Assessment of barotrauma and its mitigation measures on the behaviour and survival of snapper and mulloway

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NON-TECHNICAL SUMMARY

Assessment of barotrauma and its mitigation measures on the behaviour and survival of snapper and mulloway.

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OBJECTIVES:

1) Determine the success of different barotrauma mitigation treatments in enabling fish to return to their preferred depths following catch and release.

2) Describe the physiological responses of fish to barotrauma and its mitigation treatments.

3) Use information from the above objectives to assist in the development of a NSW policy on the best practice for releasing barotrauma affected fish.

NON TECHNICAL SUMMARY:

Fish are caught and subsequently released in almost every fishery globally as a result of fisher choice or management restrictions (e.g. size and bag limits, quotas, closed seasons), including ~20% of all commercially-caught fish worldwide and ~50% of recreationally-caught fish in Australia. Many of these released fish caught from deep water have been shown to subsequently die, many as a result of a condition known as barotrauma.

Barotrauma are physical injuries to fish caused by the effects of decreasing pressure when a fish is caught at depth and brought to the surface. Expanding gas in the swimbladder of the fish causes the swimbladder to overinflate crushing and twisting internal organs. If the swimbladder bursts, the sudden release of gas can cause further damage to internal organs, the eyes to bulge out of the head, the stomach to turn inside-out and protrude from the mouth and the intestines to be forced out of the anus. Other injuries result from the formation of bubbles when gases come out of solution in body tissues as pressure is decreased en route to the surface from depth causing similar issues to those which occur in SCUBA divers suffering 'the bends', including the blockage of blood vessels in vital organs. Death may occur as a result of these injuries, even if the fish swims off after it is released. In addition, the fish may not be able to return to depth independently due to excess swimbladder gas which makes the fish extremely buoyant causing it to float upside down on the surface, where they are susceptible to predation, overheating and sunburn.

Snapper *Pagrus auratus* and mulloway *Argyrosomus japonicus* are two of southern Australia's most important recreational fish species. They are also often caught from deep water (100-150 m) and therefore are affected by barotrauma. Management arrangements for snapper and mulloway include minimum legal lengths and bag limits. These result in ~78% of snapper and ~25% of mulloway being released following capture by recreational fishers. In addition, voluntary catch and release (C&R) has become an extremely popular practice in recreational fisheries due to the increasing recognition of the importance of maintaining healthy fish stocks for improved recreational fishing opportunities. The two most commonly used methods for reducing the effects of barotrauma work by allowing the fish to return to capture depth: (i) 'venting' air from the body cavity or swimbladder using a hollow needle or similar, and; (ii) using heavy release weights to lower the fish back to depth. There is therefore a critical need to understand the effects of barotrauma on these species and the best ways to maximising the chances of survival of barotrauma-affected individuals when released, including the effectiveness of barotrauma-mitigation treatments.

Custom-built hyperbaric chambers were first used to estimate the rates of gas exchange into and out of the swimbladder so that we could be confident that fish used in experimental simulations of C&R were physiologically in equilibrium at simulated pressure as a fish would be at actual depth in the ocean. Snapper were found to have a similar density to that of seawater and so neither float nor sink when stationary in the water column (neutrally buoyant), whereas mulloway were found to be denser than seawater and therefore sink when stationary in the water column (negatively buoyant). The rates of gas exchange into and out of the swimbladder were also substantially faster in snapper than in mulloway. The consequences of these differences in buoyancy and gas exchange rates between the two species results in differing behaviour and ecology with the mid-water-dwelling neutrally buoyant snapper able to quickly change its depth and the negatively

buoyant mulloway restricted to slower changes in depth and generally remaining closer to the bottom.

The hyperbaric chambers were then used to perform experiments which simulated the pressure changes experienced by fish during various C&R scenarios. For mulloway, survival following C&R was shown to be influenced mainly by capture depth. Death rates of ~50% were found for mulloway subjected to simulated C&R from 30 and 50 m depth, but only ~13% from 10 m. The swimbladder of mulloway was also show to burst if caught from water deeper than 17.5 m resulting in injuries such as a bloated abdomen, the stomach being forced out the mouth, bulging eyes, internal bleeding, damage to the liver and spleen and gas escaping via a tear in the body wall. The majority of mulloway landed from <10 m deep therefore, should survive if handled and released carefully. For depths >10 m, the amount of time a fish spent at the surface after capture also affected survival after release, with an increase from 2 to 10 min before release decreasing survival from ~50% down to ~10%. Time at the surface also influenced how quickly death occurred with mostly immediate death occurring when fish were kept at the surface for 10 min, whereas death occurred up to 219 d after simulated C&R when kept at the surface for 2 min.

Hyperbaric chamber experiments showed snapper to be much less susceptible to the effects of barotrauma than mulloway. Even though the swimbladder of snapper burst when capture was simulated from depths >14 m and despite using the greatest pressures the chambers were capable of simulating (~70 m water depth), survival was 100% for C&R with 2 min at the surface. The only experimental C&R simulation which resulted in the death of any snapper, albeit at low levels (~16%), was when fish were left at the surface after simulated capture from depth. Death as a result of this treatment was immediate (<15 min) and likely caused by emboli in vital organs. Survival was 100% when snapper were repressurized (returned to depth) indicating that all snapper should make a full recovery after C&R if promptly returned to depth provided other factors which could affect survival other than barotrauma are absent (e.g. deep hooking, handling, air exposure).

Depth profile trials showed that all three release methods examined (no treatment, venting and release weight) allowed fish to return to depth in both species, however the increased handling required by venting and use of release weights make untreated release the preferred method for releasing barotrauma-affected snapper and mulloway. However, depth profile trials did indicate that the use of release weights or ‘shot lines’ to return fish all the way to the bottom is an appropriate release technique if the fish is unable to submerge as it mimics the natural post-release behaviour of the fish to return all the way to the bottom and avoids any potential damage resulting from puncturing the body cavity when venting.

In order to maximise the post-release survival of mulloway, the following is therefore recommended:

- Avoid C&R fishing in water deeper than that which causes swimbladder rupture (i.e. ~17.5 m).
- If mulloway caught from water deeper than this must be released, it is recommended that the fish be released untreated with the minimum amount of time at the surface possible.
- If the fish is unable to submerge by itself, the fish should be returned to depth using a release weight or similar device.

In order to maximise the post-release survival of snapper, the following is therefore recommended:

- Release the fish untreated as quickly as possible in order to minimise handling-related stress and the amount of time the fish spends at the surface.
- If the fish is unable to submerge by itself, the fish should be returned to depth using a release weight or similar device.

1. INTRODUCTION

1.1. Background

Fish are caught and subsequently released in almost every commercial and recreational fishery globally. In fact, approximately 20% of all commercially caught fish worldwide are subsequently discarded (Cook, 2003; Hall & Mainprize, 2005). These fish may be discarded for many reasons, but most are a result of personal choice - the species or size may be economically or personally undesirable, or regulated - quotas, minimum size limits, bag limits, or closed seasons - in order to limit catches or to protect some segment of the population. All these regulations assume that most discarded fish survive without detrimental effects as result of the catch and release (C&R) process (Diamond & Campbell, 2009).

The incorporation of discard mortality into estimates of fishing mortality (Mesnil, 1986) and finding technical measures to alleviate it (Suuronen & Sarda, 2007) are important to sustainable fisheries management. Total discarded bycatch has been estimated to be approximately one-quarter of the worldwide fisheries catch, with discard mortality therefore representing a large source of uncertainty in estimates of fishing mortality (Alverson et al., 1994; Pascoe, 1997). Clearly, estimates of C&R-associated mortality are critical not only to gauge the effectiveness of regulations but also for setting unbiased catch quotas (Diamond & Campbell, 2009).

The level of discard mortality has been recognised to be a global problem with significant consequences for both fish stocks and marine ecosystems (Votier et al., 2004). Many discarded fish subsequently die as a result of capture and gear effects and their interaction with environmental factors (light conditions, temperature, air exposure, anoxia, sea conditions, and pressure changes) and biological factors (fish size and species, behaviour, physiology, and potential mortality) (Davis, 2002). An important source of post-release mortality in fish caught from deep water is caused by a condition known as barotrauma.

Barotrauma are physical injuries to fish caused by the effects of progressively decreasing pressure as a fish is brought up through the water column from depth in the course of fishing activities. The most obvious result of decreasing pressure is on the expansion of gas within a fish's swimbladder. The swimbladder is a gas-filled organ found within the body cavity of most teleost fish species and is used primarily for buoyancy control, as living tissue (i.e. the fish) is heavier than the water in which it lives (Strand et al., 2005). According to Boyle's Law, as pressure decreases (~1 atm for every 10 m water depth), gas expands exponentially. As a fish undergoes forced decompression on its way from the depth of capture to the surface, the expanding gas inside the swimbladder may cause the swimbladder to overinflate or burst, releasing the gas into the peritoneal and cranial cavities (Hannah et al., 2008a). Depending on the degree of pressure change, the excess swimbladder gas can result in abdominal bloating, crushing or torsion of organs, eversion of the stomach into the buccal cavity or mouth, forcing the intestines out of the cloaca and exophthalmia (bulging eyes) (Gotshall, 1964; Rummer & Bennett, 2005; Hannah & Matteson, 2007; Hannah et al., 2008b; Jarvis & Lowe, 2008). Increased depth of capture increases the amount of space the swimbladder occupies during decompression, decreases

the body cavity space available to organs, and increases compaction injuries to vital organs (Rummer & Bennett, 2005). Immediate death following capture is also often found to significantly increase as capture depth increases (Gitschlag & Renaud, 1994; St. John & Syers, 2005; Rummer, 2007). Excess swimbladder gas can also result in excessive buoyancy, which makes it difficult for the fish to return to depth on their own after release. Many fish released in this condition are left floating upside down on the surface, where they are susceptible to other potential deleterious impacts including predation, thermal shock, and sunburn (Jarvis & Lowe, 2008).

Other less obvious barotrauma injuries occur as a result of Henry's Law, which states that the solubility of a gas in a liquid is directly proportional to the partial pressure the gas above the liquid. This results in the formation of bubbles as gases come out of solution in body tissues as pressure is decreased en route to the surface from depth. Gas bubble formation as a result of Henry's Law also causes decompression sickness, or 'the bends', in human SCUBA divers. Dissolution of gases can cause the formation of gas bubbles and haemorrhaging in the eyes, skin and fins, haemorrhage and bleeding from the gills, and embolism in almost any internal body tissue, such as capillaries of the brain, heart, and liver (Gotshall, 1964; Lea et al., 1999; Longbottom, 2000; Hannah & Matteson, 2007). These emboli may occlude the heart and arteries, affecting circulation to the heart and gills (Beyer et al., 1976). In addition to physical trauma, fish typically display some physiological imbalance (e.g. elevated cortisol production) caused by the synergistic effects of capture, handling, change in temperature of the environment (thermocline exposure), and air exposure (Barton, 2002; Davis, 2007). The physical trauma and physiological imbalance may also result in subsequent behavioural impairment, such as a decreased ability to catch food and to avoid predators (Ryer, 2002; Ryer et al., 2004).

Susceptibility to barotrauma varies between species and is dependent upon various factors such as the relative volume of the swimbladder (Rogers et al., 1986), blood physiology (Stephens, 2001), environmental conditions (Muoneke & Childress, 1994), and the natural habitat of the fish. Release mortality of barotrauma-affected fishes can be either immediate with death occurring after fish are released at the surface, or delayed, with death occurring minutes, hours, or days after release (Schirripa et al., 1999).

1.2. Need

About half of the Australian recreational catch of line-caught fish (by number) has been estimated to be subsequently discarded or released (Henry & Lyle, 2003). The effectiveness of C&R fishing relies on the assumption that a large proportion of released fish survive without detrimental effects (Diamond & Campbell, 2009). However, in C&R fisheries involving deep water-dwelling species, this assumption may be violated as a result of high incidences of barotrauma sustained during capture (Rummer & Bennett, 2005). Voluntary C&R is becoming increasingly common in recreational fisheries due to the increasing recognition of the importance of maintaining healthy fish stocks for improved recreational fishing opportunities and the importance of the large individuals often targeted by recreational anglers to stock sustainability (Cooke & Sneddon, 2007). Several high profile tournaments (e.g. the annual "Dave Irvine Memorial Snapper Classic" held in Coffs Harbour) in Australia are becoming exclusively C&R events. Naturally, anglers, tournament organizers and managers need to know whether the fish targeted in these events survive following release.

Considerable research has been done in New South Wales (NSW) during recent years into the post-release survival of recreationally captured fish. However, almost all of this research has been done in shallow estuarine waters or in captivity (e.g. Broadhurst & Barker, 2000; Broadhurst et al., 2005; Butcher et al., 2007; McGrath et al., 2011; Broadhurst et al., 2012) and the fate of fish captured and released in deeper offshore waters remains largely unknown (but see Butcher et al., 2012). Recognition of the severity of barotrauma has resulted in other Australian states investing into understanding the effects of barotrauma on their important recreational species and into ways of maximising the chances of survival of barotrauma-affected fish when released (e.g. St. John & Syers, 2005; Sumpton et al., 2008; Brown et al., 2010, Sumpton et al., 2010). There has been little or no research on local species in NSW (but see Stewart, 2008; Butcher et al., 2012) which has inhibited informed management to the extent that NSW DPI does not have a policy and associated advisory messages on the best practice for releasing barotrauma-affected fish.

The most popular methods used by anglers for reducing the effects of barotrauma involve allowing the fish to re-pressurize by returning to capture depth. The two most commonly used techniques in Australia are: (i) 'venting' air from the body cavity or swimbladder using a hollow needle or similar, and; (ii) using heavy release weights to lower the fish back to depth. Recent research has shown that these techniques are successful in improving the survival of some Australian species, but not others. For example, tag-recapture studies in Western Australia and Queensland have shown no improvements in recapture rates by using either treatment method in many species and for dhufish *Glaucosoma hebraicum*, breaksea cod *Epinephelides armatus*, saddletail snapper *Lutjanus malabaricus* and red emperor *L. sebae*, venting was found to result in lower chances of survival (Lenanton et al., 2009; Brown et al., 2010). A recent international review on venting fish (Wilde, 2009) provided virtually no support for the practice as a means of increasing survival of captured and released fish and concluded that the practice should be discouraged by fishery management agencies because of the potential adverse effects on survival of released fish. No information exists on the effectiveness of barotrauma treatments for NSW species.

Snapper *Pagrus auratus* and mulloway *Argyrosomus japonicus* are two of the most highly-prized recreational and commercial fishing targets in coastal waters of southern Australia. Both species use estuaries as nurseries with adults moving offshore into depths of 100-150 m (Kuiter, 1993; Kailola et al., 1993). Both species are physoclists, which means that they have a closed swimbladder from which gas cannot escape unless the swimbladder is perforated or ruptured. This physiology functions well for these species which inhabit deep water because they can inflate their swimbladder at depth and are not required to gulp air from the surface before diving as physostome species must do (Pelster, 2004). However, as described above, when physoclist fish are captured and undergo a forced ascent, gas in the closed swimbladder expands as pressure is decreased, and with no way to escape, causes various injuries (Pribyl et al., 2012). Scientific assessments for these species indicate that both are overfished in some parts of their distributions and that recovery programs need to be developed (Rowling et al, 2010). In NSW, where both species are assessed as being overfished in some form, management arrangements for snapper and mulloway include minimum legal lengths and recreational bag limits. These management arrangements result in approximately 78% of snapper and 25% of mulloway being released following capture by recreational fishers in NSW (Henry & Lyle, 2003) and consequently research into the survival of these released fish is paramount. Limited work has been done to investigate the

effects of barotrauma on these species (but see Stewart, 2008; Butcher et al., 2012) with field-based assessments proving difficult.

1.3. Objectives

1) Determine the success of different barotrauma mitigation treatments in enabling fish to return to their preferred depths following catch and release.

2) Describe the physiological responses of fish to barotrauma and its mitigation treatments.

3) Use information from the above objectives to assist in the development of a NSW policy on the best practice for releasing barotrauma affected fish.

2. ESTIMATING SWIMBLADDER GAS EXCHANGE RATES AND BUOYANCY CONTROL

2.1. Introduction

Many teleosts possess a gas-filled swimbladder that acts primarily as a hydrostatic organ and, in some species, secondarily for sound production (Harden Jones, 1951; Alexander, 1959; Hallacher, 1974). Teleosts may be either physoclists, having a closed swimbladder, or physostomes, having a swimbladder which is connected to their oesophagus via a duct, allowing uptake and release of gas through the mouth.

