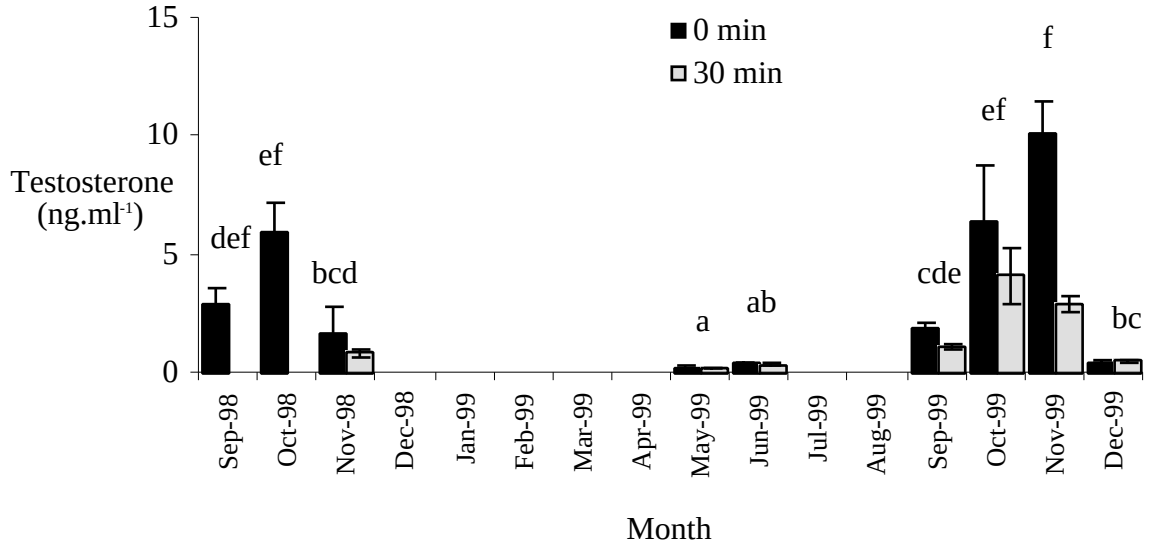

Figure 52. Effect of season and recovery time (Basal (0), 30 min) on plasma testosterone. Values are mean ± S.E., letters indicate homogenous subsets for data pooled over recovery time.

DISCUSSION

Our data indicate that there is a seasonal variation in the cortisol levels in Coral trout. The values measured in this study increased from September to October each year with increasing circulating testosterone and reproductive development. Coral trout are a protogynous fish which change sex with larger fish being males. Consequently, the fish taken in the fishery and the samples here are skewed towards female (77% of the fish were female). This was confounded by differences in stage of reproductive development between fish within time samples and there were, therefore, too few samples to identify sex differences in the stress response. There was also an effect of sampling and holding for 30 min on circulating cortisol and the absence of a significant interaction in the statistical analysis (Table 17) indicates that this effect was consistent throughout the season.

There have been few studies of the effect of reproductive status on the stress response of fish. Seasonal changes in reproductive status are believed to modify the responsiveness of the HPI axis (Kubokawa *et al.*, 1999) with female coho salmon having higher circulating cortisol which did not change with stress compared to males that responded normally. Androgen levels in both sexes were decreased after acute stress (Kubokawa, 1999) indicating that females were not refractory to stress as has been reported in some bird species (Wingfield, *et al.*, 1992). In contrast to the pattern reported for sockeye salmon, a reduction in the stress responsiveness of male rainbow trout and brown trout has been reported during sexual maturation (Pottinger *et al.*, 1995). Coral trout taken during the spawning season do not change their cortisol response, but are more likely to be compromised by high levels of cortisol occurring apparently in association with reproductive development.

As in other species, circulating testosterone is negatively impacted by the stress associated with wild capture. This observation has probably little relevance to enhancing survival in the live trout trade but will be of interest to the aquaculture industry attempting to develop rearing protocols for Coral trout.

PART F. STRESS AND PARASITES

INTRODUCTION

Parasite load is considered to be a potential cause of stress in fish and parasitic infection may have an influence on mortality of fish captured for the live fish trade. The presence of parasites on moribund Coral trout have been previously reported by fishers. Similarly, a major source of mortality is considered to be due to infections on the skin described as “burns” by the fishers.

The objective of this part of the study was therefore to investigate the pathology of parasite infections and “burns” on Coral trout and to determine if there was a relationship between parasite load and the stress response of the fish.

MATERIAL AND METHODS

This work was conducted in two parts. The first was an investigation of the pathology of parasite infections. Tissue samples were obtained from fishing boats during trips described in Part A of this study. Fish were dissected at sea and samples of lesions and body organs were placed in Bouin’s fixative. The samples were dehydrated and embedded in paraffin wax. Six µm sections were stained with haematoxylin and eosin and examined using light microscopy. A total of 4 fish were examined.

The second part of the study involved an evaluation of the stress response relative to parasite load. Ten fish were captured by hook and line at Heron and Wistari Reefs, Heron Island. Fish were sampled immediately and at 2 hours post capture for blood. Cortisol, glucose and lactate were measured in the blood samples as previously described. Fish were killed and dissected and the intensity of infection was determined.

RESULTS

Parasites

Adult and pre-adult male and female *Lepeoptheirus plectropomi* (Copepoda: Caligidae) attach to the upper palate of *P. leopardus*. There is no obvious pathology associated with attachment or feeding. There was a significant correlation between body mass of the fish and *L. plectropomi* burden ($y = -30.9 + 0.087$*fish mass; $r^2 = 0.714$; $p<0.01$).

Adult and larval stages of the copepod *Dissonus manteri* (Copepoda: Dissonidae) attach to the gills of *P. leopardus*. Both larval and adult stages cause marked pathology. The copepodid and chalimus larvae attach in the region of the secondary lamellae and cause deep feeding lesions in the gill filaments, resulting in epithelial hyperplasia, fibrosis and vascular damage. Adult *D. manteri* attach to the efferent edges of the gill filaments using large claw-like maxillipeds. This causes the formation of tumours of attachment up to 1.2 mm in diameter, resulting from marked epithelial hyperplasia and stromal fibroplasia with leucocyte infiltration. There was a significant correlation between body mass of the fish and *D. manteri* burden ($y = -29.4+ 0.10$*fish mass; $r^2 = 0.764$; $p<0.01$).

The capsalid monogenean *Trochopus plectropomi* is found on the gills. It attaches using the relatively small haptor to the afferent edge of the gill filaments where there are no secondary lamellae and feeds on both host mucus and epithelial cells. Little or no pathology is associated with this parasite, though a footprint impression is left in the host epidermis at the site of

attachment. There was no significant correlation between body mass of the fish and load of monogeneans.

Adults and juveniles of the *Hatschekia plectropomi* (Copepoda: Hatschekiidae) are also found on the gills. Adults attach near the base of the gill filaments in the region of the secondary lamellae. This results in localised epithelial hyperplasia and damage to the blood vessels of the secondary lamellae. There was a significant correlation between fish body mass and *H. plectropomi* burden ($y = -52.9 + 0.27*$fish mass; $r^2 = 0.620$; $p<0.05$).

Diploplectanum plectropomi (Monogenea: Diplectinidae) is a small (0.3 – 0.4 mm long) monogenean which occurs on the gills. It causes little or no pathology, attaching to one or two secondary lamellae. Adult and pre-adult *Dentrigryps litus* (Copepoda: Caligidae) are found moving freely over the body surface in small numbers. No obvious pathology could be associated with these copepods. These latter two parasites were not counted during this study.

Pathologies

Metacercariae of unidentified trematode parasites and adult nematodes were present in high incidence in gut tissues and in other body organs. (Figure 53 and 54). Copepod parasites were also present in high incidence in gills (Figure 55). Little or no host defence reactions were observed in these sections apart from in the gill tissues and the hepatopancreas.

Biopsies of the superficial skin lesions described as “burns” showed areas of inflammation in the connective tissue sheaths surrounding the underlying muscle bundles (Figure 56) and perivascular cuffing was observed. A muscle myopathy was present in the muscle bundles underlying the skin. Muscle fibres were disorganised and necrotic (Figure 57) and some inflammatory cells were present.

Areas of perivascular inflammation were observed in liver tissue around hepatic blood vessels (Figure 58). Encapsulated parasitic structures were observed in the hepatopancreas (Figure 59), spleen and kidney (Figure 60) and inflammatory structures were observed around these structures.

Effects of parasites on the stress response

There was no significant effect($p>0.05$) of parasite load on the cortisol, glucose or lactate at the time of capture or at 2 hours post capture.

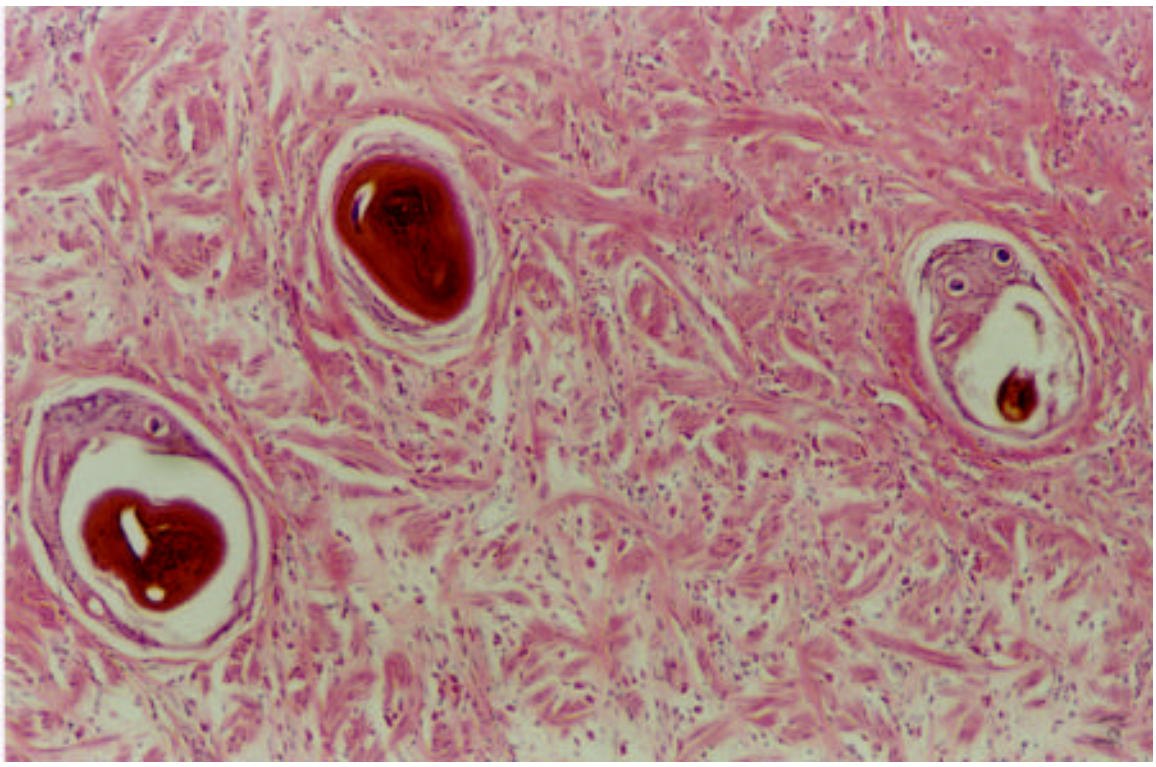

Figure 53. Parasitic infection of gut tissue (x 200).