Two simple laws of physics affect buoyancy control in species with air-filled swimbladders. Firstly, Pascal's Law states that hydrostatic pressure increases with water depth, such that in the marine environment pressure increases by ~1 atm for every 10 m of depth. Secondly, Boyle's Law states that at constant temperature the volume of a gas is inversely proportional to the pressure, for example when pressure is halved the volume of the gas doubles. As fish ascend through the water column the decrease in hydrostatic pressure causes the swimbladder to increase in volume and the fish to become positively buoyant. Conversely, as fish descend through the water column the increase in hydrostatic pressure causes the swimbladder to decrease in volume and the fish will become negatively buoyant. Physoclist fish secrete gas into the swimbladder in response to negative buoyancy and resorb gas from the swim bladder in response to positive buoyancy but both processes take time (Blaxter & Tytler, 1978; Harden Jones & Scholes, 1985).

At any point in time the volume of the swimbladder determines the depth of neutral buoyancy (Stensholt et al., 2002). Around this depth of neutral buoyancy, there exists a zone through which fish can move freely, termed free vertical range (FVR), compensating for non-neutral buoyancy by swimming (Harden Jones & Scholes, 1985; Stensholt et al., 2002; Strand et al., 2005). FVR is therefore governed by the swimbladder volume, the rate of gas exchange and the pressure under which the fish is exposed (i.e. water depth). It has been hypothesized that physoclist fish approach neutral buoyancy only at the top of their FVR (Davenport, 1999), therefore minimising the risk of an uncontrollable rapid ascent causing the swimbladder gas to expand at a rate faster than it can be resorbed and risking swim bladder rupture. However, the energetic requirements of having to swim in order to maintain position when negatively buoyant must outweigh the risks associated with such a strategy if it is to be useful to the fish. In fact, the position within a fish's FVR that it prefers to occupy will be species-specific and determined by ecologically-driven behavioural patterns (Lea et al., 1999; Parker et al., 2006; Hannah & Matteson, 2007). There exists very little information concerning the FVR of fish, but for Atlantic cod *Gadus morhua* the FVR has been estimated experimentally (Harden Jones & Scholes, 1985) and confirmed via acoustic profiles (Stensholt et al., 2002) to be approximately between a 25% pressure decrease and a 50% pressure increase, from the depth at equilibrium. The FVR in terms of distance therefore increases with increasing pressure as a result of Pascal's Law.

Knowledge of buoyancy control in fish, including the FVR and the rate at which it changes, is important in understanding restrictions to vertical movements and in

developing hypotheses regarding fish behaviour, including diel migrations for activities such as feeding and spawning. Such understanding is of particular importance for species which are important to fisheries, both in terms of knowing when and where fish may be susceptible to different fishing gears, but also in interpreting acoustic survey data to estimate relative abundance (Stensholt et al., 2002). Another emerging area of research that requires information on the rates at which fish are able to adjust swimbladder volume to achieve neutral buoyancy at a given depth (termed acclimation) is that of assessing barotrauma. This is particularly important in manipulative experiments where fish need to be acclimated to certain water pressures to accurately simulate capture from depth. Many of these experiments use custom-built hyperbaric chambers in which fish are acclimated to different pressures before being depressurized to simulate capture and their survival rates quantified (e.g. Gaspin et al., 1978; Harden Jones & Scholes 1985; Rummer & Bennett, 2005; Parker et al., 2006; Pribyl et al., 2009). Clearly, time for complete acclimation needs to be known if experiments are to accurately simulate the effects on a fish of capture from a chosen depth.

Physoclists secrete gas from the blood into the swimbladder through the multitude of parallel capillaries of the *rete mirabile* contained within the gas gland which contacts the swimbladder lumen (Wittenberg et al., 1964). The arterial flow of blood through the counter-current arrangement of the *rete mirabile* multiplies blood gas tensions in the gas gland so that it may exceed that of the arterial blood several-fold and allows gas to be secreted against large pressure gradients into the swimbladder (Strand et al., 2005). Gas is removed from the swimbladder via passive diffusion into the blood through a capillary bed known as the resorption chamber or oval (Ross, 1979). It is important to quantify these rates of gas exchange with the swimbladder of fish in order to understand their buoyancy control and limitations to their vertical distribution in the water column (Holbrook & de Perera, 2010). Unfortunately, there have been relatively few studies that have attempted to quantify the rates of swimbladder gas secretion and resorption and, with the exception of only a couple of species (e.g. cod: Harden Jones & Scholes, 1985; black rockfish *Sebastes melanops* & China rockfish *S. nebulosus*: Parker et al., 2006), this aspect of teleost physiology is largely unknown.

In fact, studies quantifying swimbladder gas exchange rates are virtually non-existent in the literature. The majority of studies have estimated fish acclimation times from observations of fish behaviour whilst pressurized in hyperbaric chambers – fish with positive buoyancy tend to be oriented head-down and to rise when motionless, fish with neutral buoyancy tend to hover with a horizontal orientation, and fish with negative buoyancy tend to have a head-up orientation and sink when motionless (Gaspin et al., 1978; Harden Jones & Scholes, 1985; Rummer & Bennett, 2005; Parker et al., 2006; Pribyl et al., 2009). The resulting acclimation rates are given in units of pressure per time (e.g. atm/h – Parker et al., 2006) or simply as days to acclimate to a particular depth (e.g. Gaspin et al., 1978). Wittenberg et al. (1964) used a syringe to extract all gas from the swimbladders of a range of species and reported the time taken in hours for the swimbladders to fully re-inflate. However, fish size is related to gas secretion rate as bigger fish have a larger gas gland surface area. Gas secretion rates are more usefully reported as a mass-specific rate (Harden Jones & Scholes, 1985). In addition, one of the most comprehensive studies on swimbladder gas exchange rates (Harden Jones & Scholes, 1985), and the only one to report actual gas exchange rates in a marine teleost, may have misreported these exchange rates because calculations were based on the volume of the swimbladder in Atlantic cod being 5% of the total fish volume when at equilibrium.

However, Davenport (1999) has since shown this to be inaccurate and that in *G. morhua* the swimbladder occupies on average <4% of fish volume.

So, while several studies have estimated acclimation rates of various species to certain water pressures, recognizing that it is acclimation rates that are generally needed, none have directly measured changes in swimbladder gas volume under different water pressures and times to estimate actual rates of swimbladder gas secretion and resorption. Here, we therefore describe a technique that directly measures changes to swimbladder volumes of both snapper and mulloway that enables calculation of actual gas exchange rates with the swimbladder for each. These gas exchange rates may then be used in designing future hyperbaric chamber experiments that require fish to become acclimated to known water pressures or depths.

2.2. Materials & methods

2.2.1. Experimental fish collection

Snapper used in these experiments were captured using hook and line from shallow (<10 m) water within the Port Hacking estuary (34º04'24"S, 151º07'43"E) during 2010. They were taken from shallow water to minimize any barotrauma injuries and maintained in a 35,000 L flow-through aquarium for between 6 and 9 months prior to being used.

Mulloway used in these experiments were purchased from an aquaculture facility (Clearwater Marine Farms, Pty Ltd) to ensure that they had no history of barotrauma. They were maintained in a 1 million L flow-through seawater pond for between 6 and 9 months prior to being used. The fish averaged 45.2 cm total length (TL) (range: 41.1-51.5 cm) and were considered an ideal size because mulloway must be legally released if <45 cm TL (their minimum legal length - MLL).

2.2.2. Swimbladder size & fish buoyancy

The relationships between swimbladder volume and various fish metrics were estimated using the experimental fish which were housed at sea level. Fish were euthanized using a lethal dose of AQUI-S anaesthetic (Aqui-S New Zealand, Ltd) before being measured (fork length – FL for snapper and TL for mulloway) to the nearest mm, patted dry with absorbent paper and weighed to the nearest 0.1 g. Each fish's volume was estimated by weighing the amount of seawater displaced when the fish was fully immersed and dividing this value by the density of seawater (1.026 g/ml). Fish density was calculated as body weight/fish volume. The volume of gas within the swimbladder was extracted using an 18 gauge hypodermic needle and syringe.

The increase in swimbladder gas volume that occurs before the swimbladder ruptures was investigated for snapper. Snapper were euthanased, patted dry with absorbent paper and weighed to the nearest 0.1 g. The body wall of each fish was then cut away using scissors to expose the swimbladder. A syringe with 18 gauge hypodermic needle was inserted through the side of the fish into the swimbladder and air forced in using pressure on the syringe plunger. The quantity of air inserted before rupture was recorded. The relationship

between swimbladder volume and fish weight determined above was used to estimate the amount of gas already in the swimbladder and the relative increase in pressure before rupture calculated from these data.

The decrease in water pressure before swimbladder rupture occurred in mulloway was investigated using the hyperbaric chambers (described below) rather than by forcing air into the swimbladddder using a needle and syringe because the mulloway swimbladder was found to be so flexible and strong that it was impossible to force in sufficient air to cause rupture - the air continually escaped through the small hole made by the needle. Four mulloway were therefore acclimated to each of 10, 15, 20 and 25 m water depths (using the gas secretion rates calculated below), before being depressurized at a rate of 1 m/s to sea level. The fish were euthanased as described above and dissected to examine whether swimbladder rupture had occurred.

2.2.3. Gas secretion rates

Swimbladder gas secretion rates were estimated using two custom-built hyperbaric chambers. The chambers consisted of a 1000 L-capacity fibreglass pool filter (SMD1050; Waterco, Ltd), with the internal plumbing modified (Fig. 2.1). The chambers were capable of simulating depths of up to 70 m (8.08 bar). Seawater was pumped into the chamber from a flow-through seawater pond using submersible borehole pumps (J725D-3; Davey Water Products, Pty Ltd) capable of pumping water at a flow rate of 40.0-22.5 L/min under pressures of 0-8 bar, respectively. Internal pressure in the chambers was controlled by a diaphragm valve (50 mm PVC; GEMÜ Valves, Inc.) which restricted the flow of water out of the chamber. Internal pressure was measured using a digital pressure gauge (XP2i-AX; Crystal Engineering Corp.).

Manipulative experiments were done in these chambers to quantify the amount of gas secreted into the swimbladders of snapper and mulloway at different water temperatures, water pressures and exposure times. The experimental procedure used was based on the understanding that under increased pressure, and subsequent reduction in swimbladder volume resulting from Boyle's Law, fish will secrete gas into their swimbladders until it occupies the same volume as when at equilibrium. Fish were placed in the hyperbaric chambers and the pressure increased to the desired level at a rate of ~1 m/s.

Snapper were exposed to two different pressures: 2 and 3 atm (equivalent to 10 and 20 m water depth, respectively). Duration at pressure varied between one and 4 h before the pressure was decreased to sea level (1 atm) a rate of approximately 1 m/s, the fish were then euthanased using a lethal dose of anaesthetic as described above, and removed from the chamber. The fish were immediately measured (FL or TL), patted dry, weighed and the volume of gas within the swimbladder extracted using a hypodermic needle and syringe as described above. This was done for 10 fish during winter (at water temperatures of 15-16 °C) and 11 fish in early summer (at water temperatures of 19-20 °C).

Mulloway were exposed to three different pressures: 2, 3 and 4 atm (equivalent to the pressures at 10 20 and 30 m water depth, respectively). Duration at pressure varied between 2 and 22 h before the pressure was decreased to sea level and the fish treated in the same manner as described above for snapper. This was done for 8 fish during spring (at

water temperatures of 15-17 °C) and 12 fish during late summer (at water temperatures of 20-23 °C).

The quantity of swimbladder gas within an individual fish at sea level equilibrium was estimated using the fish weight - swimbladder gas relationships determined for each species (see above). The quantity of gas secreted during the experiment was calculated as the volume of gas extracted using the syringe following time at pressure in the chamber, adjusted for its expansion between the pressure at which it was secreted and sea level, minus the estimated volume of gas at sea level equilibrium. Gas secretion rates were reported as ml/kg fish body weight/min (Harden Jones & Scholes, 1985).

A linear model (LM) was used to test the effect of water pressure, water temperature and time at pressure, and their interaction, on gas secretion rate (ml/kg/min).

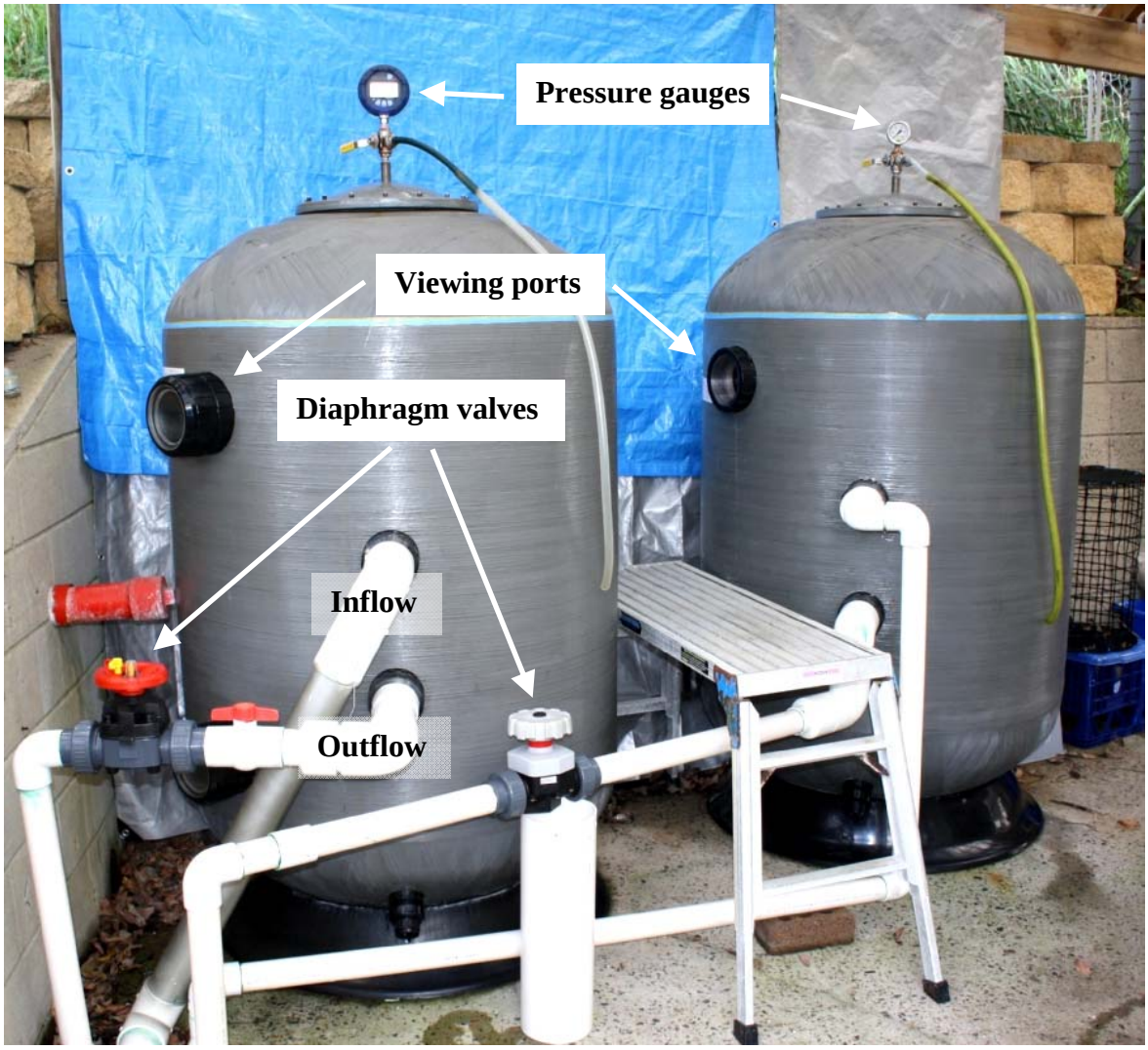
The model was:

Gas secretion rate = a + b*water pressure$_i$ + c*water temperature$_i$ + d*time$_i$ + water pressure*water temperature + water pressure*time + water temperature*time + water pressure*water temperature*time + ϵ_i

Where a to d are constants.

The model was calculated using the freeware statistical package "R" (R Development Core Team, 2006). The significance of each variable to the model was tested using the null hypothesis that they were significantly different from 0 using partial *z*-tests.

Figure 2.1. The twin custom-built hyperbaric chamber setup at the Cronulla Fisheries Research Centre of Excellence.



2.2.4. Gas resorption rates

Swimbladder gas resorption rates were estimated using the hyperbaric chamber described above. Manipulative experiments were done to quantify the amount of gas resorbed from the swimbladders of snapper and mulloway at different water temperatures and water pressures. Resorption rates are reported as ml/kg fish body weight/min (Harden Jones and Scholes, 1985).

The experimental procedure used was to place individual fish in the chamber and increase the pressure to a desired level (P1) at a rate of ~1 m/s. The fish were maintained at P1 for a period that allowed them to secrete sufficient gas into their swimbladders to achieve neutral buoyancy. These periods were determined using the rates of gas secretion estimated during this study. Following acclimation, the pressure in the chamber was decreased to a level whereby the fish were observed to be slightly positively buoyant so that they slowly rose through the water column when stationary (P2). The fish were maintained at P2 for a period estimated to be shorter than the period necessary to resorb all

excessive gas from the swimbladder based on the understanding that swimbladder gas resorbs substantially (up to 5 times) faster than it is secreted (Parker et al., 2006). The chamber pressure was then decreased to sea level (1 atm) at a rate of ~1 m/s, the fish were then euthanased using a lethal dose of anaesthetic as described above, and removed in the chamber. The fish were immediately measured (FL or TL), patted dry, weighed and the volume of gas within the swimbladder extracted using a hypodermic needle and syringe as described above.

The volume of gas within an individual fish's swimbladder at equilibrium was estimated using the fish weight - swimbladder gas volume relationships determined above for each species. This volume was then adjusted according to Boyle's Law to estimate the volume it would occupy at sea level if changed from P1. The volume (at sea level) of gas resorbed was then calculated from the difference between this estimate and the quantity extracted using the syringe, adjusted for the pressure at which it was resorbed according to the volume it would have occupied at P2, again using Boyle's Law.

Snapper were acclimated to pressure between 2 and 2.3 atm (equivalent to 10 & 13 m water depth, respectively). Our estimates of gas secretion rates suggested that snapper would acclimate from sea level to pressure equivalent to a depth of 10 m after 14 h on average and ~16 h using the mean secretion rate minus 1 SE. The fish were subsequently left in the chamber at P1 for between 18 and 22 h before being depressurized to P2. P2 was either 1.25 or 1.5 atm (equivalent to 2.5 or 5 m water depth, respectively). Snapper were maintained at P2 for between 30 and 40 min before being depressurized to sea level at a rate of ~1 m/s. This was done for 12 snapper during winter (at water temperatures of 15-16 °C) and 7 snapper during summer (at water temperatures of 19-20 °C).

Mulloway were acclimated to pressure between 2 and 2.25 atm (equivalent to the pressures at 10 and 12.5 m water depth, respectively). Our estimates of gas secretion rates suggested that mulloway would acclimate from sea level to pressure equivalent to a depth of 10 m after 46 h on average and ~53 h using the mean secretion rate minus 1 SE. The relatively protracted time needed to acclimate mulloway meant that it was possible to only do 1 run per week, and so to ensure that the mulloway were fully acclimated they were left in the chamber at P1 for at least 7 d (190-209 h) before being depressurized to P2. P2 was either 1.3 or 1.6 atm (equivalent to 3 or 6 m water depth, respectively). The mulloway were maintained at P2 for between 4 and 6 h before being depressurized to sea level. This was done for 8 mulloway during winter (at a water temperature of 18 °C) and 7 mulloway during summer (at water temperatures of 22-23 °C).

A LM was used to test the effect of water pressure, water temperature, and their interaction, on gas resorption rate (mls/kg/min).

The model was:

Gas resorption rate = a + b*water pressure$_i$ + c*water temperature$_i$ + water pressure* water temperature + ϵ_i

Where a to c are constants.

2.3. Results

2.3.1. Swimbladder size & fish buoyancy

2.3.1.1. Snapper

The relationship between fish volume and body weight for snapper (Table 2.1) was linear:

Fish volume = 0.97 × body weight + 0.85, $r^2 = 0.997$.

The linear relationship between swimbladder gas volume and body weight (Gas volume = 0.05 × body weight – 0.05, $r^2 = 0.975$) was a better fit than the linear relationship with fish volume (Gas volume = 0.03 × fish volume + 1.32, $r^2 = 0.890$).

The mean (± SE) percent of fish volume that was swimbladder gas was 4.2 ± 0.1%.

The mean (± SD) density of snapper (Table 2.1) of 1.025 ± 0.02 g/ml was not significantly different to the density of seawater (1.026 g/ml) (one sample t-test, $p = 0.79$).

Table 2.1. Data collected on fish length, body weight, fish volume, swimbladder gas volume and fish density for snapper.

Fork length (mm)	Body weight (g)	Fish volume (ml)	Swimbladder gas (ml)	Fish density (g/ml)
149	86	83.7232		1.02719
160	107.5	101.682		1.05721
162	111.9	108.382	4.8	1.03246
160	112.4	113.158	5.6	0.9933
165	120.1	122.222	5	0.98264
170	132.7	131.897		1.00609
174	142	134.503	5	1.05574
178	139.2	136.38		1.02068
181	149.2	143.226	6.5	1.04171
180	156.3	159.552		0.97962
191	168.9	164.815		1.02479
195	199.9	192.885	8.9	1.03637
197	215.8	201.267	7.9	1.07221
210	231.2	222.807		1.03767
202	232.3	223.977	10.1	1.03716
211	232.4	229.142		1.01422
211	234.8	230.994		1.01648
205	246.3	241.131	8.9	1.02144
220	247.5	244.029		1.01422
214	252.8	246.08		1.02731
220	274.3	266.01		1.03117
221	283.9	280.312	10.2	1.0128
227	306.6	298.538		1.027
250	408		17.5	
299	680.7		32	

Fork length (mm)	Body weight (g)	Fish volume (ml)	Swimbladder gas (ml)	Fish density (g/ml)
298	721.8		29	
308	787.1		34	
133	55		3.4	
155	92.28		5.8	
123	54.78		2.6	
125	50.96		2.9	
141	78.49		4.2	
139	76.25		4.2	
142	83.68		5.6	
146	79.85		4.9	
108	33.26			
125	47.21			
183	150.1		6.75	
281	561		28	
310	794		39	
301	681.5		36.1	

2.3.1.2. Mulloway

The relationship between fish volume and body weight for mulloway (Table 2.2) was linear:

Fish volume = 0.94 × body weight + 19.78, $r^2 = 0.999$.

The linear relationship between swimbladder gas volume and body weight (Gas volume = 0.04 × body weight + 2.79, $r^2 = 0.973$) was a similar fit to the linear relationship with fish volume (Gas volume = 0.04 × fish volume + 1.23, $r^2 = 0.973$).

The mean (± SE) percent of fish volume that was swimbladder gas was 4.9 ± 0.1%.

The mean (± SD) density of mulloway (Table 2.2) of 1.039 ± 0.009 g/ml was significantly higher than the density of seawater (1.026 g/ml) (one sample t-test, $p < 0.001$).

Table 2.2. Data collected on fish length, body weight, fish volume, swimbladder gas volume and fish density for mulloway.

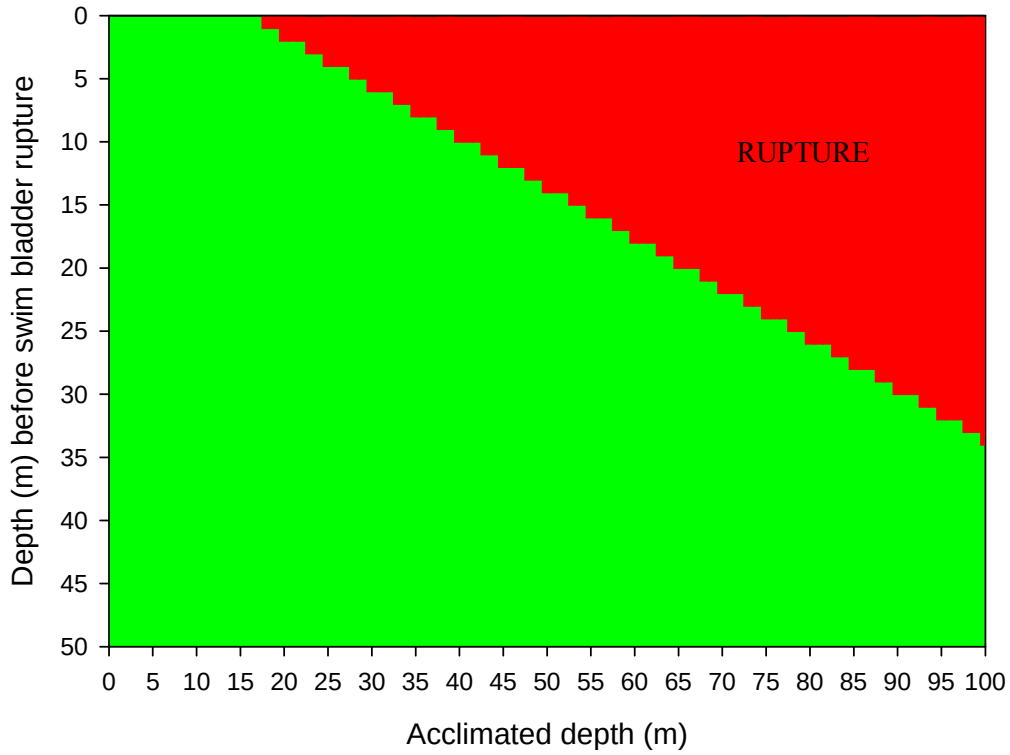
Total length (mm)	Body weight (g)	Fish volume (ml)	Swimbladder gas (ml)	Fish density (g/ml)
394	612.1		29.6	
406	648.8	627.78	32.2	1.03349
407	658.7	633.24	28	1.04021
411	712.9	677.00		1.05303
413	671.7	649.81		1.03369
417	761.2	738.99		1.03006
420	691.6	667.93	28.5	1.03543
420	751	721.35		1.04111
425	744.8	719.49		1.03517
430	855.1	841.13		1.01661

Total length (mm)	Body weight (g)	Fish volume (ml)	Swimbladder gas (ml)	Fish density (g/ml)
435	826.3	794.15		1.04048
435	857.8	826.61	40	1.03773
439	790.4	759.36	32	1.04088
440	812.9	777.97	37	1.04490
440	814.3	794.87	38	1.02445
443	868.1	841.52	36	1.03159
454	822.8	795.91	33.5	1.03379
454	942.9			
461	924.3	896.20		1.03136
463	955.8	926.41		1.03172
473	1122.4	1079.82	44	1.03943
478	1044.4			
480	1142.3	1094.25		1.04391
483	1045.5			
483	1085.3	1045.81		1.03776
485	1166.4			
485	1166.4			
490	1228.8			
499	1163.3	1123.20	48	1.03570
500	1333.85	1288.89	58	1.03488
504	1239.5			
505	1291.2			
515	1450.1	1404.78		1.03226
516	1352.2			
516	1352.2			
519	1504.3		60	
532	1553.8			
547	1549.8			
548	1658.9	1569.40	71	1.05703
552	1682.1			
555	1715.8	1615.01	74	1.06241

2.3.2. Change in pressure before swimbladder rupture

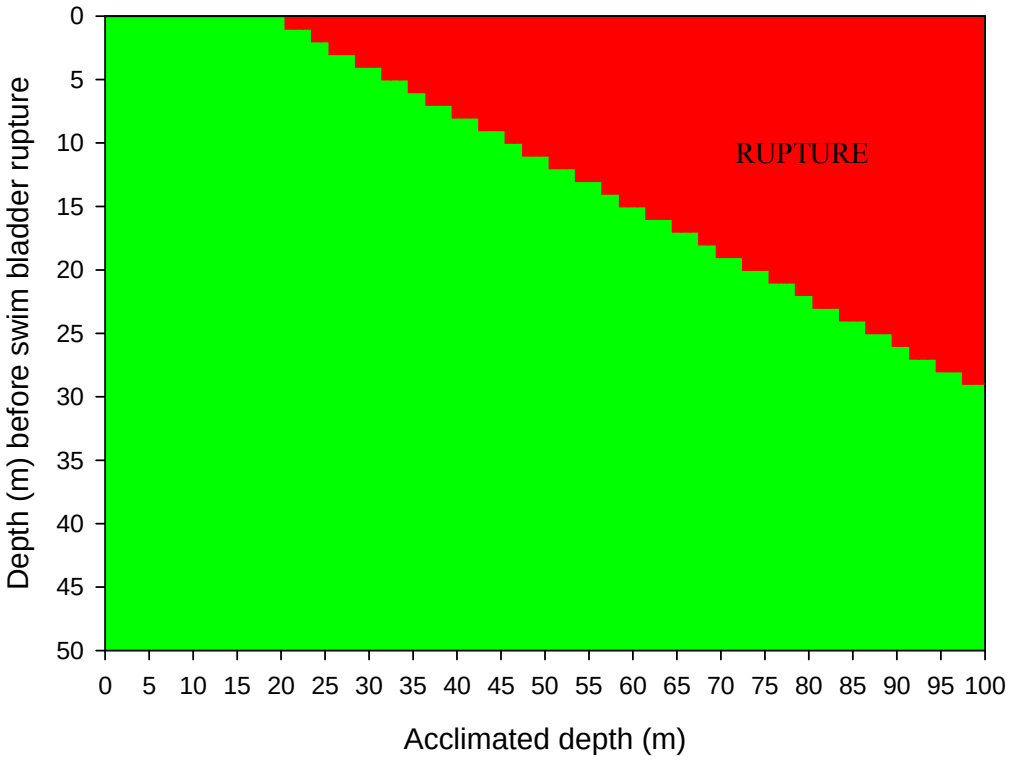
Twelve snapper were used to estimate the increase in pressure required before swimbladder rupture. The average (± SE) increase in pressure before the swimbladder ruptured was 2.49 (± 0.14) times the pressure at equilibrium. This change in pressure indicates that any fish caught from deeper than 14.9 m water depth will suffer a ruptured swimbladder if brought directly to the surface. The vertical range in terms of meters before swim bladder rupture occurs increases with increasing depth at equilibrium (Fig. 2.2). This is due to the decline in relative pressure increase with depth. As an example, a snapper could potentially ascend from 30 m water depth to 7 m depth before swimbladder rupture - a vertical distance of 23 m; however a snapper at 100 m water depth could ascend to a depth of 35 m before swimbladder rupture - a vertical distance of 65 m (Fig. 2.2).

Figure 2.2. Vertical ascent range for snapper before swimbladder rupture.



None of the mulloway acclimated to pressure equivalent to either 10 or 15 m water depth ruptured their swimbladders when depressurized to sea level in the hyperbaric chambers. However, all mulloway acclimated to pressures equivalent to 20 and 25 m water depth ruptured their swimbladders. The average increase in pressure before the swimbladder ruptured was therefore 2.75 times the pressure at equilibrium (equivalent to the pressure change from 17.5 m water depth to the surface). Consequently it is estimated that a mulloway at 30 m water depth could potentially ascend to 4 m before swimbladder rupture occurred - a vertical distance of 26 m; however a mulloway at 100 m water depth could ascend to a depth of 29 m before swimbladder rupture - a vertical distance of 71 m (Fig. 2.3).

Figure 2.3. Vertical ascent range for mulloway before swimbladder rupture.