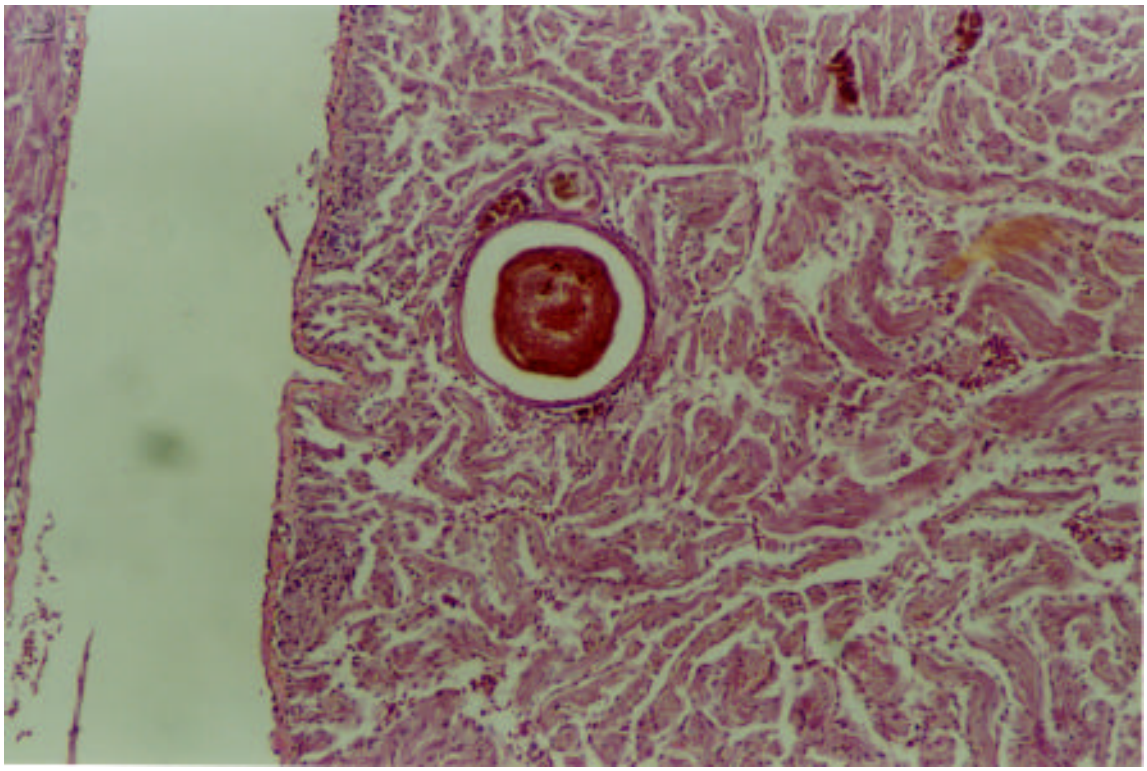

Figure 54. Parasitic infection of heart tissue (x 100).

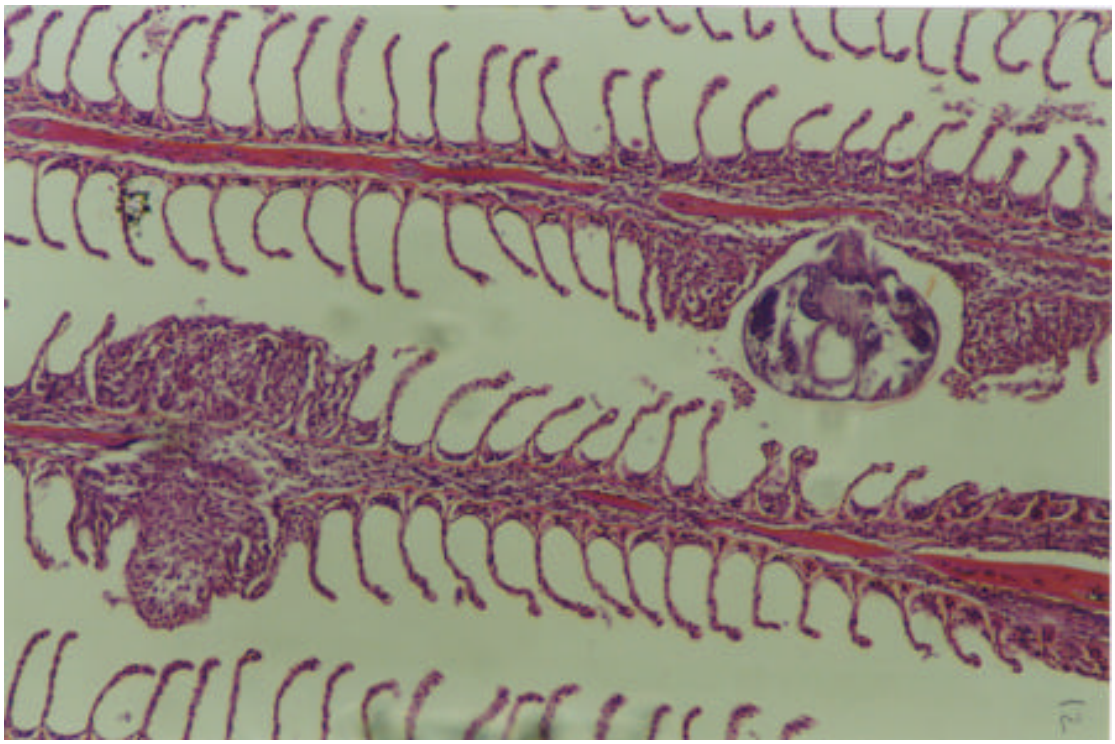

Figure 55. Copepod parasites in gill tissue (x 100). Note inflammation and hyperplasia at infection site.

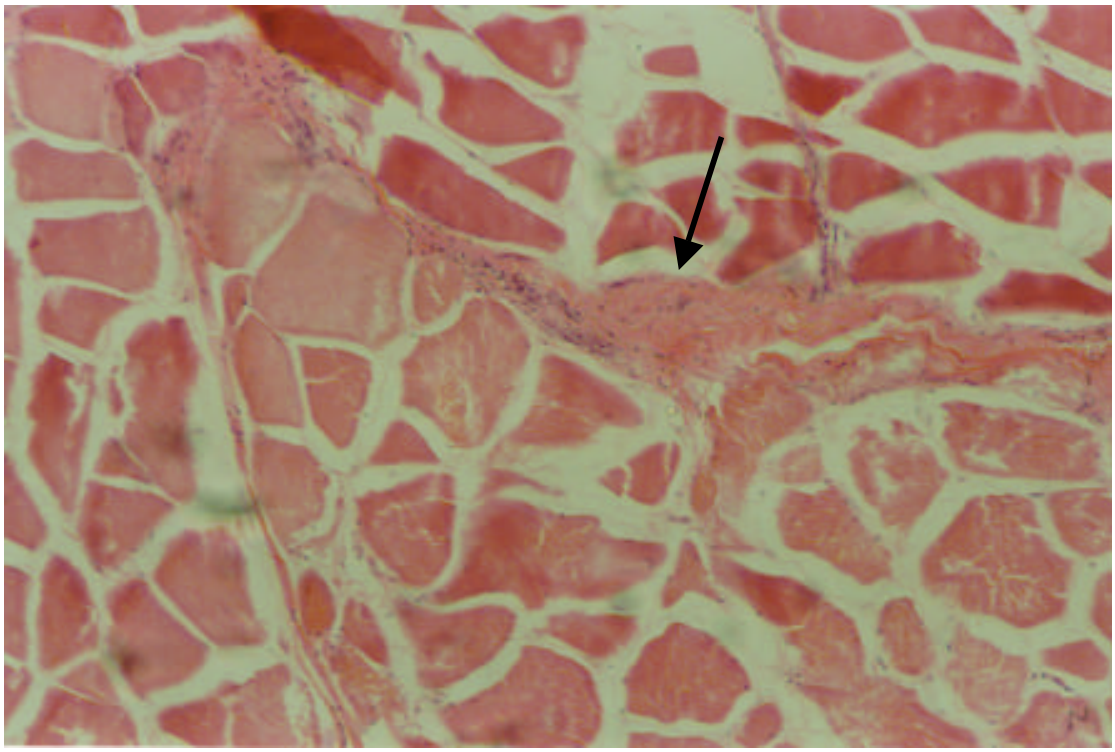

Figure 56. Inflammation in connecting tissue sheaths (arrow) surrounding muscle bundles underlying skin lesions (x 200).

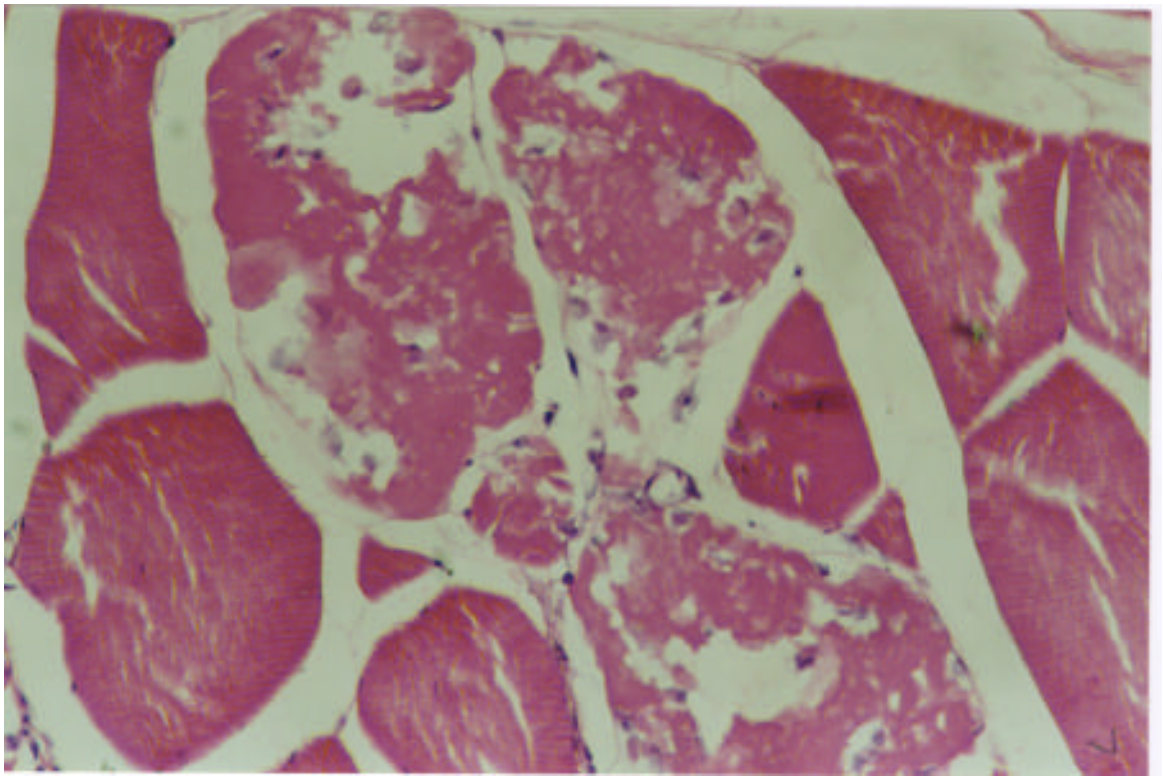

Figure 57. Muscle myopathy (x 100).