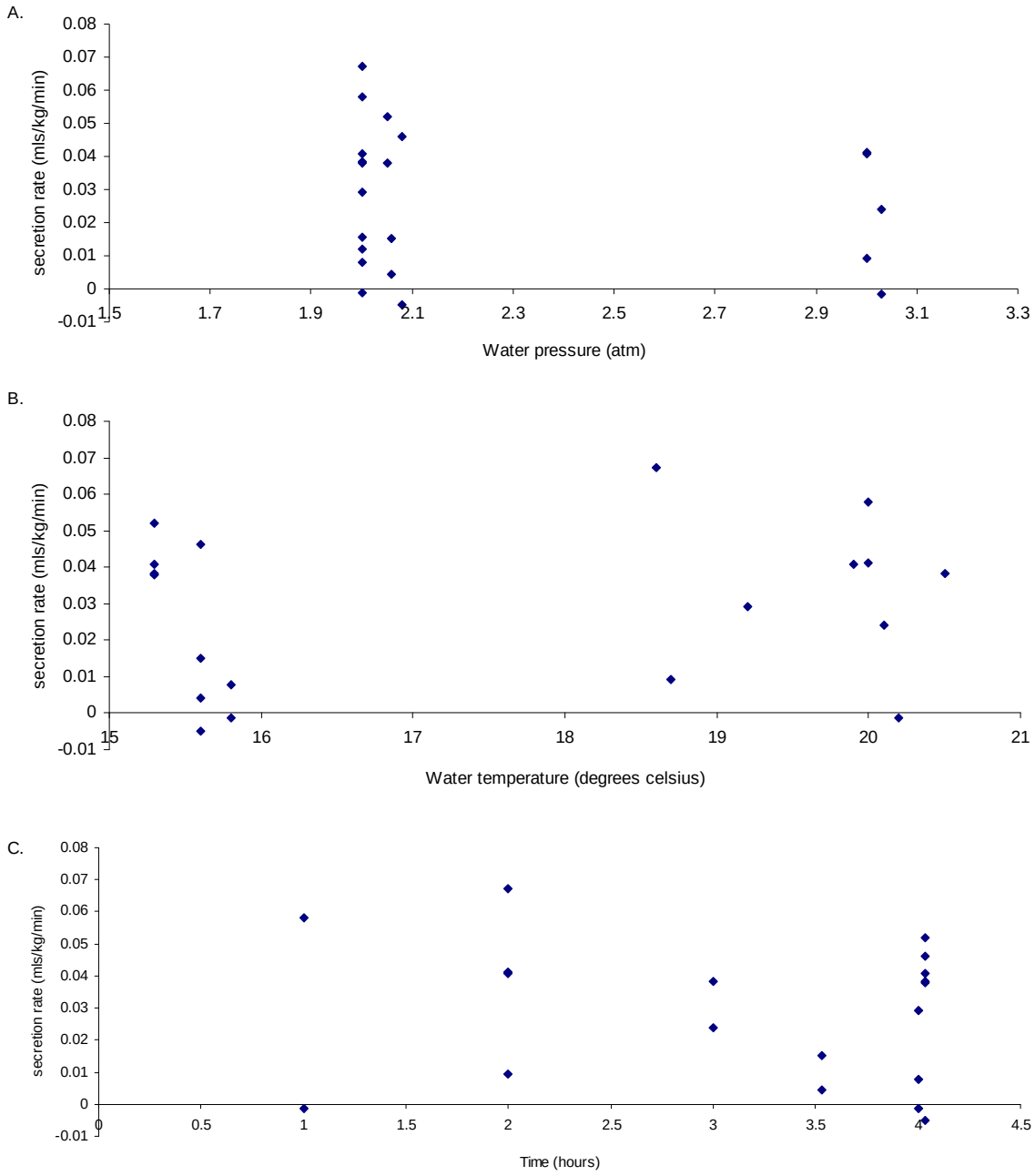
2.3.3. Gas secretion rates

2.3.3.1. Snapper

The rate of gas secretion into the swimbladders of snapper was not significantly affected by water pressure, water temperature, time at pressure or interactions between these factors ($p > 0.05$) (Fig. 2.4).

The mean (± SE) rate of snapper swimbladder gas secretion was therefore calculated to be 0.027 (± 0.005) ml/kg/min.

Figure 2.4. Relationships between snapper swimbladder gas secretion rate and **A.** water pressure; **B.** water temperature and; **C.** time.

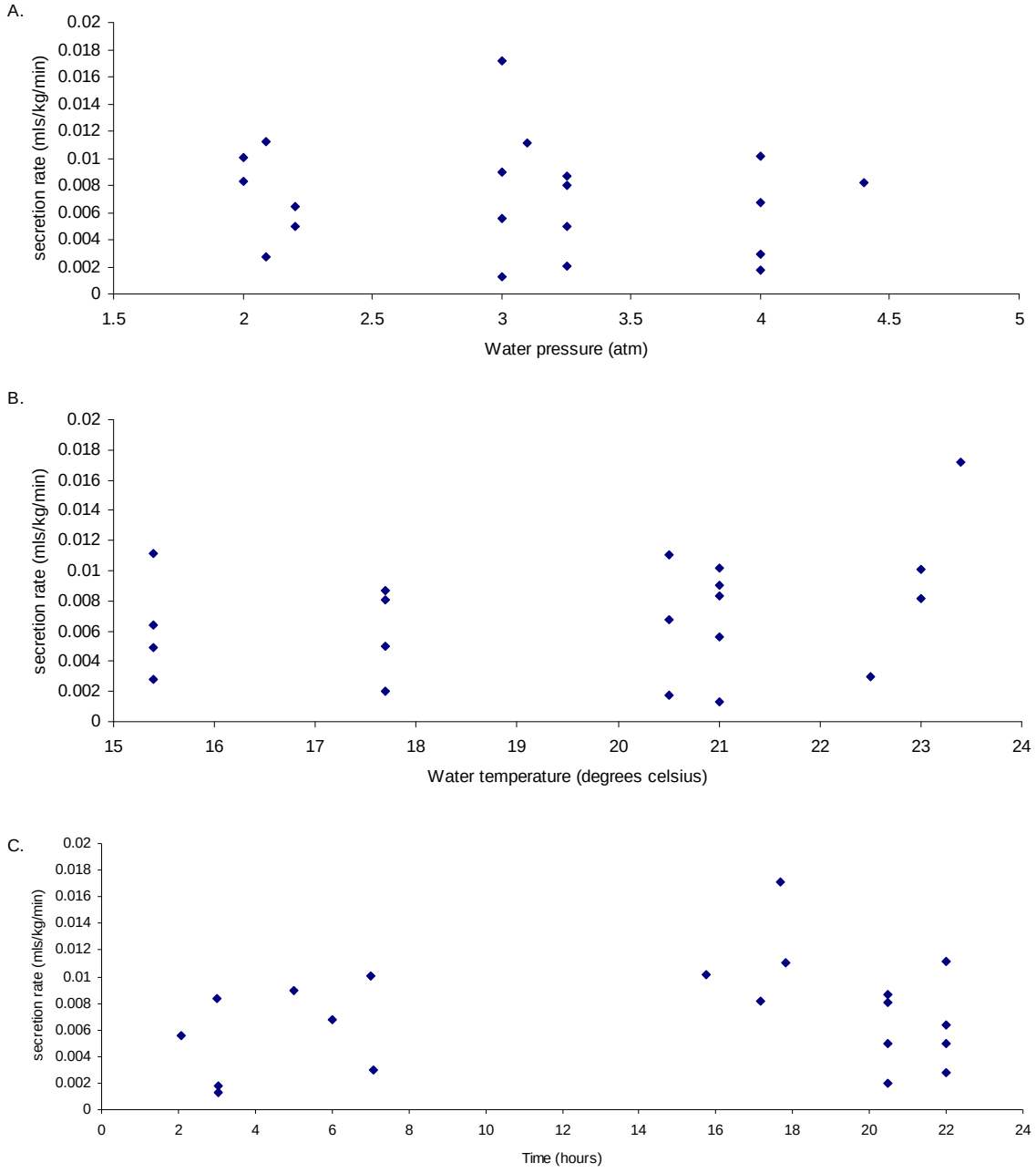


2.3.3.2. Mulloway

Similarly, the rate of gas secretion into the swimbladders of mulloway was also not significantly affected by water pressure, water temperature, time or interactions between these factors ($p > 0.05$) (Fig. 2.5).

The mean (± SE) rate of mulloway swimbladder gas secretion was therefore calculated to be 0.007 (± 0.001) ml/kg/min.

Figure 2.5. Relationships between mulloway swimbladder gas secretion rate and **A.** water pressure; **B.** water temperature and; **C.** time.



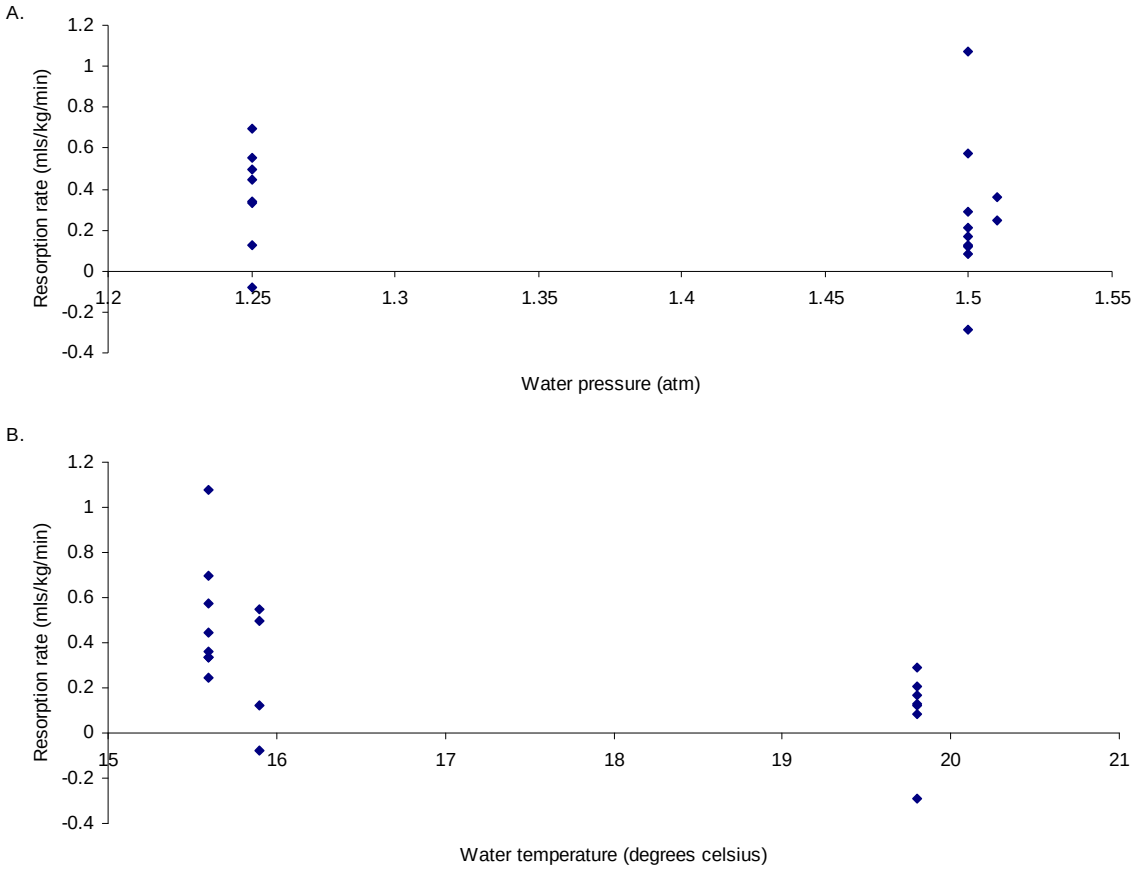
2.3.4. Gas resorption rates

2.3.4.1. Snapper

The rate of gas resorption from the swimbladders of snapper was not significantly affected by water pressure, water temperature or interactions between these factors in the full model ($p > 0.05$) (Fig. 2.6).

The mean (± SE) rate of snapper swimbladder gas resorption was therefore calculated to be 0.309 (± 0.069) ml/kg/min.

Figure 2.6. Relationships between snapper swimbladder gas resorption rate and **A.** water pressure and; **B.** water temperature.

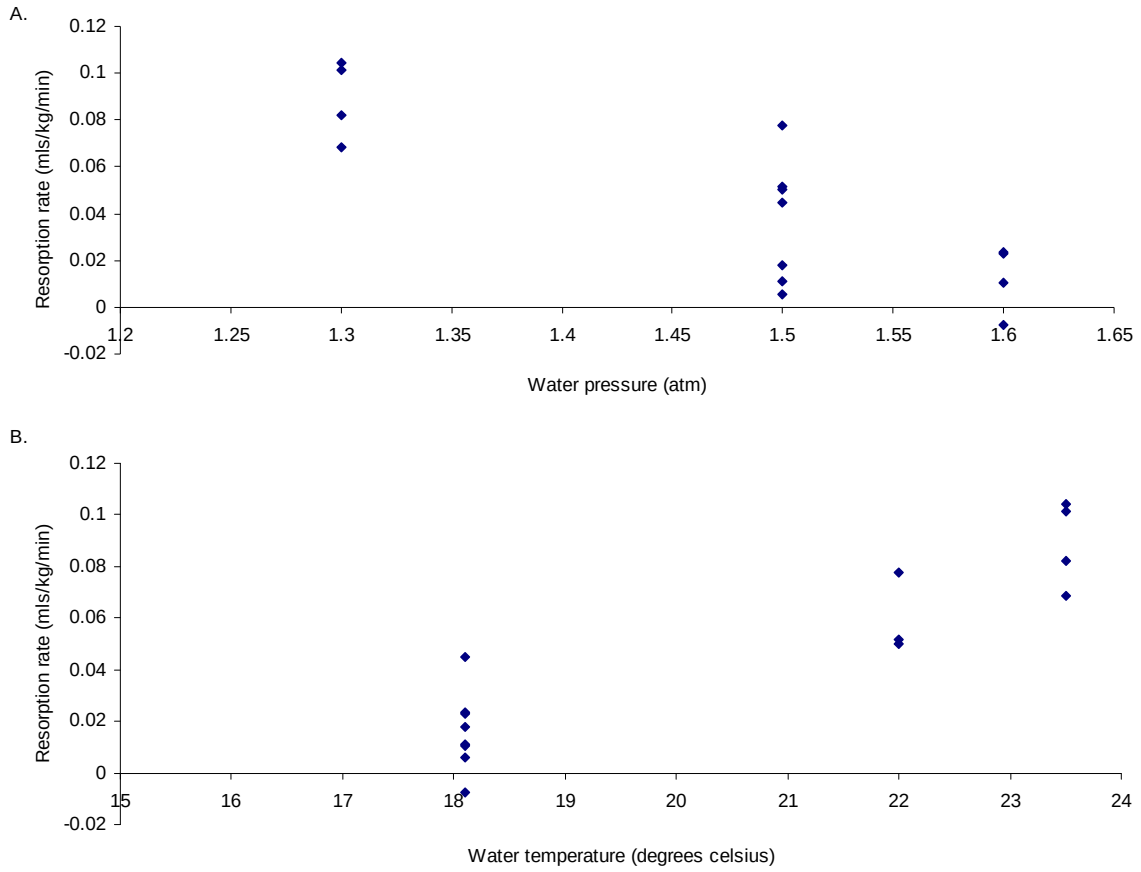


2.3.4.2. Mulloway

Similarly, the rate of gas resorption from the swimbladders of mulloway was also not significantly affected by water pressure, water temperature or interactions between these factors in the full model ($p > 0.05$) (Fig. 2.7).

The mean (± SE) rate of mulloway swimbladder gas resorption was therefore calculated to be 0.044 (± 0.009) ml/kg/min.

Figure 2.7. Relationships between mulloway swimbladder gas resorption rate and **A.** water pressure and; **B.** water temperature.



2.4. Discussion

2.4.1. Swimbladder size & fish buoyancy

Snapper have a swimbladder volume that is on average 4.2% of the total volume of the fish. In contrast, relative to body size mulloway have a larger swimbladder, averaging 4.9% of their total body volume. This aligns with the general approximation that in marine teleosts the swim bladder occupies ~4-5% of the volume of the fish (Harden Jones and Scholes, 1985), however, the relative volume of the swim bladder to total fish volume in marine teleosts is species-specific and can vary from ~1% (Harden Jones, 1951) to >5% (Parker et al., 2006). If the main function of the swimbladder is to provide buoyancy, then its volume relative to total fish volume will depend on the overall density of fish's tissue and the buoyancy required for normal energy-efficient behaviour. There are some advantages to having a relatively small swimbladder, including a lower rate of change in buoyancy with change in depth, and also generates a smaller acoustic signal and so lower delectability by predators which use echolocation to hunt (e.g. dolphins; Davenport, 1999). These advantages are weighed against a greater energy cost in maintaining position when deeper in the water column whenever negatively buoyant (Alexander, 1990; Strand et al., 2005) as fish are forced maintain position by generating hydrodynamic lift (upwards or downwards) using their fins and by tilting their bodies (Blake, 1979). The density of

snapper was not significantly different to the density of seawater, indicating that snapper at equilibrium are neutrally buoyant. In contrast, mulloway were denser than seawater and are therefore negatively buoyant at equilibrium. Given the greater relative volume of the swimbladder in mulloway, the mulloway tissue must be denser than that of snapper. The difference in buoyancy between pink snapper and mulloway may therefore be attributable to their differing behaviours and mechanisms for maintaining their preferred position in the water column (e.g. Jarvis & Lowe, 2008). The energetic costs to pink snapper of maintaining position in the water column will be less than those for mulloway; however the advantage to being negatively buoyant for mulloway likely relates to the increased behavioural choice of the position a mulloway can occupy in the water column that an increased FVR provides for a negatively buoyant mulloway compared to when neutrally buoyant.

2.4.2. Swimbladder gas secretion

The rate of swimbladder gas secretion for snapper (0.027 ± 0.005 ml/kg/min) was nearly 4 times faster than that for mulloway (0.007 ± 0.001 ml/kg/min). Swimbladder gas secretion rate was not significantly affected by the water pressure, water temperature or times at pressure experienced by either species in the current study. The finding that gas secretion rates did not vary with either water pressure or time at pressure suggests that gas is secreted at a constant rate whenever a certain level of pressure differential exists between the gas gland and the swimbladder. Harden Jones & Scholes (1985) reported a weak positive relationship ($p < 0.01$) between gas secretion rate and water pressure for Atlantic cod *Gadus morhua*, whereas Strand et al. (2005) found "filling the swim bladder to be radically slower at greater depths". Rummer and Bennett (2005) reported acclimation rates of 68, 98 and 174 hours to depths of 30, 50 and 110 m in hyperbaric chambers for red snapper *Lutjanus campechanus*. Using Boyle's law, these acclimation rates equate to ~ 91 hours to refill the swim bladder at 30 m, 118 hours to refill the swim bladder at 50 m and 189 hours to refill the swim bladder at 110 m, suggesting gas secretion (in terms of ml/h) slowed markedly with increasing pressure. Rummer & Bennett (2005) did not quantify gas secretion rates directly; rather they used observations of fish behaviour to estimate when they were fully acclimated. It is clear that more work is needed to understand the process of swimbladder gas secretion and whether it varies with water pressure.

Swimbladder gas secretion is known to be an active physiological process (Parker et al., 2006) and is therefore likely to vary with metabolic rate. It would seem logical then that swimbladder gas secretion rates would be positively related to temperature and Harden Jones & Scholes (1985) presented strong evidence to suggest this to be the case for *G. morhua*. It is possible that the temperature ranges over which we did our experiments (~5-6°C) were too narrow to allow us to detect any significant differences given the inherent variability between individual fish and measurement error. Harden Jones & Scholes (1985) detected an effect of temperature over a much greater temperature range of ~18°C. In addition, McNabb & Mecham (1971) also found that the swimbladders of the freshwater sunfish *Lepomus macrochirus* inflated faster at higher temperatures

Our results confirm swimbladder gas secretion to be a slow process in marine teleosts (Strand et al., 2005). Being one of the few studies to quantify actual rates of swimbladder gas secretion, we were able to estimate acclimation rates for fish moving between different depths (Table 2.3). We are also able to compare the acclimation rates of snapper and

mulloway with several other species based on observations of their behaviour in hyperbaric chambers and also time taken to refill swimbladders when manually emptied of gas (Table 5). We estimated that a mulloway of 40 cm would take ~99 h to refill an empty swimbladder and a snapper of 35 cm ~27 h. Wittenberg et al. (1964) used syringes to empty the swim bladders of 10 species of physoclist fish and reported the time taken to refill the swim bladder. Tytler & Baxter (1973), Gaspin et al. (1978), Harden Jones & Scholes (1985), Rummer & Bennett (2005) and Parker et al. (2006) all reported observed times for different species to appear acclimated to various water pressures within hyperbaric chambers. We used Boyle's Law to convert these rates into times needed to completely refill empty swimbladders for comparison between species (Table 5). This shows that swimbladder gas secretion rates are hugely variable, even between related species. While much of the variation reported may be due to the subjective nature of determining when a fish appears to behave as if fully acclimated within hyperbaric chambers, the study of Parker et al. (2006) showed the two species of rockfish (genus *Sebastes*) studied to have extremely different gas secretion rates, and they were able to demonstrate that this was related to their behaviour and vertical movements within the water column. Likewise Wittenberg et al. (1964) related the extremely rapid gas secretion rate in bluefish *Pomatomus saltatrix* to the species' habit of rapidly moving between the sea floor and the surface when feeding. The finding that snapper are able to secrete gas into their swimbladders four times faster than mulloway is therefore likely to enable them to move more quickly and efficiently into deeper waters.

Table 2.3. Estimated times for mulloway and snapper to acclimate to different water depths from sea level.

Water depth (m)	Mulloway Time to acclimate (h)	Snapper Time to acclimate (h)
10	47	13
20	62	18
30	70	20
40	75	22
50	78	22
60	80	23
70	82	23
80	83	24
90	84	24
100	85	24

Table 2.4. Estimated times for mulloway and snapper to acclimate to sea level from different water depths.

Water depth (m)	Mulloway Time to acclimate (h)	Snapper Time to acclimate (h)
0	150	23.6
10	67	10.6
20	40	6.3
30	26	4.1
40	18	2.8
50	12	2
60	9	1.4
70	6	10.8
80	3	0.5
90	2	0.2
100	0	0

Table 2.5. Comparative acclimation times for various marine teleosts. Acclimation rates are presented as the time needed to secrete sufficient gas to fully inflate the swimbladder.

Species name	Common name	Approximate time to refill empty swimbladder (h)	Reference
Pomatomus saltatrix	Bluefish	4	Wittenberg et al, 1964
Lagodon rhomboides	Pinfish	4 - 9	McCutcheon, 1962
Gadhus morhua	Atlantic cod	10	Scholander et al., 1956
Stenotomus versicolor	Scup	10 - 12	Wittenberg, 1958
Anguilla sp.	Eels	10 - 18	Wittenberg, 1958
Gadus morhua	Atlantic cod	20	Harden Jones & Scholes, 1985
Opsanus tau	Oyster toadfish	18 - 24	Wittenberg, 1958
Tautoga sp.	Tautog	24	Wittenberg, 1958
Pagrus auratus	**Snapper**	**27**	**Present study**
Sebastes melanops	Black rockfish	44	Parker et al., 2006
Pollachius virens	Saithe	48	Tytler & Blaxter, 1973
Prionotus carolinus	Common searobin	48	Wittenberg, 1958
Prionotus evolans	Striped searobin	48	Wittenberg, 1958
Fundulus heteroclitus	Mummichog	48	Copeland, 1952
Lutjanus campechanus	Red snapper	91	Rummer & Bennett, 2005
Sciaenidae	Croaker	93	Gaspin et al., 1976
Argyrosomus japonicus	**Mulloway**	**99**	**Present study**
Tetraodontidae	Toadfish	150	Gaspin et al., 1976
Leiostomus xanthurus	Spot Croaker	183	Gaspin et al., 1976
Sebastes nebulosus	China rockfish	236	Parker et al., 2006
Morone saxatilis	Striped bass	240	Gaspin et al., 1976
Fundulus heteroclitus	Mummichog	244	Gaspin et al., 1976
Fundulus majalis	Striped killifish	266	Gaspin et al., 1976
Morone americana	White perch	312	Gaspin et al., 1976

2.4.3. Swimbladder gas resorption

Very few studies have attempted to quantify the rate of swimbladder gas resorption. We found that the rate of swimbladder gas resorption for pink snapper (0.309 ± 0.069 ml/kg/min) was ~7 times faster than that for mulloway (0.044 ± 0.009 ml/kg/min). These rates of gas resorption were also ~11 and 6 times faster than the rates of gas secretion in each species, respectively. Both species are therefore able to maintain near-neutral buoyancy whilst ascending through the water column much faster than when descending (Table 2.4). The swimbladder gas resorption rates were not significantly affected by the water pressures or water temperatures experienced by either species in the current study. Swimbladder gas is removed via passive diffusion into the blood through the resorption chamber (Ross, 1979) and there are, therefore, no obvious reasons why such passive diffusion would be substantially affected by temperature or water pressure. However, in one of the only studies to attempt to quantify swimbladder gas resorption rates, Harden Jones & Scholes (1985) reported much faster resorption rates with increased pressure in *G. morhua*. An examination of these results however, shows that these authors did not account for the proportional increases in volume of swimbladder gas being equal to the proportional reductions in water pressure, regardless of the water pressure to which the fish were acclimated. In addition, Figure 3 (Buoyancy adjustment when subject to 25% reductions in pressure every 30 min) presented in Harden Jones & Scholes (1985) does not show any difference in acclimation times with equal decreases in pressure.

The only other study we found that attempted to quantify rates of swimbladder gas resorption (Parker et al., 2006) did not examine effects of water temperature or pressure. Their results were consistent with ours, and also those of Harden Jones & Scholes (1985) in that swimbladder gas resorption in two rockfish species (*Sebastes* spp.) were substantially (up to ~5 times) faster than the rate of gas secretion.

2.4.4. Implications for vertical range

The findings that mulloway and pink snapper have different densities when at equilibrium and very different rates of swimbladder gas exchange suggest that they utilize the water column in different ways. Snapper most often occur in pelagic schools up off the seafloor with schooling occasionally occurring at the surface (Kailola et al., 1993). The energetic costs of maintaining position in the water column is therefore reduced by the neutral buoyancy of the semi-pelagic snapper. In contrast, the more negatively buoyant mulloway are most often found in close proximity to the bottom (Kailola et al., 1993). Pink snapper also possessed much faster rates of swim bladder gas exchange and would therefore be able to more quickly change their position in the water column whilst maintaining near-neutral buoyancy than mulloway. Mulloway, having relatively slow rates of swimbladder gas exchange, will be more restricted in their FVR and in the time taken to acclimate when ascending or descending through the water column than snapper.

The finding that mulloway resorb swimbladder gas ~6 times faster than it is secreted indicates that they can acclimate faster when ascending through the water column than when descending. Mulloway have a thick, flexible swimbladder that takes up a large volume within the visceral cavity which we hypothesize to be able to contain any enclosed gases under substantial pressure prior to rupture, therefore allowing mulloway to ascend through the water column without the risk of becoming excessively buoyant or suffering

swimbladder perforation. However our hyperbaric chamber trials indicated swimbladder rupture occurred after a decrease in pressure of ~2.75 times, similar to that observed for pink snapper. Similarly, the finding that pink snapper resorb swimbladder gas at ~11 times faster than it is secreted indicates that they too acclimate faster when ascending through the water column than when descending. In contrast though, pink snapper have a much smaller swimbladder that is surrounded by the ribs and muscle tissue except where it is exposed to the visceral cavity. In addition, it is made of apparently quite thin, non-elastic material and we found that the swimbladders burst when subjected to, on average, a decrease in pressure of just ~2.5 times. Having a relatively fast rate of gas resorption will allow pink snapper to ascend through the water column gradually whilst minimising the risk of excessive buoyancy or swimbladder rupture. In fact, such a change in water pressure before rupture does not substantially affect a fish's ability to ascend through the water column in terms of vertical distance, except when nearing the surface as the greatest pressure changes occur nearest the surface. The ability to ascend from 100 m to 35 m, or from 30 m to 7 m water depth before swimbladder rupture (see Fig. 2), in addition to possessing relatively fast gas resorption rates, means that pink snapper are anatomically well-adapted to exploit the vertical dimension.

2.4.5. Implications for hyperbaric chamber experiments

The ability to estimate the time taken for fish to become acclimated is vital for any experiments that aim to investigate the effects of different experimental pressures or depths on fish physiology (Harden Jones & Scholes, 1985; Rummer & Bennett, 2005; Parker et al., 2006; Pribyl et al., 2009). Our results confirm that rates of swimbladder gas secretion and resorption are highly variable and extremely species-specific. A similar conclusion was reached by Parker et al. (2006) who found that it was not possible to infer gas exchange rates from even congeneric *Sebastes* species, such were the levels of variation. In terms of designing future hyperbaric chambers experiments using pink snapper and mulloway, we estimate that pink snapper require ~23 h and mulloway ~80 h, on average, to become physiologically acclimated to 60 m water depth after being at equilibrium at sea level (Table 2.3). Similarly, we estimate that pink snapper would require gradual decompression over ~14 h and mulloway ~89 h, on average, to acclimate to sea level after being at equilibrium at 60 m water depth (Table 2.4).

3. MULLOWAY CHAMBER EXPERIMENTS

3.1. Introduction

Mulloway *Argyrosomus japonicus* (Temminck & Schlegel, 1844) are distributed throughout coastal and estuarine waters of the Pacific and Indian Oceans. Mulloway are a valuable commercial and iconic recreational species throughout its distribution in Australian waters (around southern Australia between Brisbane in Queensland and Shark Bay in Western Australia). Juveniles are found exclusively in inshore waters while adults are mainly inshore and generally in waters <100 m deep. Mulloway can grow to very large sizes (>75 kg & >180 cm total length-TL) and live for more than 40 years (Silberschneider et al., 2009). Sexual maturity occurs at roughly 68 cm TL for females and at an age of 3+ years (Silberschneider & Gray, 2005).

In NSW mulloway are assessed as being "overfished" (Rowling et al., 2010). This is in response to research which demonstrated that mulloway were substantially growth overfished, but probably also recruitment overfished (Silberschneider & Gray, 2005). Long-term declines in landings and catch-per-unit-effort (CPUE) for the offshore handline fishery (that tends to target spawning aggregations during summer), severe age-class truncation and a spawning potential ratio (SPR) <10% of virgin levels all suggest that the spawning stock is currently very small. In NSW mulloway are mainly a recreational species, with the recreational catch estimated to be between five and 10 times the commercial catch (Stewart & Hughes, 2007). Current management arrangements for mulloway in NSW include a minimum legal length (MLL) of 45 cm TL and a recreational bag limit of five fish per person., of which only two can be >70 cm TL.

Substantial numbers of mulloway are released following capture by recreational fishers. The most recent estimate is that approximately 25% of all mulloway captured by recreational fishers in NSW are subsequently released (Henry & Lyle, 2003). In response to the overfished exploitation status of mulloway, NSW fishery managers are developing a recovery program for the species. It is highly likely that this recovery program will involve some variations to current management arrangements for recreational fishers. Any increases in the MLL from 45 cm TL and any reductions in the bag limit from five fish will see an increase in the proportion of mulloway which are legally required to be released following capture. It is therefore vital that research be done into whether mulloway survive following capture and release (C&R). To date, very little research exists concerning the survival rates of mulloway following C&R. Experiments on very small captive mulloway reported approximately 80% survival for mouth hooked fish with the hooks removed; however this decreased to approximately 27% survival for mulloway that had swallowed the hook and subsequently had the hook removed (Butcher et al., 2007). No research has been done on barotrauma-related injuries and their effects on C&R mortality for mulloway.

3.2. Materials & methods

Mulloway for use in chamber trials were sourced from a commercial aquaculture business (Clearwater Marine Farms, Pty Ltd). All experimental animals were kept at the Cronulla Fisheries Research Centre Aquarium Facility in a large mesh pen within a 1 million L recirculated seawater pond. Prior to use in experiments, fish were transferred into circular 5,000 L fibreglass tanks, a few at a time and tagged with individually numbered t-bar tags to allow individual fish to be indentified. All experiments were done using the custom-built pressure chambers described in Chapter 2 between 17 February and 26 May 2011 at water temperatures of 19 to 23 ºC. The chambers were partially filled with seawater and the fish were anaesthetised with a 1 ml/100 L concentration of AQUI-S anaesthetic. Once anaesthetised, the fish were transferred into the chambers one at a time in 20 L plastic buckets filled with seawater.