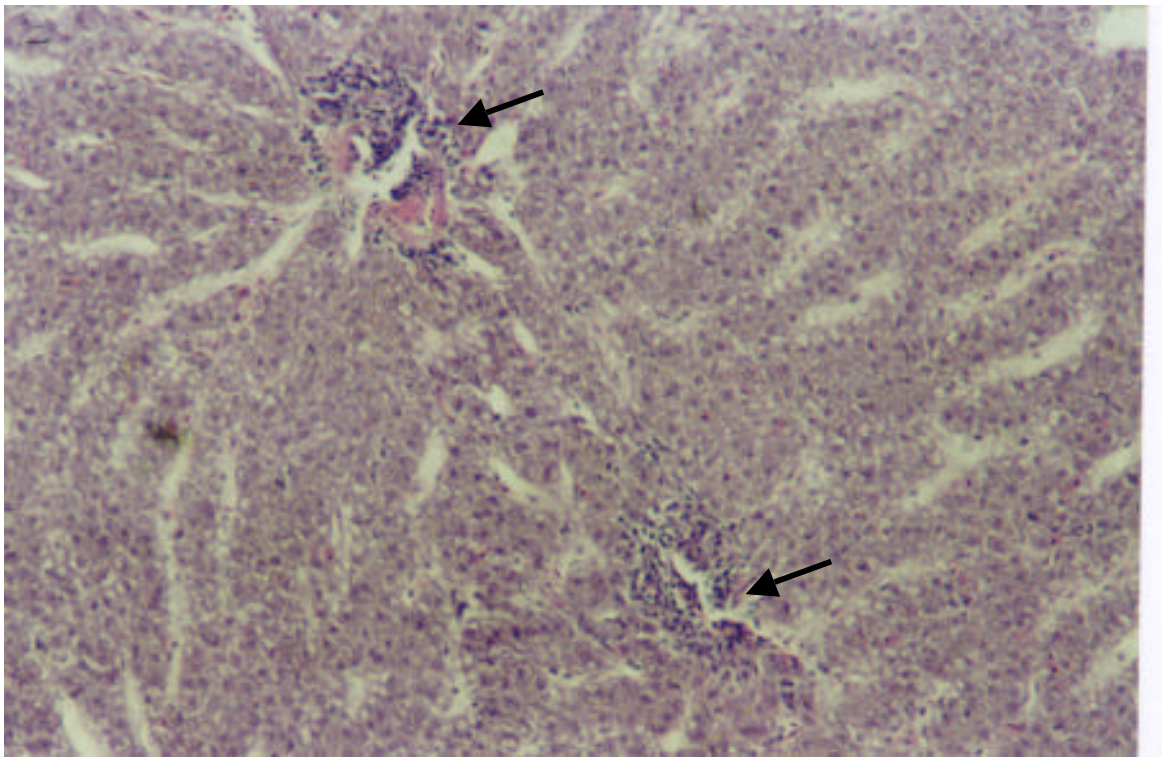

Figure 58. Perivascular inflammation (arrow) in the liver (x 200).

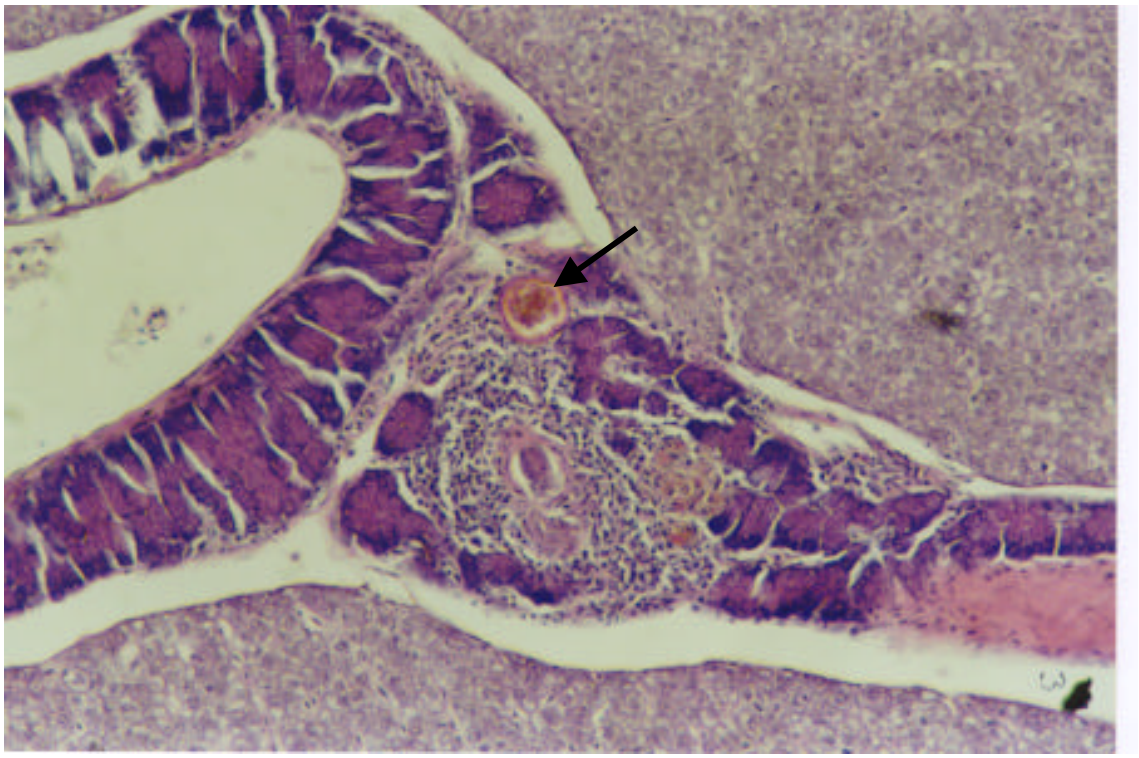

Figure 59. Encapsulated parasitic structure (arrow) in the hepatopancreas. Note the inflammatory reaction (x 200).

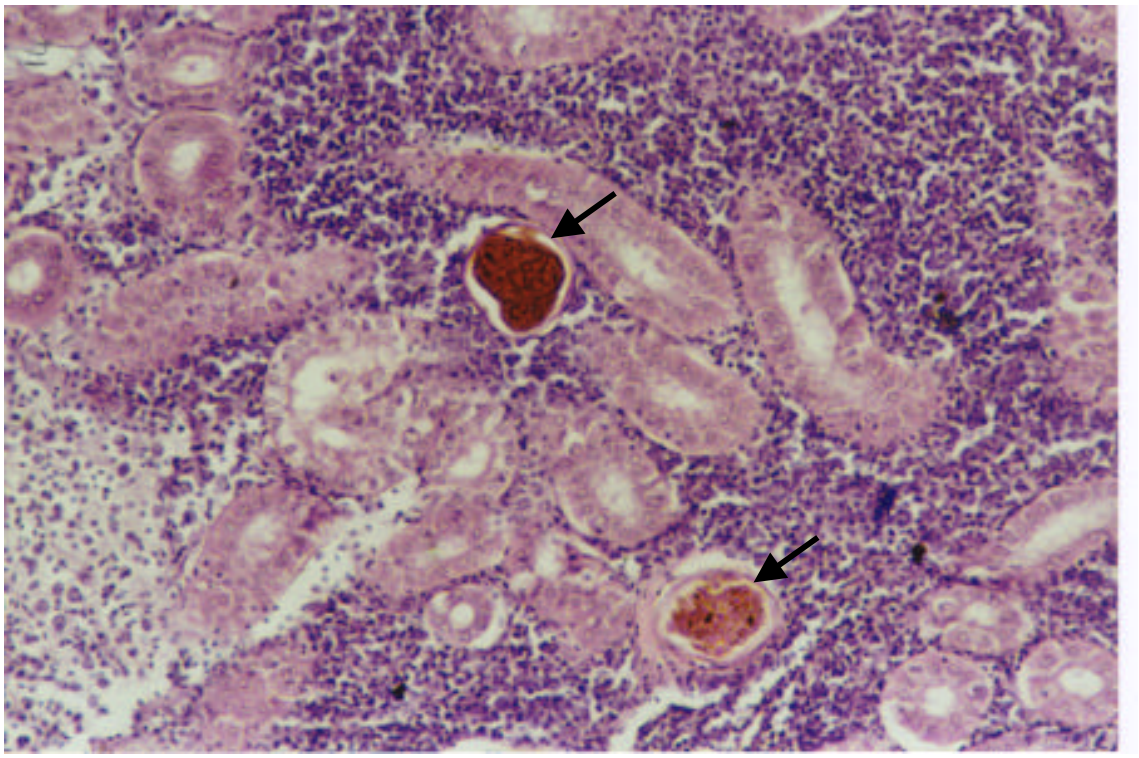

Figure 60. Encapsulated parasitic structures (arrow) in kidney (x 200).

DISCUSSION

The presence of a range of different parasites on the animal's surface, particularly in the gills, and in different tissues taken from moribund Coral trout were found in this study. High parasite loads have also been proposed to be related to increased stress responses of fish. Data from the

present study, however, refute that contention, with no correlation between the stress response immediately after capture or at 120 min after capture.

The presence of parasites may also be associated with increase likelihood of infection, particularly in animals that are immunologically compromised such as when stressed. In this regard, treatments that can reduce the parasite load of a fish after capture may result in enhanced probability of survival of that animal. Routine treatments of low salinity water are recommended to reduce the load of both copepod and parasitic protozoans in aquaculture (Rowland and Ingram, 1991) and would seem to be a useful adjunct to handling practices of live Coral trout. Baths at 10 parts per thousand salt (30% seawater) for up to 90 min (Frisch and Anderson, unpublished) has previously been shown to not result in any additional stress response.

BENEFITS

Commercial Fishers will benefit directly through improved handling methods for live Coral trout. This will reduce the mortality of fish in the industry and provide increased return on individual fish taken. Prices to the fishers of live trout in the period from 30/1/2000 to 30/1/2001 averaged $29.20/kg while prices for fish used for fillet averaged $7.15/kg (assuming a 50% fillet recovery) (G. Muldoon, unpublished data). This represents an increase in value of 409% by keeping the fish alive. Since the industry is estimated to produce over 1000 T of live fish in 2001, this represents a difference in value of approximately $22 million by selling fish live rather than as fillet. Improved methods for keeping fish alive for longer periods also removes the restriction placed on skippers to transfer fish to processors early, providing opportunities for increased management efficiencies in their operation brought about by greater flexibility in the periods spent at sea. Principals of live fish operations will benefit by access to a training video which can be used to train new employees in fish handling and dory tank management that will provide more rapid induction of staff into their businesses. The commercial fishing industry will benefit through the improvement of the Quality Assurance Procedures previously published by the Queensland Seafood Industry Association by incorporating practices shown to be of benefit to the handling of live fish.