3.2.1. Experiment 1

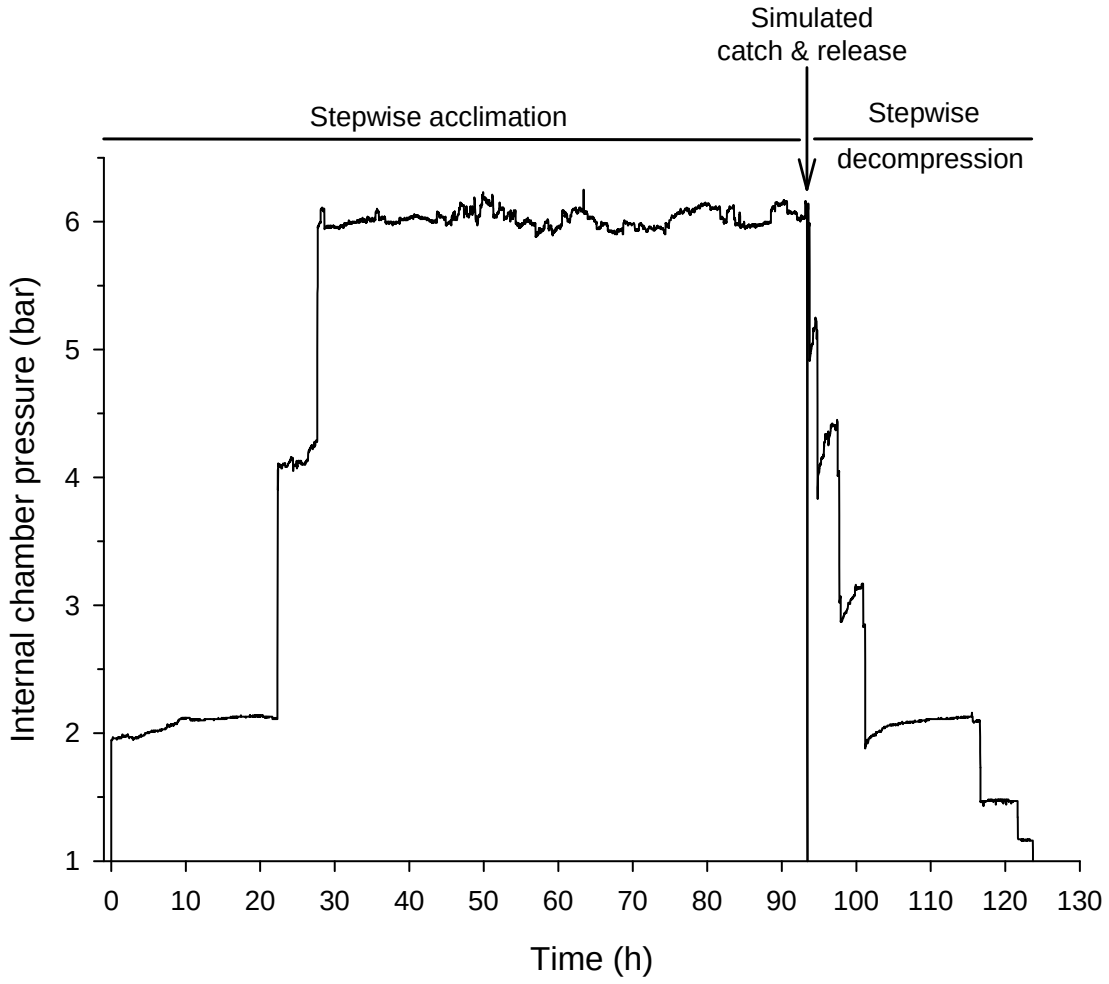
In the first experiment, we simulated capture and release and subsequent return to acclimation depth following a short surface interval of fish from three different depths (10, 30 and 50 m) and monitored their short- and long-term survival.

Following introduction of the experimental fish, the chambers were completely filled, closed and pressurized to an equivalent of 10 m water depth (2.02 bar) after approximately 10 minutes. According to our estimated swimbladder gas secretion rate for mulloway (0.007 ml/kg/min), stepwise changes to the pressure within the chambers were done so that all fish were fully acclimated to experimental depths. The pressure was changed to an equivalent of 30 m water depth (4.04 bar) after 24 h for fish being acclimated to 30 m depth, and to 50 m (6.06 bar) after a further 6 h for fish being acclimated to 50 m depth. An example of the internal chamber pressure changes which occurred during a trial is given in Fig. 3.1.

Once acclimated to depth (after a total of 95 h), treatment fish were subjected to simulated capture by reducing the pressure in the chambers to surface pressure (1.01 bar) at the rate of approximately 1 m/s. The chamber remained at this pressure for 2 mins before being repressurized to the original acclimation depth at the rate of approximately 1 m/s to simulate the release of the fish and subsequent return to capture depth. Control fish were acclimated to depth as for treatment fish, but were not subjected to this simulated capture and release.

According to our estimated swimbladder gas resorption rate for mulloway (0.044 ml/kg/min.) a stepwise depressurization schedule based on Boyle's Law was done so that all fish became fully acclimated to each new depth before further depressurization occurred, including 16 h at 10 m equivalent depth. This ensured that fish could be brought back to surface pressure without causing further barotrauma. This depressurization took 31 h from 50 m equivalent depth (6.06 bar), 26 h from 30 m equivalent depth (4.04 bar) and 7 h from 10 m equivalent depth (2.02 bar).

Figure 3.1. An example of a typical hyperbaric chamber depth profile for mulloway during experiment 1.



Once the chambers had been returned to surface pressure, the lids were removed, most of the water drained out and the fish anaesthetized as described above. Once anaesthetised, the fish were transferred into a 60 L plastic bin where they had their tag number, length (mm) and barotrauma symptoms recorded: alive/dead, exophthalmia, bloodshot eyes, bubbles in eyes, firm abdomen, stomach eversion, intestine protrusion from the cloaca, bloodshot cloaca, haemorrhaging from the gills, rippled skin, whether the fish floated or sunk whilst anaesthetised and general condition. The fish were then transferred into a circular 5,000 L fibreglass tank for short term monitoring before being released into a separate large mesh pen within the recirculated seawater pond.

When a fish died, the date of mortality and tag number was recorded and an autopsy was performed to look for internal barotrauma-related injuries which may have contributed to death. An example template of the autopsy datasheet is given in Figure 3.2.

Figure 3.2. Example of autopsy datasheet used to record internal barotrauma-related injuries which may have contributed to mortality.

Species				Treatment/Control		Date of death		
Tag #				Depth		Date of autopsy		
						Died in	Chamber	Tank	Pond

Organ	*Injury*	*Severity*		*Symptom*	*Comments*
Eyes	Exophthalmia	None	☐	Eyeball protrudes only slightly from eye socket	
		Mild	☐	<5mm	
		Moderate	☐	5-10mm	
		Severe	☐	>10mm	
	Haemorrhage	None	☐	Iris silver	
		Mild	☐	Visible under close examination	
		Moderate	☐	Ruptured blood vessels obvious	
		Severe	☐	Extensive haemorrhage in iris and conjunctiva	
	Bubbles	None	☐	Eye clear	
		Mild	☐	Visible under close examination	
		Moderate	☐	Obvious bubbles	
		Severe	☐	Extensive bubbles throughout eye/large bubbles	
Skin	Haemorrhage	Location(s)	☐		
	Bubbles	Location(s)	☐		
Fins	Haemorrhage	Location(s)	☐		
Gills	Haemorrhage	None	☐	Pink	
		Mild	☐	Visible under close examination	
		Moderate	☐	Blood in between gill filaments	
		Severe	☐	Everywhere	
	Bubbles under tissue posterior to gills		☐		
	Tear in tissue posterior to gills		☐		
Anus	Bloodshot		☐		
	Gut protruding		☐		
Abdomen		Normal	☐		
		Firm	☐		
		Bloated	☐		
	Gas released from BC when dissected		☐		
Swim Bladder	Hyperinflation	None	☐	Gas filled but flexible	
		Mild	☐		
		Moderate	☐		
		Severe	☐	Organs squashed forward and against body wall	
	Perforation	None	☐	Normal	
		Mild	☐	<5mm	
		Moderate	☐	5-10mm	
		Severe	☐	>10mm	
Viscera	Displacement	Normal	☐	Viscera below SB occupying anterior part of body cavity	
		Mild	☐	Viscera pushed slightly forward	
		Moderate	☐	Viscera only partly visible from underneath	
		Severe	☐	Viscera barely visible. L intestine stretched between S intestine and anus	
	Mesentery torn		☐		
	Hemorrhage		☐		
Stomach	Eversion	Normal	☐	Stomach below SB occupying anterior part of body cavity	
		Mild	☐	Stomach partly inside out	
		Moderate	☐	Stomach inside pharyngeal/buccal cavity	
		Severe	☐	Stomach protruding from mouth	
Liver	Trauma	Normal	☐	Lobes connected by a bridge of tissue	
		Mild	☐	Bridge slightly torn or compressed	
		Moderate	☐	Significantly torn or compressed nominal bridge of tissue	
		Severe	☐	Bridge severed completely	
Hepatic veins	Damage	Normal	☐	Veins extend from bridge of liver tissue through septum	
		Mild	☐	Slightly stretched but intact	
		Moderate	☐	Significantly stretched but intact	
		Severe	☐	Severed	
Spleen	Splenomegaly	Normal	☐	Elongated triangular shape	
		Mild	☐	Slightly enlarged but still elongated triangular shape	
		Moderate	☐	Becoming distorted	
		Severe	☐	Enlarged and distorted	

3.2.1.1. Sampling Design

Two chambers were used simultaneously with separate borehole pumps and diaphragm valves so that the pressure in each chamber could be adjusted independently. For each depth (10, 30 or 50 m), there were treatment (T) and control (C) groups making a total of 6 experimental groups (10T, 30T, 50T, 10C, 30C and 50C). Two blocks of six runs were used with three replicate treatment runs and one control run for each depth per block. The three treatments were therefore replicated 6 times in total, and the 3 controls were replicated twice. The order of treatment and control runs to chambers was allocated by a process of constrained randomisation in order to ameliorate the impact of any systematic variation between runs and chambers (Table 3.1). Each block contained one run per depth which consisted of a control and its corresponding treatment. The randomisation was also constrained to ensure that each depth control was used only once in each chamber, and remaining runs in each block were assigned so that each of the treatments were replicated three times in each chamber. However, due to misadventure, a slightly altered design had to be implemented (Table 3.1). Four fish were used in each chamber each run, so there were a total of 24 treatment and 8 control fish per depth.

Table 3.1. Experimental design for mulloway in experiment 1. Simulated acclimation depths were 10 m ("10"), 30 m ("30") or 50 m ("50"). C = control and T = treatment.

Block	Run	Chamber 1	Chamber 2
1	1	30T	10T
	2	50C	50T
	3	30T	50T
	4	10T	50T
	5	30T	30C
	6	10C	10T
2	7	30T	50T
	8	10T	10C
	9	50T	50C
	10	10T	30T
	11	30C	30T
	12	50T	10T

3.2.1.2. Statistical Analyses

To examine effects of depth and treatment, the chamber was treated as the experimental unit, to allow for possible non-independence in the outcomes for individual fish in the same chamber at the same time. Two analyses examined whether treatment or depth affected: i) mortality (the probability of a fish dying), or ii) the survival time post-trial of fish which died. All calculations and analysis were performed using the R statistical software (R Development Core Team, 2012).

In the first analysis, the data analysed consisted of counts of the number of fish which died (out of four) in each run in each chamber. The probability of a fish dying was modelled as a function of treatment (T or C), depth and their interaction on an underlying logistic scale

using a generalized linear model (GLM). To allow for additional variation in the death rates between runs and chambers above that expected from binomially distributed data with treatment and depth effects only, an overdispersion component was fitted using the quasi-binomial family with the 'glm' function in 'R'. This overdispersion component allows for lack of independence between fish in the same chamber which could result in such additional variation between runs and chambers.

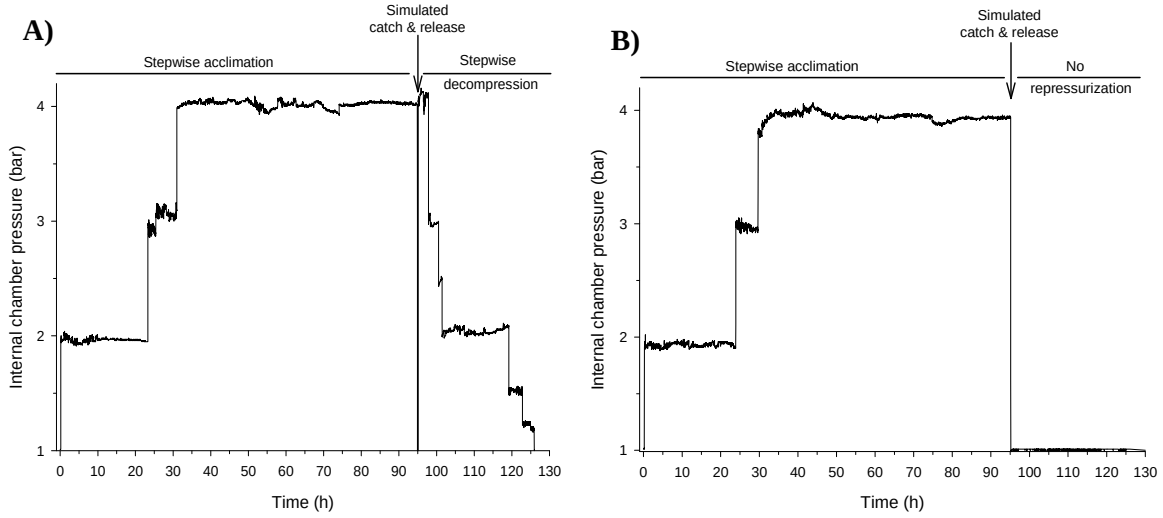
In the second analysis, the data analysed consisted of the survival time post-trial of individual fish which died (or the time from trial to the end of the monitoring period for those fish which survived up to that point). Data were analysed using the semi-parametric Cox proportional hazards approach, where the relative hazard function was modelled as a function of treatment, depth and their interaction (on a log scale). We chose this approach as exploratory analysis indicated that the distribution of survival times did not follow any of the standard distributional models used in parametric survival regression models. The 'coxph' function in the survival package in 'R' was used. To allow for non-independence between fish in the same chamber, as well as run and chamber effects, a mixed effects extension of the Cox model was used (Pankratz et al., 2005) as implemented in the 'coxme' function in the new 'coxme' package in 'R' (Therneau, 2012). Chambers, runs and chambers by runs were treated as random effects. In this way, allowance was made for any lack of independence in the survival times between fish in the same chamber simultaneously. Likelihood ratio tests using the Laplace approximated maximum likelihood, were used to test for significance of individual terms in the model (from the full model), both fixed and random.

3.2.2. Experiment 2

In the second experiment, we simulated capture and release from two experimental depths (10 and 30 m) and monitored their short- and long-term survival, but on this occasion we had two treatments: some fish were returned to acclimation depth following a long surface interval - the "repressurized" group - and others were kept at surface pressure for the remainder of the trial (to simulate a fish unable to return to depth) - the "leave at surface" group.

Following introduction of the experimental fish, the chambers were completely filled, closed and pressurized to an equivalent of 10 m water depth (2.02 bar) after approximately 10 minutes. According to our estimates of swimbladder gas secretion rate for mulloway, stepwise changes to the pressure within the chambers were done so that all fish were fully acclimated to experimental depths. The pressure was changed to an equivalent of 30 m water depth (4.04 bar) after 24 h for fish being acclimated to 30 m depth. Examples of the internal chamber pressure changes which occurred during a trial is given in Fig. 3.3.

Figure 3.3. An example of a typical hyperbaric chamber depth profile for mulloway during experiment 2 for **A)** "repressurized" and **B)** "leave at surface" treatments.



Once acclimated (after a total of 95 h), both groups of treatment fish were subjected to simulated capture by reducing the pressure in the chambers to surface pressure (1.01 bar) at the rate of approximately 1 m/s. For the "repressurized" treatment, the chamber remained at this pressure for 10 mins before being repressurized to acclimation depth at the rate of approximately 1 m/s to simulate the release of the fish and subsequent return to capture depth. The "leave at surface" group were left at surface pressure for the remainder of the trial. Control fish were acclimated to depth as for treatment fish, but were not subjected to this simulated capture and release. A video camera mounted on the outside of the chamber recorded the behaviour of the fish during experimental treatments through the lower observation port. The same depressurization schedule applied in experiment 1 was used to ensure that the fish were brought back to surface pressure without causing further barotrauma.

Once the chambers had been returned to surface pressure, the lids were removed, most of the water drained out and the fish anaesthetized as described above. Once anaesthetised, the fish were transferred into a 60 L plastic bin where they had their tag number, length (mm) and barotrauma symptoms recorded as in experiment 1. The fish were then transferred into a circular 5,000 L fibreglass tank for short term monitoring before being released into a separate large mesh pen within the recirculated seawater pond.

When a fish died, the date of mortality and tag number was recorded and an autopsy was performed to look for barotrauma-related injuries which may have contributed to death.

3.2.2.1. Sampling Design

Two chambers were used simultaneously with separate borehole pumps and diaphragm valves so that the pressure in each chamber could be adjusted independently. For each depth (10 or 30 m), there was a "repressurize" treatment (R), a "leave at surface" treatment (S) and controls (C) making a total of six experimental groups (10R, 30R, 10S, 30S, 10C and 30C). A single block of six runs were used with two replicate runs for each of the two treatments and two control runs for each depth. The order of treatment and control runs to

chambers was allocated by a process of constrained randomisation in order to ameliorate the impact of any systematic variation between runs and chambers (Table 3.2). The first run consisted of "repressurized" treatments for both depths (i.e. 10R and 30R) as it was considered useful to know whether all fish would die before being repressurized (<10 min). The randomisation was also constrained to ensure that each of the treatments and controls were used only once in each chamber. Four fish were used in each chamber each run, so there were a total of 8 fish for each of the treatments and controls per depth.

Table 3.2. Experimental design for mulloway in experiment 2. Simulated acclimation depths were 10 m ("10") or 30 m ("30"). C = control and T = treatment.

Run	Chamber 1	Chamber 2
1	30R	10R
2	10C	30C
3	10R	30S
4	30R	10S
5	10S	30S
6	10C	30C

3.2.2.2. Statistical Analyses

To examine effects of depth and treatment, the chamber was treated as the experimental unit, to allow for possible non-independence in the outcomes for individual fish in the same chamber at the same time. The data analysed consisted of the counts of the number of fish which died (out of four) in each run by chamber.

The probability of a fish dying was modelled as a function of treatment (R, S or C) and depth (10 or 30 m). In addition, treatment effects were broken down into two contrasts: i) control (C) versus treatment (S or R), and ii) 'leave at surface' (S) versus 'repressurize' (R). Since there was 100% mortality in at least one treatment by depth group (see 3.3.2.3 below), a bias-reduced GLM was used to obtain robust estimates of mortality in each treatment by depth group as implemented in the 'brglm' package in R (Kosmidis, 2007). To allow for additional variation in the death rates between runs and chambers, above that expected from binomial distributed data with treatment and depth effects only, an overdispersion component was fitted using the quasi-binomial family with the 'glm' function in R. This overdispersion component allows for lack of independence between fish in the same chamber which could result in such additional variation between runs and chambers.

As described for Experiment 1 (3.2.1.2), survival times post-trial were analysed using the semi-parametric Cox proportional hazards approach, where the relative hazard function was modelled as a function of treatment, depth and their interaction (on a log scale).

3.3. Results

3.3.1. Experiment 1

3.3.1.1. In situ observations

Video footage recorded during experimental treatments revealed that the most common behaviour exhibited by fish whilst in the chambers was slow swimming (Fig. 3.4). This was consistent over periods when the pressure was steady as well as during stepwise pressurization to, and depressurization from, acclimation depth for both treatment and control fish.

During simulated capture and release, however, behaviour was very different. Fish became increasingly agitated as the pressure progressively decreased and they became excessively buoyant and they erratically rushed around the chamber attempting to remain near the bottom (Fig. 3.5). For fish acclimated to 30 or 50 m simulated depth, a distended abdomen, flaring of the gill covers (Fig. 3.6) and head shaking (Fig. 3.6) was also observed. This was often followed by the eversion of the stomach into the buccal cavity protruding from the mouth (Figs 3.6 and 3.7) as pressure approached 1 bar.

Figure 3.4. Normal mulloway behaviour in the chambers.

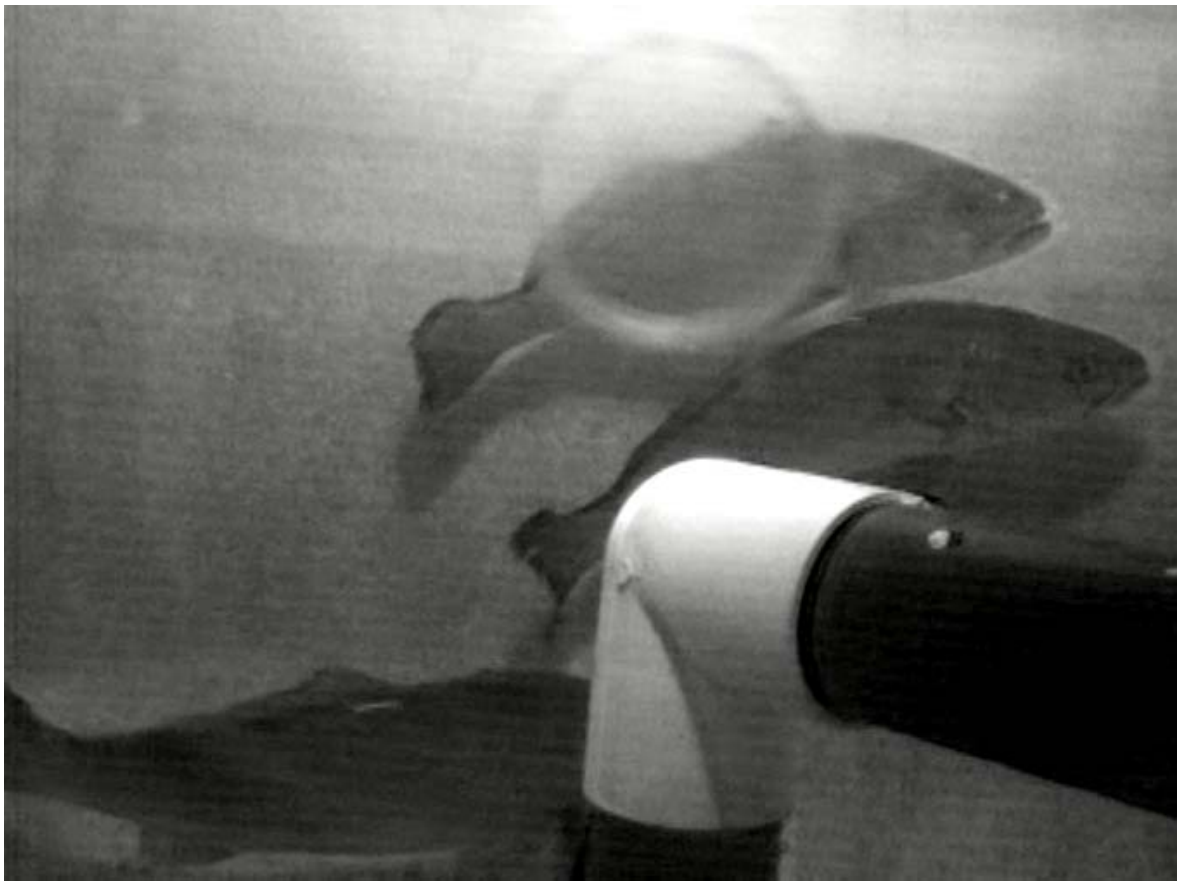


Figure 3.5. Two examples of mulloway attempting to overcome excess buoyancy by swimming downwards towards the bottom of the chamber taken from video footage captured during simulated catch (depressurization).

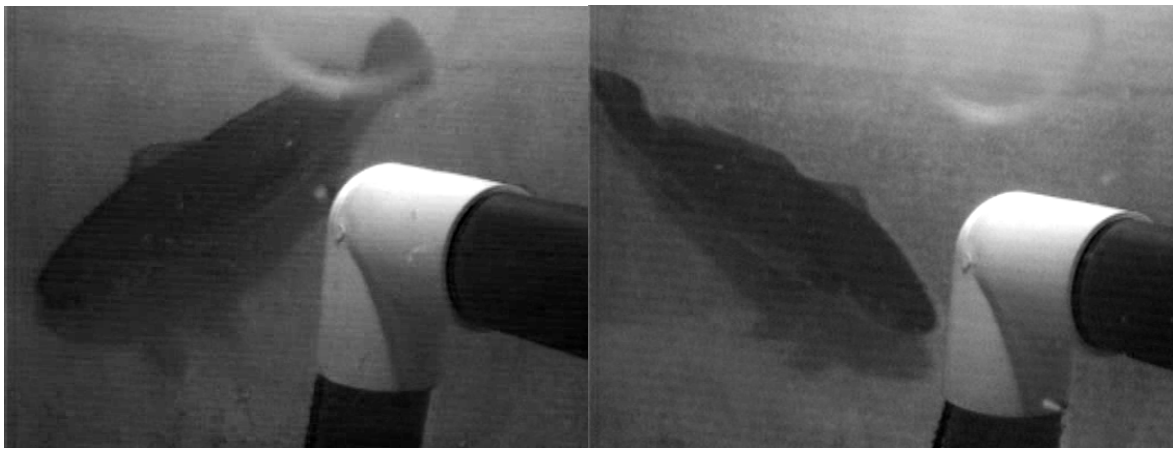


Figure 3.6. A sequence of stills from video footage captured within the chamber during simulated catch (depressurization) of mulloway showing head shaking and gill flaring followed by stomach eversion (circled).

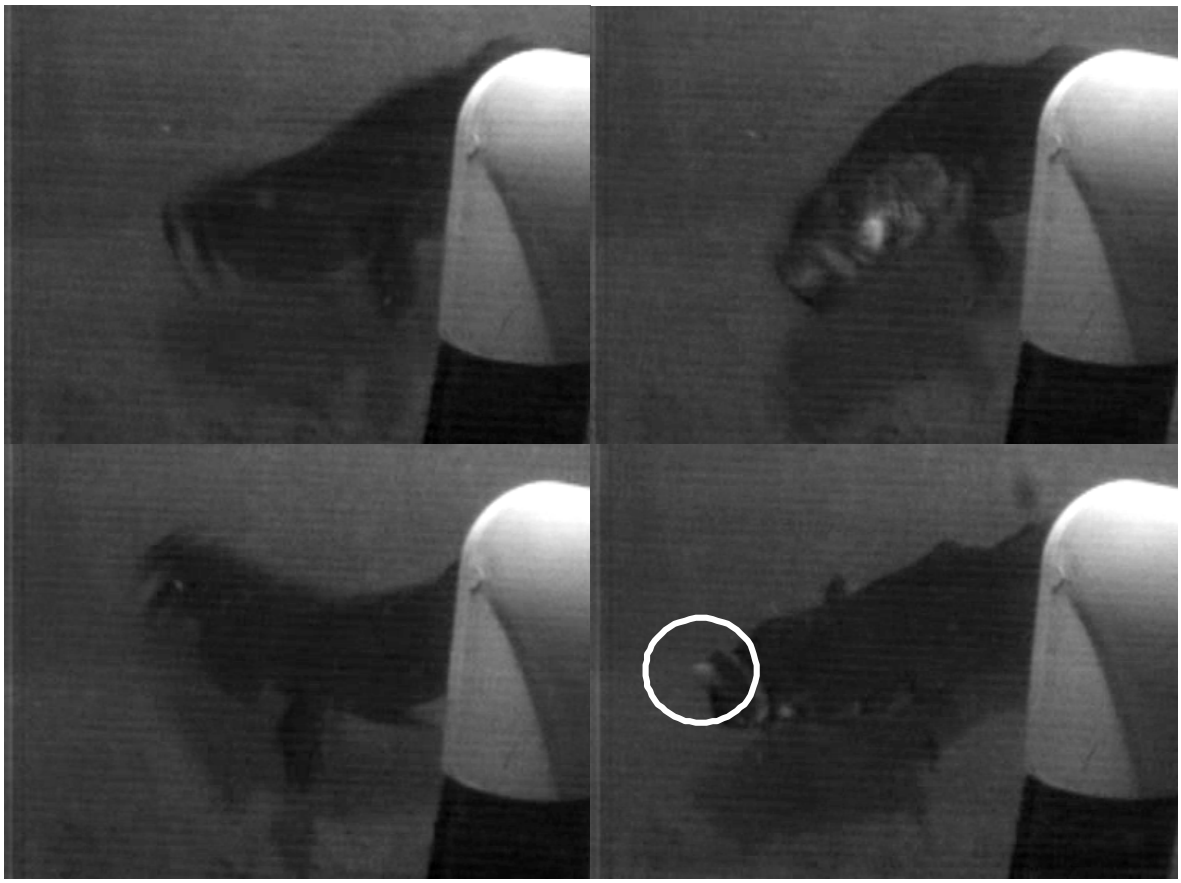
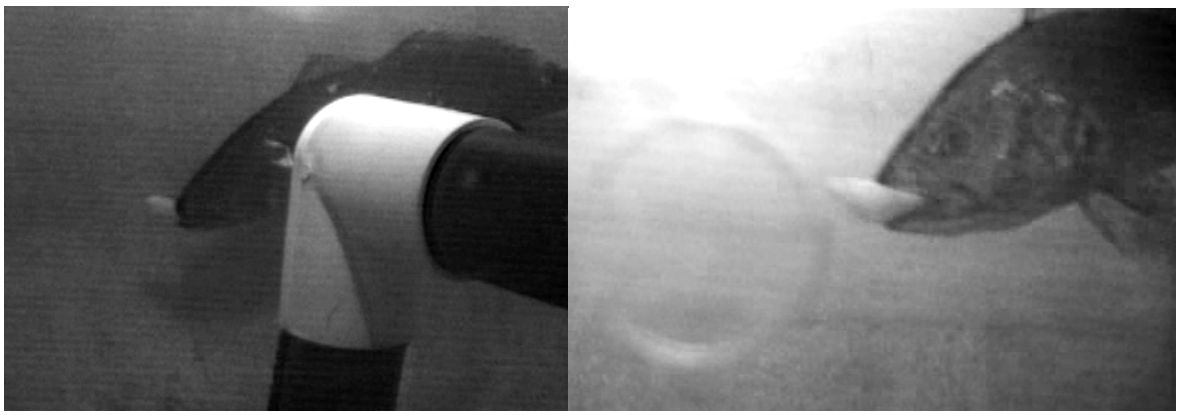


Figure 3.7. Two examples of stomach eversion in mulloway from video footage captured within the chamber during simulated catch (depressurization).



For at least one treatment fish out of the four in each chamber acclimated to 30 or 50 m, bubbles were observed exiting the body cavity of the fish via the pharyngo-cleithral membrane (tissue immediately posterior to the gills underneath the opercula; Fig. 3.8) during the 2 min surface interval before recompression. Immediately prior to recompression at the end of the surface interval many fish were not visible to the camera because they were floating upside down against the roof of the chamber. Upon repressurization to acclimation depth, the buoyancy of these fish decreased and they slowly descended to the bottom of the chamber (Fig. 3.9); many rested on the bottom ventilating quickly whilst others attempted to maintain neutral buoyancy in the chamber by swimming constantly with a head-up attitude (Fig. 3.10).

Figure 3.8. Two stills from video footage captured within the chamber during simulated catch (depressurization) of mulloway showing head shaking and gill flaring followed by release of gas bubbles (arrows) from the pharyngo-cleithral membrane. Circled are clusters of gas bubbles which have already exited the body cavity of this fish via the pharyngo-cleithral membrane.

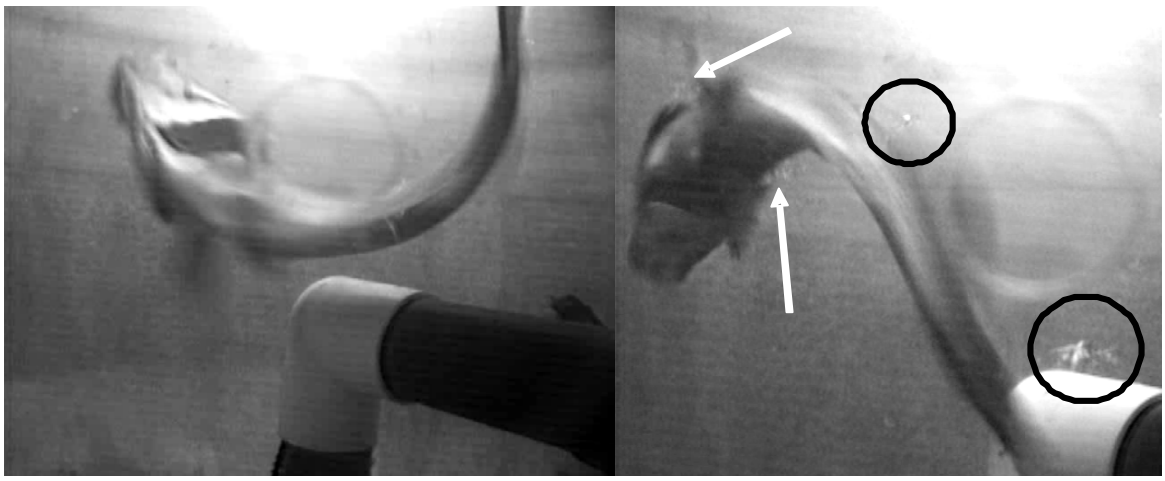


Figure 3.9. A) Still taken from video footage captured within the chamber following simulated release (repressurization to 30 m equivalent depth) of mulloway after 10 mins at surface pressure showing slow descent to the bottom of the chamber and cessation of ventilation. **B)** Dead mulloway with severely distended abdomens and flared gills floating on the surface within the chamber ~20 mins after simulated capture (depressurization) from 30 m simulated depth.