Live fish processors will benefit from this project through improved quality and supply of live product and increased information about transport methods.

This project will increase the ease with which commercial fishers can move from dead to live fishing through overcoming technical limitations. In so doing, benefits will accrue to the environment, fisheries managers and other competing users of the resource such as recreational fishers as a result of the reduction in fishing effort documented by Mapstone *et al*. (2001).

Improved methods developed by this project for handling live Coral trout will further benefit recreational fishers will further benefit from this project by information regarding handling procedures utilised when returning undersize or un required fish to the water.

These benefits are largely as described in the original application.

DISSEMINATION OF THIS INFORMATION

A copy of the training video produced in this project was sent by mail to 240 commercial line fishing license holders in 2002 by the Queensland Fisheries Service.

FURTHER DEVELOPMENT

Until recently, live fish have largely been traded in Hong Kong and immediately adjacent southern China. While anecdotal reports of fish being sold into mainland China have been made, this trade has reportedly been occurring through smuggling of fish by Hong Kong based traders. Recently however, China has begun to open trade to other countries. This, with a reported increase in disposable incomes in parts of China has provided increased market possibilities for the live fish trade from Australia. Further development of these markets is constrained by transport arrangements which mean that times to market are greater than to Hong Kong and may be up to 48 hours. Current transport arrangements are satisfactory for the current transport time of 24 hours, but fail when extended to 48 hours. Thus, further development opportunities exist for developing transport technologies that can limit the stress of the fish and maintain them alive for extended periods.

This work is applicable to other species, but may also require tuning of practices to identify species specific responses.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Summary

This study found that major areas of stress for Coral trout taken in the live trout fishing industry are largely associated with practices in the dory. Many of these stressors are associated with poor water quality resulting from poor dory tank design or operation. These stressors result in significantly increased levels of cortisol and blood glucose which provide clear physiological indicators of Coral trout stress.

Experiments to determine levels of the key water quality parameters that appear to cause stress found 0.5 $mg.L^{-1}$ ammonia and 70% dissolved oxygen saturation to be minimal criteria for water quality. It should be noted that these two factors may also act in concert and so fishers should maintain water quality with lower ammonia and higher oxygen concentrations than these levels.

In order to provide a mechanism to reduce the likelihood of poor water quality in dory tanks, a design for a cylindrical tank with the inlet at the top and the outlet at the bottom providing plug or laminar flow through the tank was developed. This tank, of 185 L and sized to fit into a dory, was able to maintain high flow velocities with limited mixing at an exchange rate of 6 $exchanges.h^{-1}$, a volume easily achieved with off-the-shelf 12V bilge pumps used in the industry.

The maximum stocking density for this type of tank was determined to be 20 $fish.tank^{-1}$ or approximately 100 $kg.m^{-3}$ which is equivalent to the maximum average catch rate per session achieved in the earlier study of dories in the fishery.

A number of other husbandry parameters were investigated. Feeding Coral trout were found to have lower circulating cortisol than animals that were starved. Coral trout found an increase in temperature of 5°C to result in significant stress but moving to water up to 10°C cooler did not. Handling induced a stress response, but this did not differ between fish held out of water for up to 6 min. By 6 min however, the animals had lost their righting response indicating that they were severely hypoxic (had very low blood oxygen) and holding them out of the water for this period should be avoided. Tank colour (light vs dark) had no effect on the stress response of the fish.

Anaesthetic served to lessen the stress response of handling but the chemical used in this study is not registered for food use so this fact is of little use for fish destined for human consumption unless food grade anaesthetics can be demonstrated to be of similar value. This information is of relevance to the collection of broodstock for aquaculture, however.

Significant seasonal variation was observed in the circulating levels of cortisol in Coral trout immediately post capture. Levels were highest in the spawning season between October and November indicating that at this time of year, Coral trout are more likely to be physiologically compromised. This may explain why fishers report greater problems maintaining fish alive during the spawning season and suggest that they must pay particular attention to husbandry practices at this time.

Numerous parasites were found on Coral trout. While levels of parasitism are not related to overall levels of stress, their presence during levels of stress when immune function is reduced by stress may result in opportunistic infections. These may be controlled by low salinity (10 ppt) bathing of fish immediately upon capture to reduce the parasite load.

Other factors in the normal harvest cycle of Coral trout were also evaluated during this study. These were the effect of using a dehooker, swim bladder deflation and the effect of depth of capture. While all of these were found to be stressful, they were also considered to be unavoidable. The conclusion reached is that the animals should handled as gently and as rapidly as possible. Deflation through the dorsal wall of the rectum was not related to infection and was most easily achieved. Rimmer and Franklin, 1997) described a method of deflation involving passing a needle through the body wall of the fish. While this is effective, it requires the fish to be placed and held firmly on a flat surface, a process that itself inflicts damage to the fish. If the animal is cradled upside down in one arm and the head is restrained by holding the line taught at the same time, the fish tends towards quiescence. In this position, deflation can be rapidly achieved with minimal stress to the fish. An alternative is to immediately release the animal into the dory tank. When it loses it's balance and floats to the surface, the fish is also usually quiescent and can be deflated at that time. Failure to immediately deflate the swim bladder will result in drying of the ventral surface and subsequent infection, usually with mortality. Previous criticism of this method by Squire and Johannes (1999) suggesting that it would lead to enhanced rates of infection have proved not to be the case in this study.

Use of a dehooker is also recommended. This allows rapid release of the fish into the dory tank and does not add to the stress response. Fish captured at depth (20 m) are more likely to suffer swim bladder extrusion from the mouth or other swim bladder problems. Fish taken from this depth either appear healthy or moribund when placed into a dory tank. Moribund fish should be killed immediately for ethical and food quality reasons and placed on ice since they will not recover. Fish that appear healthy upon release into the dory tank are likely to remain so if good water quality in the dory tank is maintained.

Recommendations

- Water quality in all tanks used to hold fish throughout the harvest cycle should be below 0.5 $mg.L^{-1}$ ammonia and above 70% DO saturation.
- This can be achieved by ensuring good water exchange in the tank (at least 6 $exchanges.h^{-1}$ even in a well designed tank) and good design such that wastes are rapidly removed from the water.
- The maximum stocking density of Coral trout in a dory tank should be 20 $fish.tank^{-1}$ or approximately 100 $kg.m^2$. This also serves as a useful indicator of maximum stocking densities in other tanks.
- Coral trout held in main tanks should be fed.
- When moved into a different tank, Coral trout should be placed in water at or below the temperature of the water from which they are being moved.
- When being moved, fish should be kept out of water for as short a time as possible and certainly for not more than 5 min.
- Tank colour (light vs dark) is of no consequence. Individual colours (eg red, blue etc) may be however.
- If health regulations and product acceptability allow it, fish should be anaesthetised when handled.

- Particular attention must be paid to ensuring good water quality when fishing during the spawning season.
- Parasites may be controlled by low salinity (10 ppt) bathing of fish immediately upon capture.
- Use of a dehooker is recommended to reduce the overall stress period the animal is physically handled.
- Swim bladder deflation should be conducted quickly and with minimal physical force to restrain the fish. Deflation through the dorsal wall of the rectum with an 16 gauge needle provides an easy and efficient way to achieve this.
- Fish captured at depth (20 m) should be assessed to determine if they are moribund or healthy. Moribund fish should be removed from the live harvest process and killed immediately.

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APPENDIX 1: INTELLECTUAL PROPERTY

This project has led to the development of a training video entitled “Live trout, not dead”. Distribution of a free copy of this video to licensed Coral trout fishermen has, and is, providing a mechanism for disseminating the findings of this study to the industry. However, it may prove to be of interest to other parties both in Australia and internationally and provides a marketable product.

No other patentable or marketable products have arisen from this research. All results will be published in scientific and non-technical literature. Intellectual property arising from the measurement, analysis and interpretation of raw data vest jointly with the Fisheries Research and Development Corporation, James Cook University, CRC for Sustainable Development of the Great Barrier Reef, The University of Queensland, Curtin University, and the authors of this report.

APPENDIX 2: STAFF

Principle Investigator:	Trevor Anderson
Co-Investigators:	Bruce Mapstone Peter Appleford Campbell Davies Louis Evans Michael Bennett Susan Bennett
Project Staff:	Kevin Kane Anthony Hart
PhD students:	Tracey Turner

APPENDIX 3: ORIGINAL SCRIPT FOR THE VIDEO "LIVE TROUT, NOT DEAD".

Note: Some variations from the script may have occurred during production.

SUMMARY OF THE THEME OF VIDEO

Commercial operators currently catch Coral trout for the Asian live fish market. The value of this product is critically dependent on the fishing and handling practices which fishers employ to maximise survival. Fishers catch Coral trout from a range of depths, and the animals are hauled onto the vessel and placed in a storage tank. At present the fishing, handling and stress management protocols employed during this process are many and varied. The research however, has identified and quantified best and worst practices, and our objective is to train the commercial operators in a standardised 'stress-minimization' methodology so as to encourage them to change their fishing practices and develop 'optimum' habits. In essence, we are seeking a combination of footage of both "best" and "worst" practices at sea, accompanied by a narrative explaining the reasons and methodology behind the prescribed protocols, backed up by graphics showing the scientific research upon which the recommendations are based.

Shot	Script	Shotlist	Source
INTRODUCTION			
1	Music and title: "Live trout, not dead"	Various aerials of the fishery, i.e. reefs, main vessels and dories in action.	Sea
2		Footage of happily swimming underwater trout in a tank and underwater	Land/ Sea
3	Written Text: 'this is an information video for Coral trout fishers on handling and holding Coral trout for the live fish market'	dorymen catching and handling bright healthy specimens with care and enthusiasm.	Sea
4		Shots of happy processors receiving good quality live Coral trout, perhaps discarding those of dubious presentation	Land
ENTER PRESENTER: DETAILS NATURE AND CONTEXT OF PROBLEM			
5	Hello, and welcome to the program Let's talk about live Coral trout.	PTC (presenter to camera) Presenter with graphic subtitle	Land
6	"In 1999, the value of live the fish trade in Coral trout is conservatively estimated at A$***10*** million	ptc Main vessels and dories	Sea
7	However the demand for live Coral trout is not being met and it is clear that the market could accept additional product."	markets – restaurants in South East Asia,	Land
8	Brett asks "So, where's the fish?"	Empty tank	Land
9		Restaurateur shakes head	Land

10	"The major limitation preventing the live Coral trout fishery from expanding is the occurrence of injury and disease in the fish between capture and processing.	fish with pathology symptoms – fin rot, white spot disease,	Sea
11	Fish showing any signs of stress are immediately sacrificed to obtain fillet."	Diseased fish pulled out and subject to filleting process	Sea
12	The leakage of this product from the high value live market to the lower value fillet market results in significant loss of value in the fishery, and a resulting loss in income to all sectors of the industry."	Doryman's paycheck - live fish @ $25 per kg whole fish. (make sure year is written on paycheck)	Land
13		Doryman's paycheck - dead fish @ $13 per kg fillet. (make sure year is written on paycheck)	Land
14	As a result of these concerns, the live reef fish industry made a request for research to try and solve these problems of disease and mortality in the live fish trade.	Presenter	Land
15	In 1997, a grant from the FRDC, the Fisheries Research and Development Corporation, was awarded to Dr Trevor Anderson and colleagues of James Cook University.	FRDC, CRC, and JCU corporate logos	Land
16		FRDC contract. Shot of Trev as researcher	Land
GENERAL NATURE AND CONTEXT OF RESEARCH			
17	"In conjunction with FRDC and Industry, these researchers investigated sources of stress in Coral trout during the catching, handling, and holding process.	Fancy looking research laboratories	Land
18	"They tell me that all of this has been achieved by looking at the blood of live Coral trout. Is that right, Trev?	Presenter speaking	Land/ Sea
19	Absolutely, Brett. We went to sea with a number of fishing vessels and took blood samples from Coral trout at various stages of the capture and holding cycle.	Trevor talking	
20	Within the blood there are biochemical components, which indicate the level of stress in the animal.	researcher extracting blood from live Coral trout	Sea
21		Laboratory technician (white coat) transferring blood to a vial	Land
22	These need to be isolated from the blood using modern technologies.	Inserting vial into a centrifuge and turning on.	Land

23	"Some of the indications of stress in Coral trout are cortisol, glucose and lactate. (y axis). These change over time (X axis), rising quickly to 30 minutes (ascending curve), before recovering if animals are held in good conditions (descending curve).	Build Y axis. Build X axis Build ascending curve Build descending curve	Land
24	"Stress has been measured under a range of conditions. These include level of oxygen and ammonia in the water,…"	fancy looking probe immersed in a dory tank to measure oxygen and ammonia	Land/ Sea
26	"….the practice of deflating the swimbladder, …"	needle inserted into a swim bladder (cradle position)	Sea
25	"..the effect of de-hookers,….'	doryman de-hooking a trout	Sea
27	"..and stress at different stocking densities."	tanks with different stocking densities of Coral trout.	Sea
28	"The outcomes of this research were to identify problem areas, and ways in which practices can be improved to minimize stress. Back to you, Brett."	Trevor talking	Land
29	"Thanks Trev. So there you have it. Topics to be covered in this training video are: handling, dehooking, deflation of swim bladder, fish caught from deep water, water quality, dory tank design, stocking densities, feeding regime, and seasonal variation."	Brett talking appropriate vision graphics over	Land/ Sea
30		Text: List of topics, read by Brett.	Land
GRAPHIC: HOW IS STRESS PRODUCED IN FISH?			
31	"Before summarising the findings of the research, lets examine the question, how is stress produced in fish?	Brett (on the boat deck?)	Sea
32	Is it the same as stress in humans?	Boat skipper looking stressed, i.e. cursing, yelling, thrashing about.	Sea
33	Trevor:.	Brett VO	
34	Put simply, stress in Coral trout is produced from biochemical reactions which run out of control when fish are removed from their normal environment,…	Trevor	Land/ Sea
35		Trout pulled up over the side of dory.	Sea
36	…..and placed in a dory tank."	Trout placed in dory tank	Sea
37	These biochemical reactions produce	Shots of trout thrashing	Sea

	significant amounts of circulating hormones and sugars in the blood.	around in the water and looking decidedly pale.	
38		Schematic diagram. hormones, sugars with large upward arrow near them.	Land
39	Increased concentrations of hormones and sugars cause trout to suck large amounts of oxygen from the water.	Trout with a significant gill action (i.e. opercular plates moving or expanded)	Sea
40	Trout also produce ammonia at a fast rate by vomiting and excreting.	Trout vomiting up a pilchard (could be similar to above shot).	Sea
41	This degrades the water quality, as ammonia is very toxic to marine animals, causing further stress to the trout and the vicious cycle continues.	Trout lying in shallow water of a dory tank surrounded by all sorts of muck and crap.	Sea
42	One of the interesting things we found was that, although all trout get stressed,…	Trevor	Land/ Sea
43	….some individuals appear to handle it a lot better than others, much like humans".	Skipper and doryman talking together. Skipper – on main vessel, stressed, doryman – in dory, relaxed "No worries mate".	Sea
44	Back to you on the boat, Brett.	Trevor	Land/ Sea
45	No worries, Trev. So the take home message is, try not to cause stress.	trout and "stressed" skipper- flushed angry face etc.	
GRAPHIC: WHO CAUSES CORAL TROUT TO BE STRESSED??			
46	"Let's have a look at a doryman at work and see if we can identify any practices which may be thought stressful to the Coral trout."	Dory leaves main vessel and finds a hang, all excited about getting out there and fishing (he's a fast driver!). (All these scenes through to 64 with graphic indicating wrong practice if appropriate)	Sea
47		Screams to a stop (almost falls over), makes anchor	Sea
48		Fisherman baiting hook	Sea
49		Throwing out hook	Sea
50		Hauls in fish	Sea
51		De-hooks incorrectly, i.e. with fingers, squeezes fish hard	Sea
52		Drops fish accidentally on floor.	Sea
53		Picks fish up	Sea
54		Throws fish in dory tank,	Sea

		which has no water or bilge pump operating (include sound effects).	
55		Quickly throws in some water from a bucket, splashing and knocking the trout about.	Sea
56		Turns bilge pump on, quickly fills up tank.	Sea
57		The fish is floating upside down with a distended swim bladder, and looking sick. (will need to catch fish from deeper areas to get this shot)	Sea
58		Doryman looks confused	Sea
59		Rushes to deflate swimbladder (i.e. lifts fish up incorrectly to pierce swimbladder),.	Sea
60	Doryman “Curses!”	Pricks himself accidentally (curses!),	Sea
62		On final inspection, the fish is extremely moribund and possibly dead	Sea
63	Fish sacrificed to ice bucket, first bled then thrown in bucket.	Doryman holds up knife, a guilty look on his face, turns to look at the skipper, raising his shoulders as if “wincing”.	Sea
64	Skipper “Curses!”	Shot pans to the skipper, looking through binoculars, gets stressed (shaking his head, arms folded), uttering a silent curse.	Sea
65	Like we said earlier, “Live trout, not dead.” This could be repeated in bold letters to remind the audience of the theme of the video.		Land
GRAPHIC: MINIMIZING STRESS IN CORAL TROUT			
66	“Right, that is obviously not the correct procedure. Let’s now have a look at some of the various ways in which stress can be minimized when handling Coral trout.”	Presenter with appropriate background (somewhere on the boat)	Sea
67	“One thing we can’t avoid is stressing the trout by catching them.	Presenter watching the fishing (perhaps fishing?)	Sea
68	A hook in anyone’s mouth is bound to cause discomfort and heartache. However, we can avoid stressing	Coral trout being bought to the surface.	Sea

	them any further once in the boat. Here is how you might do it."		
GRAPHIC: USE A DE-HOOKER AS MUCH AS POSSIBLE			
69	The very first thing is to make sure there is good clean water in the dory tank before releasing the trout into it.	Dory tank full of water	Sea
70	"Release trout as quickly as possible. Use a de-hooker at all times. Minimize touching and avoid grabbing the fish in any way as this breaks the protective mucous coat on the trout.	Doryman dehooking the trout quickly and efficiently into the dory tank.	Sea
71	Now the pearling industry in Western Australia suffered huge disease problems in the late 1970's because they treated the pearl oysters like bricks.	Presenter holding a brick, throwing it in the tank.	Sea
72	A Coral trout is not a brick. Try not to handle it like one. If you must handle it, be gentle.	Presenter holding a Coral trout, gently putting it in tank.	Sea
GRAPHIC: DEFLATE THE SWIM BLADDER			
73	One of the common side effects of quick hauling, is swim bladder expansion.	Shot of floating trout with distended swim bladder	Sea
74	If the fish is caught with a distended swim-bladder, deflation must be carried out as quickly and efficiently as possible.	Presenter	Sea
75	This is best done whilst the fish is still on the hook, and can be held in an effective and supportive manner	Fish on hook, being cradled in the right arm of the dorymen, thumb and forefinger holding the line	
76	With support, deflation can be achieved by the insertion of a hypodermic needle at a point near the anus	Close-up of needle inserted within swim bladder near anus (fish being cradled).	Sea
77	Once the needle has been inserted, the air should escape quite quickly.	Close-up of the deflating process as air escapes quickly.	Sea
78	Then it is a simple task to dehook .	De-hooking fish into tank.	Sea
79	An appropriate needle to use is the 19 gauge hypodermic needle, like this one. These can be purchased at any chemist	Close-up of type of needle	Land
GRAPHIC: FLOATING FISH FROM DEEP WATER			
80	Coral trout are often caught from water greater than 10m depth. The research found that if the fish was inactive or dead looking after being hauled from depths greater than 10m, it was likely to remain that way.	Fish caught from deep water being hauled onto deck	Sea
81	So, rather than try to revive it by	Trout looking very sick –	Sea

	deflating the swim bladder and placing in the dory tank, it is better to put it on ice immediately.	decision to place on ice.	
82		Trout being placed on ice.	Sea
83	Also, if you don't place the trout in the dory tank, it can't pollute the water as it dies.	Presenter	
GRAPHIC: DORY TANK WATER QUALITY			
84	The next major source of stress in Coral trout is the dory tank itself. The research looked at characteristics of water quality and how these could be improved to the benefit of the Coral trout.	Shot of a typical dory tank (square, designed for holding fish).	Land/ Sea
85	There are two aspects to good water quality - Dissolved Oxygen level and ammonia level. Dissolved Oxygen has to be maintained high, and ammonia has to be kept low,	Shot of fisherman anchoring, turning bilge pump on, filling tank with water. Text overlay with Dissolved Oxygen high, Ammonia low	Sea
86	These two things can be accomplished by a constant flow-through of water.	Dory tank with a constant flow through indicated by water gushing through outlet pipe	Sea
87	The shape of the tank plays an important part in water quality. One of the things that happens with square dory tanks is the occurrence of dead spots as the water flows through. This may hinder overall water quality.	Schematic figure of square water flow dynamics, indicating how dead spots are created.	Land
88	The dory tank bilge pump should be turned on before you start fishing and left on at all times when fish are in the tank.	Fisherman adjusting flow speed to maintain at above 6 tank volumes an hour.	Sea
90	The research discovered that keeping the dory tank water exchange rates above 6 tank volumes an hour will achieve desired water quality levels.	Text: Water flow greater than 6 tank volumes per hour	Land/ Sea
89	Ammonia will be kept down by flushing or removing pilchards and other waste out of the tank as often as possible.	Putting hand in tank and fishing out pilchards and crap.	Sea
91	As well as good flow through, water quality can also be improved by additional aeration.	Aeration put in dory tank and bubbling away.	Sea
92	Airstones like these are useful and can be purchased from any aquarium shop.	Still shot of make and type of aerator	Land
GRAPHIC: DORY TANK REQUIREMENTS			
93	Another way to achieve good water quality is to design a tank specifically for this purpose. Most dory tanks have	Shot of the entire circular tank siting inside a dory with all the inlet/outlet	Sea

	been designed to hold lots of dead fish, not for good water quality.	pipes properly set up	
94	Borrowing on ideas from aquaculture, the researchers have identified some requirements for dory tanks in order to achieve good water quality.	Side view of round dory tank	Land/ Sea
95	The water inlet pipe is situated at the top of the tank, which has a circular design in order to generate a steady water flow.	Close-up of water inlet at top.	Land/ Sea
96	A sloping conical bottom to allow accumulation of waste materials at a central point so they can be easily flushed out.	Close-up of the bottom of the conical tank, presenter pointing to the design feature	Land/ Sea
97	Water level is maintained at a constant level via an outlet pipe, which rises up to just below the inlet pipe before dog-legging to flow over the side.	Close-up of the outlet pipe design	Sea
98	The narrow neck also reduces water movement or free-surface effect caused by the rocking of the dory. The end result is lots of tiny waves across the top, and little movement in the rest of the tank.	Shot of little waves at top of dory	Land
99	Compare this with what happens in a square tank when the dory is rocking. Large waves are generated which move the trout unnecessarily.	Shot of big waves across top of dory tank	Land
100	Use of round tanks also results in efficient water flow and no dead spots.	Schematic figure of round tank water flow dynamics.	Land
101	Cleaning the tank of pilchards and other wastes is accomplished by periodic cracking of the valve on the outlet pipe.	Opening of valve on outlet pipe	Sea
102	This quickly flushes the waste onto the dory deck where it can be later washed away.	Water flowing onto the deck, filled with crap.	Sea
103	As we can see, there are a number of advantages of this tank, which include: no dead spots, no clogged outlets, no overflowing, no flow adjustments, constant water level, all waste easily removed.	Shots of each advantage, text overlay	Land
104	In summary, serious thought ought to be given to designing dory tanks for good water quality, and use of round tanks is one way to achieve this.	Presenter – points to round tank	Sea
GRAPHIC: SCIENTIFIC RESULTS FOR WATER QUALITY			
105	Here are some scientific results from measuring water quality with currently used dory tanks in the fleet.	Graphs of oxygen and ammonia set together	Land

106	For dissolved oxygen, we can see that the range observed in current dory tanks was quite variable, but always below desired levels.	Dissolved Oxygen graph. Each component highlighted as being addressed by the narrative.	Land
107	For ammonia levels we see that the variation in dory tanks was very large, and almost always above the recommended maximum level.	Ammonia graph. Each component highlighted as being addressed by the narrative.	Land
108	What the researchers concluded is that there is certainly room for improvement in water quality of dory tanks	Presenter	
GRAPHIC: STOCKING DENSITIES			
109	Another factor influencing water quality, and hence stress, is stocking density.	Presenter in a dory, surrounded by lots of people, very squishy	Land/ Sea
110	Many fish in a confined space will quickly degrade water quality by lowering dissolved oxygen, and increasing ammonia levels.	Square dory tank with a lot of Coral trout swimming around, looking not so good.	Sea
111	Researchers measured stress in Coral trout from stocking densities ranging from 10 fish per 200 litres to 30 fish per 200 litres	Dory tank with a few Coral trout swimming around, looking relaxed	Sea
112	They found that when stocked at 30 fish, there was a significant stress response, but not at lower densities.	Text: 30+ fish per 200l?? "Stress, Stress, Stress" repeated in pulsating letters	Land
113	So they recommended a maximum stocking density of 20 fish per 200l in dory tanks. This means, if your're catching a few, offload them more often.	Text: maximum stocking density – 20 fish per 200l. Range of tank sizes with volumes + #s for recommended holding.	Land Sea, port
GRAPHIC: FRESHWATER WASH			
	There are a couple of preventative measures we can take to help the fish deal with stress.	Large tank in main vessel	
	The first of these is a low salinity wash.		
	Marine parasites and disease organisms are killed by freshwater, so washing the fish in low salinity water helps prevent disease.	Moving fish from freshwater wash to holding tank.	
	Placing the fish in clean 1/3 seawater, 2/3 freshwater for 15 to 20 minutes does not stress Coral trout and can be applied on the vessel.	Graphic: 1/3 seawater, 2/3 freshwater for 15 to 20 minutes.	
GRAPHIC: HOW ABOUT FEEDING?			
118	One of the inconsistent industry practices identified by the research	Large tank in main vessel, fisher throwing pilchards	Sea

	team was that of feeding on the main vessel.	in.	
119	Some operators fed their captive trout, whilst others starved them. The research has shown that fed fish cope better with stress.	Scientific graph, two feeding averages, feeding lower than non-feeding	Sea
120	Stress in fed Coral trout was about half that, in those which were starved.	Each treatment in graph highlighted as narrative moves over it.	Land
121	Therefore, to keep trout healthy and un-stressed, they should be fed whilst in the main tank.	Presenter, pointing to main tank	Sea
GRAPHIC: DOES SEASON AFFECT STRESS IN CORAL TROUT?			
122	An issue raised by industry was that at certain times of the year, for example, spawning, trout seemed highly likely to be stressed out.	Presenter	Sea
123	In order to test this idea, researchers examined the blood of Coral trout for an 18 month period to December 1999.		Land
124	What they found confirmed the suspicions of industry. Namely that stress was highest in trout caught in October, both in 1998 and 1999.	Text: Conclusion: October is the season of stress.	Land
125	So you need to be extra careful during October when handling trout.	Presenter	Sea
126	Well, we seemed to have come to the end of our story. Hopefully by now you and your fish will be nice and relaxed. But just to make sure you've taken in these stress-minimization techniques, let's have a summary.	Presenter, sipping bundy rum	Sea
GRAPHIC: SO HOW DOES IT ALL WORK?			
127	Handle fish gently	Trout being cradled	
128	Release caught trout in a full tank of water	Trout dropping into water	Sea
129	Use a de-hooker	De-hooker being used	Sea
130	Deflate the swimbladder	Swimbladder deflation	Sea
131	Keep dissolved oxygen high. Why? Trout need it to breathe.	Clean water tank with bubbling water.	Land/ Sea
132	How? Water flow greater than 6 tank exchanges per hour; stocking density 20 fish per 200L or less; dory tank designed for water quality, not just holding of fish, additional aeration	Accompanying text – summarized in dot points	Land
133	Keep ammonia low! Why? It is toxic to fish! How do you keep it low? High water flow, flush wastes regularly, keep stocking densities low, use dory tanks designed for better water	Accompanying text – summarized in dot points	Land

	quality.		
135	Feed the trout while in the main tank	Feeding in the main tank	
136	Be extra carefull during the season of stress, around October.	Text: “Beware the season of stress – October”	
138	Remember, the prime objective at all times is to keep fish alive and in top condition.	Healthy trout swimming	Sea
139	This can be accomplished with care, patience, and the best possible water quality.	Text: Care, patience, best water quality.	Land
140	Operating under these conditions will improve the financial value of the catch, and provide a more humane way of treating Coral trout.	Presenter	Sea
GRAPHIC: WHAT HAVE WE REALLY LEARNT?			
141	Well, the proof of the pudding is in the eating. Let’s see if our doryman has learnt anything from this video.	Dory leaves main vessel and finds a hang.	Sea
142		Dory making anchor	Sea
143		Fills tank, switches on bilge pump	Sea
144		Baits hook	Sea
145		Throws out hook	Sea
146		Hauls in fish	Sea
147		Lifts fish up to pierce swimbladder in cradle position.	Sea
148		Deflates swimbladder correctly.	Sea
149		De-hooks with de-hooker quickly into tank	Sea
150		Shot of healthy fish in water of dory tank	Sea
150a		Dory alongside main vessel, unloading	
151		Turns to look at the skipper, demanding acknowledgment of his best efforts	Sea
152		Shot pans to the skipper of the boat who gives a laconic smile, thumbs up	Sea
153		Shot pans back to doryman, who mimics the skipper’s response, then mouths the same silent curse given to him earlier.	Sea
154	Well done! My stress levels are definitely way down. Time for an afternoon siesta, I reckon. But before that, I’m hoping “Billy” will shout me	Presenter leans back in deck-chair.	Land

	lunch after his big new paycheck.		
155	How about that one Brett, I kept it especially happy for you.	“Billy” the doryman pointing to a beautiful trout in a tank (can computer graphics touch up the smile?)	Land
156		Asian restaurateur says “Certainly Sir” and whisks it out. Dynasty restaurant	Land
157	“On behalf of Trev, “Billy”, and myself, we’d like to wish you happy fishing. Remember, the secret to live fish is care, patience, and good water quality.	Presenter about to eat Coral trout at a nice restaurant	Land

APPENDIX 4: ISO BEST PRACTICE MANUAL: CATCHING AND HANDLING OF LIVE REEF FISH ABOARD THE VESSEL.

Best Practice: Catching and handling of live reef fish

Preface to the current manual

This Best Practice manual is a modification of the Best Practice manual prepared as a result of consultation by John Sumner (Sumner, 1997). It has been modified in accordance with the findings of the Fisheries Research and Development Corporation funded project 97/341 "Enhancement of ship-board survival of coral trout destined for the live fish market" which are described in Anderson (2003).

In addition to this manual, a training video entitled "Live Trout, Not Dead" is available to assist current and new entrants to the live reef fish fishery with developing expertise in handling live reef fish.

The success of the project owes much to a number of people and organizations:

Kevin Kane, Tony Hart, Peter Appleford, Louis Evans, Mike Bennett, Tracey Turner, Sue Bennett, Campbell Davies and Bruce Mapstone were involved with the project.

Salaries for staff were provided by James Cook University, the Cooperative Research Centre for the Ecologically Sustainable Development of the Great Barrier Reef, The University of Queensland and Curtin University.

James Cook University and University of Queensland provided aquarium facilities.

The project was also greatly assisted by the support and cooperation of many commercial line reef fishers who gave time, access to fish and to their vessels for sampling, information and their encouragement.

Trevor Anderson
May, 2003.

Preface to the previous manual

The Best Practice manual has been synthesised from the range of procedures used by the two fishing vessels and the two processors which participated in the Australian Seafood Industry Project (ASIQAP). This project has been funded by the Department of Industry, Science and Tourism (DIST), Queensland Department of Primary Industries (QDPI), the Queensland Commercial Fishermen's Organisation (QCFO) and by the more than 20 companies which participated.

The manual is in two parts:

- catching and handling live reef fish aboard the vessel and
- storage of live reef fish on shore and their delivery.

An important aspect which is included in this Best Practice manual is the way the business is run. All four operations have successfully achieved ISO 9002 certification which reflects their ability to meet the international standard. Each of the companies has an ISO Manual which details how they operate a number of aspects crucial to enhancing the profitability of their

specific operation. For obvious reasons I have not included market-sensitive information on how companies deal with contracts, customer complaints, their subcontractors, controlling key documents, staff training and statistical techniques. These and other elements of the ISO 9002 standard are pivotal to the way a company runs its affairs and makes money.

Each company has become more or less familiar with the Hazard Analysis Critical Control Point (HACCP) concept. Prior to beginning this project each had a broad awareness of food safety and its practical, everyday control. I believe that their progress to ISO 9002 has helped accentuate the role of food safety in their operations.

This Best Practice manual owes much to the following companies and people:

· Jo Trewavas (QA Manager/Owner), Bruce Trewavas (Owner) and Mike Matteschеck (Skipper) of the FV Crested Tern in Bundaberg.

· David Caracciolo (QA Manager) and Jim Wallace (Skipper) of the FV Dorothy B in Mackay.

· Jo Trewavas (QA Manager), Gerald Dawson (Operations Manager) and Bruce Trewavas (Managing Director) of Satellite Seafoods, Bundaberg.

· Graham Caracciolo (Export Manager) and David Caracciolo (General Manager), Searaker Pty. Ltd, Mackay.

John Sumner

May, 1997

Contents

Introduction

Issues for Best Practice in catching and handling live fish aboard the vessel

The matter of Best Practices in reef fish catching and processing was considered by an expert panel during the sector workshop held in conjunction with the QA Summit in Brisbane on 8-9 May 1997.

The panel comprised:

- Participants in the ASIQAP.
- A reference panel from suppliers of services to the industry and customers who purchase reef fish.

Further discussions were held with fishers in preparation of the proposal to the FRDC for funding.

There were some general issues surrounding Best Practice:

1. Catching and handling aboard the dory to eliminate abrasions through the scales of the fish.

2. Handling and holding conditions which keep the fish strong and healthy through the duration of the voyage.

3. Handling procedures at unloading to eliminate damage.

These, together with other specific issues which contribute to best practices, are presented in the following manual.

1. Job descriptions of staff carrying out QA-related tasks

1.1 Owner

Responsible for:

1. Overall management of company and responsible for export.
2. Formulate and direct Vessel policy on quality management and quality assurance.
3. Formulate and direct policy on personnel training.
4. Be available for briefings by the Skipper and QA Manager.
5. Review all serious incident reports to ensure regulatory bodies and customers are informed and remedial action has been instituted.
6. Maintain an understanding of Vessel's Quality Manual requirements.
7. Chair the Quality Team meetings.

1.2 QA Manager

Responsible for:

1. Dealing with customer complaints and surveys and passing on this information to the owner and Skipper.
2. Ensure that in-house and independent audits are performed as prescribed.
3. Be a member of the Quality Team.
4. Assume the owner's responsibilities and authorities during his absence.
5. File documentation relating to the quality system.
6. Prepare summary and trend data for the owner on matters of quality.
7. Ensure that the Quality Manual is updated promptly each time an amendment is approved.
8. Implement, organise and monitor the QA program.
9. Develop draft amendments for consideration at performance review meetings.

1.3 Skipper

QA-related duties are:

1. Ensure the QA program as written is adequately performed at all times on the Vessel.
2. Report serious defects to the owner.
3. Monitor the effectiveness of personnel training.
4. Document load-out and shipping to Satellite Seafoods.
5. As necessary, be a member of Quality Team.

1.4 Fishers

Responsible for:

1. Knowing quality requirements as written in the manual.
2. Implementing quality requirements as written in the manual.

2. Vessel

2.1 Description of vessel

Construction and equipment details of the Vessel:

- Day tanks (1/fisher) and 2 spare tanks to accommodate high catching rate.
- Two aerated tanks each capable of holding14 t water and 1 t fish.
- Four water pumps to give complete tank water replacement in less than 10 minutes.
- Desk snap freezer capable of freezing 2000 kg of product to -18^{o}C in around 6 hrs.
- Brine tank capable of crust freezing product in 2 hrs.

2.2 Species captured

1. The Vessel has dories from which fishermen catch from near the reef in two sessions (am and pm).
2. The main species is coral trout (*Plectropomus leopardus*) but other species are also taken.

3. Capture of live reef fish

Best practice issues

1. Dory handling procedures aimed at preventing physical damage to the fish and minimising the stress response:

· Knotless net to reduce abrasion of surface.
· Smooth apron to prevent abrasion of the scales.
· Deflation of a distended swim bladder
· Use of a de-hooker at all times to remove hook without damage.
· Optimum water quality in the dory tank, achieved by:
- Water flow greater than 6 tank exchanges per hour.
- Regular flushing of bait and waste products.
- Dory tank designed specifically for good water quality – e.g. sloping or conical bottom to collect wastes for quick flushing.
· Maximum stocking density at 20 fish per 185 L (100 $kg.m^{-3}$). Initial handling on the dory is aimed at preventing physical damage to the fish and to minimising stress:

2. Similar precautions are used on board the main vessel

3.1 Preparation of the dory

1. The dory is fitted with a tank designed to eliminate dead spots (i.e. where flow is minimal or negligible, as in the bottom corners of square tanks). Dory tank design will include:

· A pump capable of pumping the volume of the tank in less than 10 minutes.
· Circular corners to enhance water flow
· Sloping and/or conical bottom to allow accumulation of waste materials at a central point where they can be flushed out.
· Installation of a 'crack valve' to instantly flush wastes from the bottom of the tank
· Narrow neck to reduce free-surface area, thus nullifying the potentially stressful rocking motion of the dory. A double-lid system (small lid for the narrow neck, large lid to enable fish to be removed easily) works best with this feature.

2. The dory is set up and the tank is cleaned of old bait and dirty water.

3. The tank is washed out with non-toxic foaming detergent.

4. Each fisher carries:

· Bait
· Hook line and sinkers
· Safety equipment
· 16 gauge surgical needle

3.2 Fishing technique

1. The fisher travels to the reef and anchors, usually near a pressure point on the reef. In this context, a ‘pressure point’ is where tidal currents make contact with a coral bommie or other section of the reef. This is generally perceived as the area of most bait activity.

2. The baited line is set up to 25m deep.

3. The fish is bought to the surface as quickly as possible.

4. If necessary, the swim bladder is deflated, prior to de-hooking, as described in Section 3.3.

5. The hook is removed using a de-hooker in the manner described in Section 3.4.

6. If required, the fish is held without causing damage or removing scales by cradling upside down in one arm.

3.3 Swim bladder deflation

1. The swim bladder is deflated so as to enable the fish to achieve neutral buoyancy and swim upright.

2. A 16 gauge needle inserted through the anus at a 45° toward the top of the head and held for sufficient time to allow depressurisation.
3. The size 16 gauge needle can be cleared of blockages by quickly blowing air by mouth through the needle..

3.4 De-hooking

1. De-hooking is accomplished via the use of a special tool known as a “de-hooker”. It is a stainless steel rod upturned into a hook at the bottom end. The handle is usually made of wood. The de-hooker is held by the right hand, and used to grab the hook in the fish’s mouth, while the left hand pulls the fishing line into a horizontal position, with the trout positioned over the dory tank. With a quick flick of the right wrist, the de-hooker twists the hook out of the fish’s mouth, and it drops into the dory tank full of water.

2. De-hooking is accomplished within 5 seconds of the live fish being pulled onto the dory.

3.5 Storage aboard the dory.

1. Live fish are stored aboard the dory in water with greater than 70% dissolved oxygen and less than 0.5 $mg.L^{-1}$ of ammonia.

2. The fish are placed in the dory tank through which seawater is continuously circulated by a bilge pump which puts a minimum of 6 tank volumes per hour through the tank.

3. Dory tank bilge pump to be left on at all times when fish are in the tank.

4. Flush pilchards (bait) and other wastes out of tank regularly (every 30 minutes).

5. Up to 20 fish per 200 L (100 kg.m^3) of fish are stored until the dory returns to the mothership.

6. A battery powered aerator is used to keep dissolved oxygen levels high.

3.6 Unloading from dory to mothership

1. Main vessels will have a number of recovery tanks that hold live fish for a day or so before transfer to the main holding tanks. Water flow in the recovery tanks is to be maintained at 6 or more exchanges per hour.

2. Fish are passed from the dory to mothership by net or perforated plastic crates.

3. Prior to transfer to the recovery tanks, fish are soaked in freshwater to kill parasites. Either:

    - Soak fish in pure freshwater for 3 minutes.
    - Soak fish in 33% sea water (1/3 seawater, 2/3 freshwater) for 15 – 30 minutes.

3.7 Recovery of fish

1. Fish in the recovery tank are monitored by the Skipper.

2. Dead, damaged or moribund fish are removed for processing.

3. Payment to fishers is made on the basis of species, size and whether dead or alive after holding in the day tanks.

4. Payment is made to the boat based on the quality of the live fish, i.e. whether suitable to be sold as live fish or rejected from being kept live and either gilled and gutted or filleted. If any live fish are rejected, then the fisher is paid based on the percentage of his live catch.

4. Storage of live fish aboard Vessel

Best practice issues	
1.	On ‘open’ systems, the live storage tanks must provide sufficient flow-through to simultaneously aerate water and to remove waste products.
2.	On ‘closed’ systems the aeration and scrubbing systems must maintain a suitable environment for survival.
3.	The Skipper must operate procedures to regularly void physical contaminants from tanks.

4.1 Live storage tanks

Either:

1. The Vessel has a number of large tanks with ‘open’ systems which are capable of holding live fish.
2. During operations the tank is completely filled with water and has a pound board which baffles water movement at the surface. Tank construction results in minimal water surge.
3. The tank has a maximum loading of 100 $kg.m^{-3}$ live fish.
4. Except when in estuaries (see 4.2) the tank has a flow rate giving a complete water exchange every 10 minutes.
5. Venturis allow turbulence to be introduced to the incoming water.

Or:

1. The Vessel has a number of large tanks with ‘closed’ systems which are capable of holding live fish.
2. During operations the tank is completely filled with water and has a pound board which baffles water movement at the surface. Tank construction results in minimal water surge.
3. The tank has a maximum loading of 100 $kg.m^{-3}$ live fish.
4. Normally, the tank has a flow rate giving a complete water exchange every 10 minutes.
5. Water is collected and passed through a particulate filter capable of filtering particles greater than 20 μm.
6. Water is passed through a biological filter of at least 20% of the main storage tank volume.
7. The main tank is aerated vigorously such that dissolved oxygen remains above 70% saturation.
8. Ammonia in the water of the holding tank is measured at least daily to ensure that it remains below 0.5 $mg.L^{-1}$.

9. Nitrite in the water of the holding tank is measured at least daily to ensure it remains below 0.1 $mg.L^{-1}$.

10. Nitrate in the water of the holding tank is measured at least daily to ensure it remains below 20 $mg.L^{-1}$.

11. If necessary, water is exchanged with fresh seawater to ensure nutrient concentrations remain within acceptable values.

4.2 Water management in live tanks in the estuary

1. Since freshwater for long periods is lethal for reef fish, the seawater suction is turned off when the vessel enters the river mouth.

2. A circulation aeration system is arranged using multiple tanks to ensure circulation at a rate of at least 6 exchanges per hour and the suction pump off.

3. The exit overflow on the main tank is capped.

4. Oxygen uptake is enhanced by use of splashing into tanks and additional aeration or oxygenation.

4.3 Cleaning of the tanks

1. When filled with fish, venturis are usually operated just cracked open, but are opened completely to remove foam and scum.

2. A submersible pump and non-toxic foaming detergent is used to clean the main tank when in port.

3. The detergent is completely washed from the tank.

5. Unloading the vessel

Best practice issues
1. Handling procedures must minimise stress and damage to the fish by:
· Preventing threshing of fish.
· Having them out of water for no more than a few (5) minutes.
2. Weighing of individual fish must be replaced by payment based on size and number of pieces.

5.1 Unloading live fish

1. The water level in the main tank is lowered to facilitate removal of the fish.
2. Fish are netted from the main tank and transferred to fish boxes.
3. Each box is transferred to the factory or lorry and each fish loaded into the storage tank.
4. A count is made of fish according to species and mass and recorded on the Live Fish Received Record.

5.2 Unloading frozen product

1. Product is unloaded from the boat to the factory freezer, weighed, and recorded on Filleting Record.

6. Process flow and HACCP diagrams

Symbols used in process flow diagram

Symbol	
O	Operation
	Inspection
♣	Combined operation/inspection
>	Transfer
∇	Storage

There are no food safety considerations in the catching and handling of live reef fish and this is reflected in the HACCP treatment of this process.

6.1 Process Flow Diagram: Capture, storage and unloading of live fish

	Process	Symbol	Frequency	Check	Responsibility
1	Bait storage	∇	Every carton	Bait hard frozen until used	Skipper

Catching

2	Dory setup	♣	Every trip	All equipment aboard Storage tank clean Aeration system working	Fisher
3	Landing fish	o	Every fish	Handling live fish when captured to prevent damage and stress	Fisher
4	Depressurising swim bladder	o	Every fish	Careful puncture of swim bladder behind the pectoral fin	Fisher
5	Storage	∇	Every fish	Water circulation and aeration in operation	Fisher
6	Transfer to vessel	>	Every trip	Handling during transfer process to larger boat so as to minimise stress and damage to fish	Fisher

Handling aboard vessel

7	Recovery	♣	Every fish	Condition of fish in each recovery tank Remove dead, damaged or moribund fish	Skipper
8	Transfer to main tank	>	Every fish	Handling during transfer to minimise stress and damage to fish	Skipper
9	Storage	∇	Every fish	Water conditions in tank Check condition of water and fish Remove dead, moribund or damaged fish	Skipper

Transfer ashore

10	Transfer ashore	>	Every load	Remove dead, moribund or damaged fish Handling technique Number of pieces	Skipper

6.2 HACCP Chart: Capture, storage and unloading of live fish

	Critical operation	Potential hazard	Critical control point	Preventative control and monitoring measures	Corrective action
1	Capture	Damage to fish	Netting fish Handling	Net of knotless manufacture Fisher trained to grip fish to minimise stress and scale removal	Dispose of damaged fish for filleting and freezing Train fisher in catching and handling techniques
2	Depressurising swim bladder	Death of fish	Syringing	Fisher trained to insert syringe behind pectoral fin and to withdraw air until swim bladder reduced to normal size	Dispose of damaged fish for filleting and freezing Train fisher in syringing
3	Storage on dory	Death, damage to fish	Tank construction and operation	Tanks constructed without protrusions which may damage fish Fisher checks water circulating and aerated	Dispose of damaged fish for filleting and freezing Train fisher in sound practices aboard dory
4	Transfer to vessel	Death, damage to fish	Netting and handling	Net of knotless manufacture Fisher trained to grip fish to minimise stress and scale removal	Dispose of damaged fish for filleting and freezing Train fisher in handling techniques
5	Recovery	Stress, infection	Skipper inspection	Skipper inspects each recovery tank at regular intervals	Dispose of damaged fish for filleting and freezing
6	Transfer to main tank	Death, damage to fish	Netting and handling	Net of knotless manufacture Fisher trained to grip fish to minimise stress and scale	Dispose of damaged fish for filleting and freezing Train fisher in handling techniques
7	Storage in main tanks	Death, damage to fish	Tank construction and operation	Tank constructed without protrusions which damage fish Pounds boards inserted to eliminate surging Aeration working correctly Filters working correctly Pumps working correctly	Modify tank to eliminate protrusions and introduce pound boards. Transfer fish to backup tank. Dispose of dead, moribund or damaged fish for filleting and freezing
8	Passage up estuary	Death of fish	Water system	Skipper changes water system from straight-through to recirculation by involving rear tank as aeration/recirculation reservoir	Dispose of dead, moribund or damaged fish for filleting and freezing Retrain skipper in water management system
9	Unloading	Death, damage to fish	Netting and handling technique	Skipper drops water level in tank to facilitate removal of fish Crew handle fish carefully	Dispose of dead, moribund or damaged fish for filleting and freezing
10	Checking catch	Loss of product at factory	Inspection	Driver checks quality of fish before accepting	Dispose of dead, moribund or damaged fish for filleting and freezing

7. Processing and packaging of reef fish

Aboard the Vessel, product caught in the morning is processed after lunch by the Skipper and one Fisher. In the evening, all of the crew assist in filleting, packing and freezing.

Best practice issues

1. Numerous quality issues such as removal of bones and trimming fillets.
2. Overpacking is a problem with 'round' packs and crushed fillets. The saving on carton price is more than lost in the reduced price paid for the product.

7.1 Gill and gutting

1. The following species are gilled and gutted:
   - Coral trout up to 2.5 kg, emperor, wrasse, reef cod, barramundi cod.
2. Processing waste is discarded over the side.
3. The cavity is scrubbed to remove blood line (kidneys).

7.2 Filleting

1. The following species are filleted:
   - Coral trout > 2.5 kg, stripey bass, hussar, mackerel, large cod, parrot.
2. Each fillet is removed and washed in water.
3. The washed fillet is trimmed, re-washed and drained.
4. Coral trout wings are removed for separate freezing.
5. Fillets are packed into 10 kg shatterpacks.
6. If the appearance is OK, fish are gilled and gutted.
7. G&G fish are packed in a tube bag, blast frozen, graded boxed and labelled for domestic sale.

7.3 Individual wrapping

1. Each G&G fish is individually wrapped in a bag.
2. Each bagged fish is packed into a carton, stomach-down.
3. A total weight near to 10 kg is packed into the carton, which has been pre-labelled with date, species and grade.
4. The carton is stapled and loaded into the snap freezer.

7.4 Shatterpacking

1. Each fillet is laid on a sheet of plastic film in the carton.
2. Layers of fillets are packed on interleaving plastic film to achieve a shatterpack.
3. Between 10-11 kg is packed into each carton, which has been pre-labelled with date, species and grade.
4. The carton is stapled and loaded into the snap freezer.

8. Process flow and HACCP diagrams

8.1 Process Flow Diagram: Processing of frozen reef fish on Vessel

	Process	Symbol	Frequency	Check	Responsibility
1	Transfer to brine tank	>	Every fish	Fish stored in brine tank until processed	Skipper
2	Gill and gutting	o	Every fish	Gut contents, membranes and blood line removed	Fisher
3	Filleting	o	Every fish	Fillet, trimmed and washed	Fisher
4	Packing G&G fish	o	Every fish	Fish packed stomach down	Fisher
5	Packing fillets	o	Every fillet	Interleaved without overpacking	Fisher
6	Labelling	♣	Every carton	Correct species, grade, date	Fisher
7	Snap freezing	o	Every carton	Time in snap sufficient for hard freezing	Fisher
8	Storage	∇	Every fish	Freezer maintains product colder than -18ºC	Skipper
9	Transfer ashore	>	Every load	Product handled quickly without damage to carton	Skipper

8.2 HACCP Chart: Processing of frozen reef fish on Vessel

	Critical operation	Potential hazard	Critical control point	Preventative control and monitoring measures	Corrective action
1	Storage on vessel	Production deterioration	Brine tank	Fish quickly loaded to brine tank while crew rest after morning shift	Process 'warm' fish first and quickly into deck snap freezer
2	Processing	Damage to product	Knife work of fishers	Crew trained in gill and gutting and filleting techniques to maximise yield	Rework product Retrain filleters
3	Packing	Product description Product damage	Labelling Mass in carton	Crew trained in species identification Crew trained to not overpack carton	Repack product "Round" cartons disposed at discount Crew trained in packing
4	Snap freezing	Product quality loss	Time in snap	Crew allow sufficient time for product to hard freeze in centre of pack Skipper probes pack if doubt	Replace in snap until completely hard frozen
5	Frozen storage	Product quality loss	Freezer management	Crew load freezer only with hard frozen product Freezer stacked to minimise quality loss	Skipper retrains crew in management of stock
6	Unloading	Product damage	Handling	Rapid unload without damage to cartons	Product which has softened dealt with first and quickly into shore freezer

9. Freezing and frozen storage

9.1 Snap freezing

1. Cartons are loaded into the snap freezer.
2. Snap freezing is carried on until the next day, unless the catch has been large, when the freezer is unloaded the same evening to the freezer room.

9.2 Frozen storage

1. Cartons are loaded from the snap freezer into the freezer room.
2. Product is stacked in the freezer until unloaded.
3. The freezer temperature is capable of maintaining product at colder than -18^{o}C.

10. Unloading the vessel

The freezer room aboard the Vessel holds 500 cartons (6 T) of product, which is unloaded at the wharf at the end of the trip.

10.1 Moving from boat to wharf

1. Crew unload the freezer by handing up cartons onto the deck of the Vessel.
2. At high tide a conveyor is set up from boat to wharf.
3. At low tide the crew load cartons into a net.

10.2 Sorting and weighing

1. Processing staff sort cartons onto pallets according to species and grades.
2. A bulk weight is made and recorded in the weigh-in book. This is the basis for payment of Skipper and crew of the Vessel.
3. Product is quickly transferred to the shore-based freezer store where it is stored until picked for distribution.

11. Bait storage and preparation

1. Pilchards are used, kept frozen in freezer until required.
2. Bait is thawed and stored aboard the dory in a bucket.

12. Hygiene and sanitation on the Vessel

12.1 Chemicals used for hygiene

The following chemicals are used:

Category	Trade name	Company address
Acid cleaner	Industrial fallout	Symbio Products. 50 Johnson Street Bulimba. 4171
Detergent	Teepol	Shell Australia
Detergent	Sodi-Chlor	Symbio Products 50 Johnson Street Bulimba. 4171

12.2 Application of cleaning chemicals

1. Detergent solution (Sodi-Chlor) is made up in buckets and applied manually to the following areas on a daily basis:
   - Bins and tubs
   - Avery scale pan
   - Knives and other implements
   - Aprons and gloves
2. Soil and detergent are removed by rinsing in potable water.
3. Where possible, equipment is inverted to drain and dry.

12.3 End-of-trip cleandown

1. Tanks are washed out after unloading and again before new load of fish are put into storage with Jupiters foaming detergent.
2. Walls and floors on the main deck are cleaned with Jupiters foaming detergent.

12.4 Daily hygiene on deck

1. Loose soils are removed manually from equipment
2. All contact surfaces are rinsed with seawater between each catch.

13. Testing methods

The following testing methods are used on the vessel:

13.1 Measurement of chamber temperatures

Procedure:

1. Gauges on temperature-controlled rooms are checked daily and temperatures recorded in the Log Book.

13.2 Testing of tank water

Seawater is passed directly through all tanks systems so no testing is carried out.