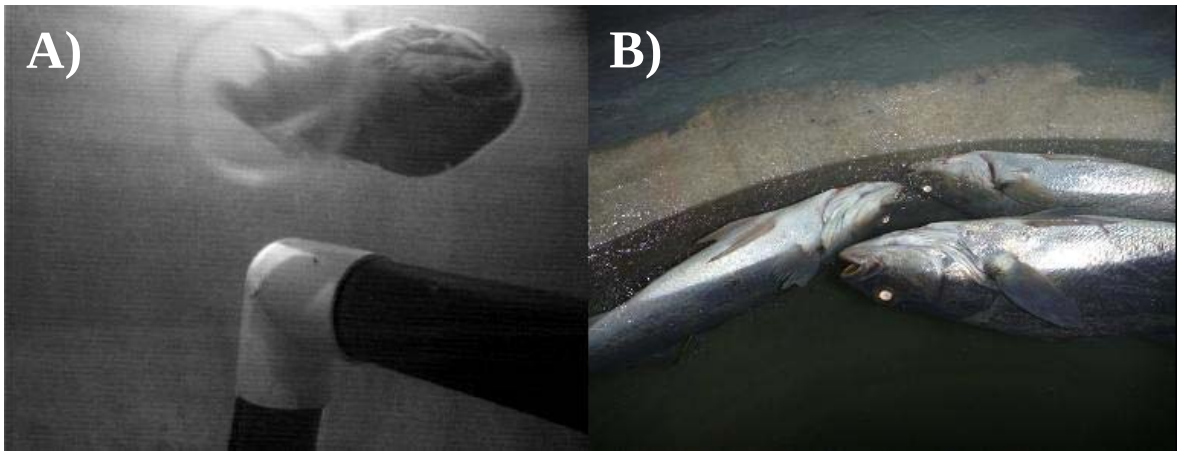
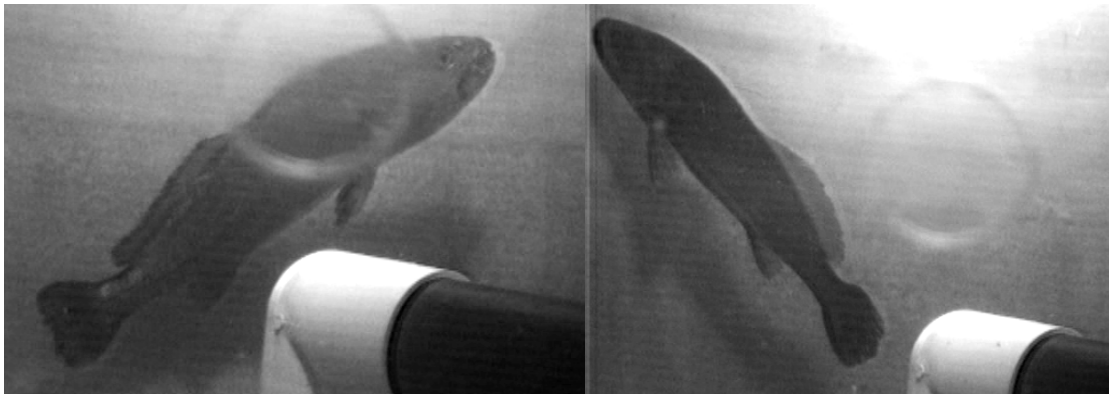


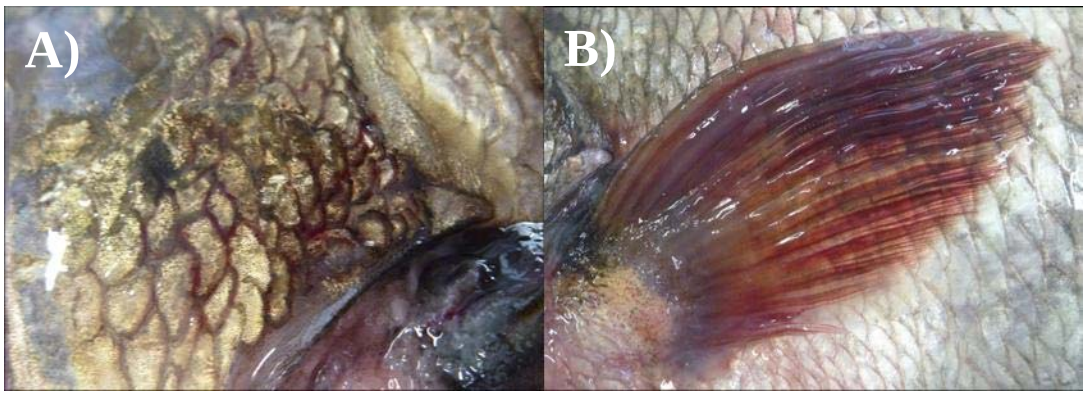
Figure 3.10. Two examples of mulloway attempting to maintain neutral buoyancy by swimming upwards towards the top of the chamber taken from video footage captured following simulated release (repressurization).



3.3.1.2. Barotrauma symptoms

Upon removal from the chamber at the cessation of the trials, the only mortality was a single treatment fish acclimated to pressure equivalent to 50 m depth (Table 3.3). The most common symptom seen in control fish was haemorrhaging on the skin (Fig. 3.11) and occurred across fish acclimated to all simulated depths.

Figure 3.11. A) Haemorrhaging of the skin and **B)** haemorrhaging of the fins, in mulloway following simulated capture from depth.



In treatment fish, a distended abdomen (Fig. 3.12) occurred in 33% of fish acclimated to 10 m, slightly less (21%) in fish acclimated to 30 m and in just 4% of fish acclimated to 50 m equivalent depth (Fig 3.13). In contrast, the percentage of treatment fish which were negatively buoyant (i.e. sank when anaesthetised) increased from 0% for fish acclimated to 10 m to 17% for fish acclimated to 30 m up to 29% of fish acclimated to 50 m simulated depth.

Figure 3.12. Excessively buoyant mulloway with distended abdomen following capture from ~14 m depth.

Figure 3.13. Percent occurrence of external symptoms of barotrauma observed in mulloway during experiment 1 following removal from the chambers. Hatched bars are controls

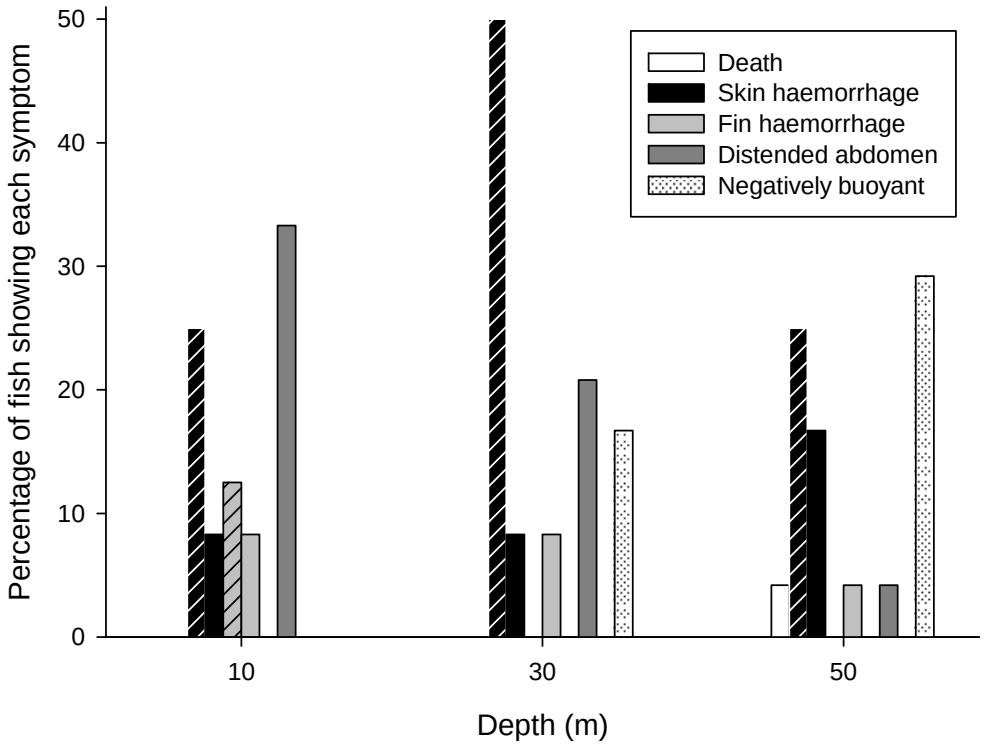


Table 3.3. Percent occurrence of external symptoms of barotrauma observed in mulloway during experiment 1 following removal from the chambers for each depth and treatment. *n* is sample size.

	Controls				Treatments			
Simulated Depth (m)	**10**	**30**	**50**	**overall**	**10**	**30**	**50**	**overall**
n	*8*	*8*	*8*	***24***	*24*	*24*	*24*	***72***
Symptom								
Mortality							4.2	**1.4**
Exophthalmia								
Corneal haemorrhage								
Corneal emphysema								
Skin haemorrhage	25.0	50.0	25.0	**33.3**	8.3	8.3	16.7	**11.1**
Fin haemorrhage	12.5			**4.2**	8.3	8.3	4.2	**6.9**
Gill haemorrhage								
Bloodshot cloaca								
Distended abdomen					33.3	20.8	4.2	**19.4**
Stomach eversion								
Rippled skin								
Negatively buoyant						16.7	29.2	**15.3**

3.3.1.3. Mortality

Using the quasi-binomial GLM to analyse death rates, there was a significant interaction between treatment and depth (Table 3.4). The unexpected deaths of 38% of 10 m control fish and 25% of 30 m control fish (Table 3.5) impaired the ability of the model to statistically tease apart the factors influencing mortality as the model failed to find significant differences between treatments or among depths leaving the interaction between the two as the only significant result. Nonetheless, obvious trends were clearly evident: overall, 36.1 (± 5.8) % of fish died as a result of simulated C&R from depth compared with only 20.9 (± 11.9) % for controls; and mortality varied substantially with depth for fish after simulated capture and release (Fig. 3.14A) with a much higher percentage of fish dying from 30 m (45.8 ± 10.0%) and 50 m (50.0 ± 6.1%) than from 10 m (12.5 ± 5.6%). The estimated dispersion component was 0.95, suggesting no evidence of over-dispersion and therefore independence between individual fish.

Figure 3.14. Results of the first hyperbaric chamber experiment on mulloway where fish were acclimated to 10, 30 or 50 m simulated depth and depressurized to surface pressure, before being repressurized to acclimation pressure after 2 mins: **A)** mean percent mortality (± SE), **B)** the mean number of days post-treatment (± SE) mortality occurred. Treatment bars (■), control bars (■).

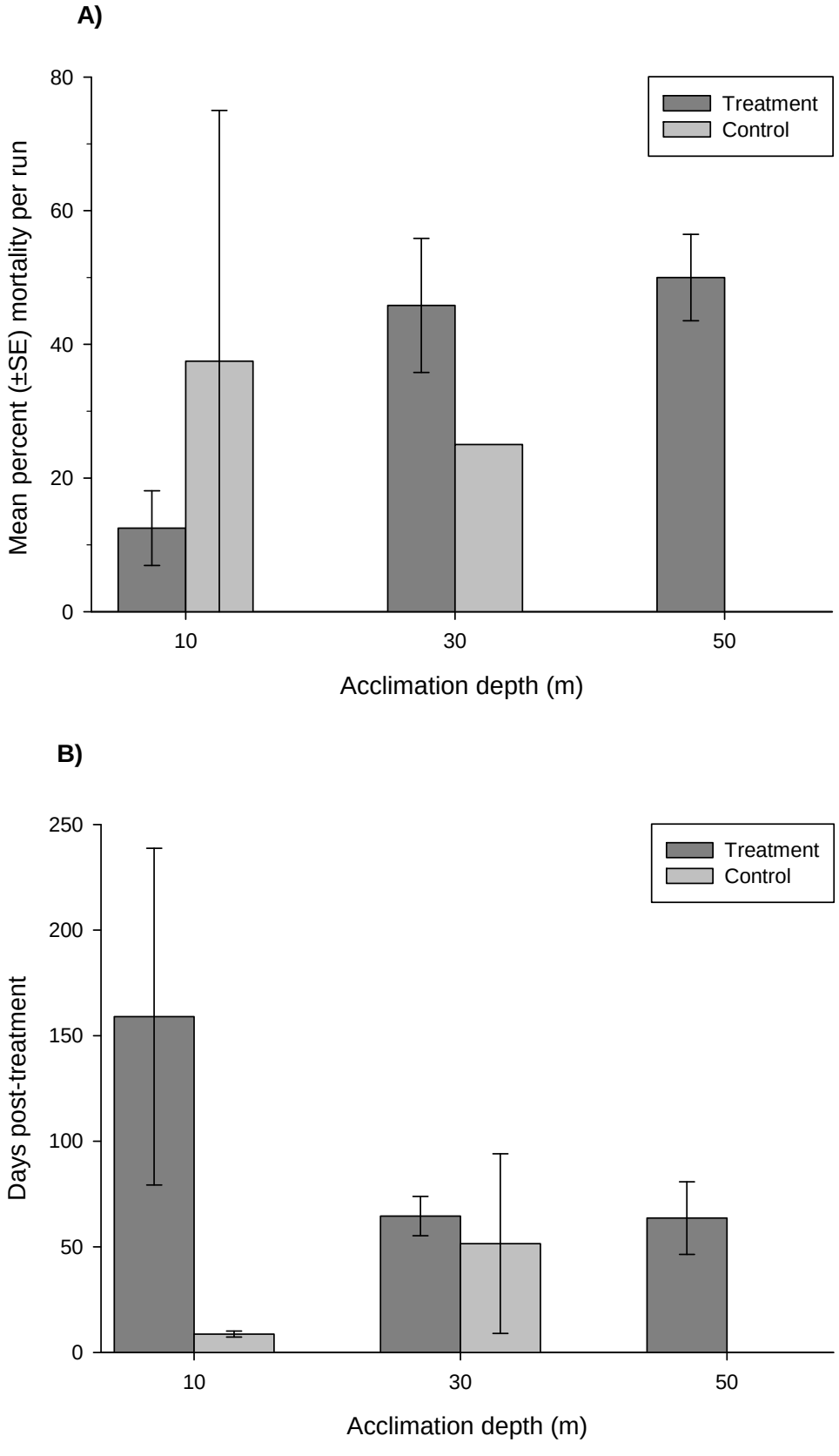


Table 3.4. Analysis of deviance table for the quasi-binomial GLM relating to the probability of a fish dying model for the first mulloway chamber experiment. The estimated overdispersion was 0.95. df is degrees of freedom.

	df	Deviance	Residual df	Residual Deviance	F	*P*-value
			23	37.539		
Treatment	1	1.703	22	35.836	1.795	0.195
Depth	1	3.672	21	32.163	3.871	0.063
Treatment × Depth	1	9.616	20	22.547	10.137	0.005

3.3.1.4. Post-mortems

Post-mortems carried out on mortalities showed that all fish that died had both external and internal injuries, some of which were consistent with rapid decompression (Table 3.5). The most common external injuries sustained by fish which died following chamber trials were haemorrhaging on the skin and fins (Fig 3.11) and occurred in both control (67 and 33% respectively) and treatment fish (72 and 48% respectively). Corneal haemorrhaging (Fig. 3.15) was recorded only in fish which had been subjected to simulated C&R from 30 (10%) or 50 m (25%) depth (Table 3.5). Internal injuries occurred exclusively in treatment fish, but only treatment fish which had been subjected to simulated C&R from 30 or 50 m depth. A range of internal injuries occurred in these fish including viscera haemorrhaging (Fig. 3.16A), torn mesentery (Fig. 3.16B, stomach eversion (Fig. 3.17), liver trauma (Fig. 3.18), damage to the hepatic veins (Fig. 3.19) and splenomegaly (Fig. 3.20).

Table 3.5. Percent occurrence of injuries revealed by post-mortems performed on mulloway mortalities during experiment 1. *n* is the number of mortalities out of the original sample size for each depth and treatment. PCM is pharyngo-cleithral membrane.

	Controls				Treatments			
Simulated Depth (m)	**10**	**30**	**50**	**overall**	**10**	**30**	**50**	**overall**
n	*3/8*	*2/8*	*0/8*	***6/24***	*3/24*	*11/24*	*12/24*	***26/72***
Symptom								
Exophthalmia								
Corneal haemorrhage						10.0	25.0	**16.0**
Corneal emphysema								
Skin haemorrhage	33.3	100.0		**66.7**	66.7	80.0	66.7	**72.0**
Fin haemorrhage		66.7		**33.3**	33.3	60.0	50.0	**48.0**
Gill haemorrhage								
PCM emphysema								
PCM perforation							8.3	**4.0**
Bloodshot cloaca								
Distended abdomen								
Swimbladder empty						72.7	66.7	**61.5**
Swimbladder hyperextension								
Swimbladder perforation						10.0	8.3	**8.0**
Swimbladder scar tissue						63.6	50.0	**50.0**
Viscera displacement								
Viscera haemorrhage							25.0	**12.0**
Torn mesentery							16.7	**8.0**
Stomach eversion						10.0		**4.0**
Liver trauma						10.0	33.3	**20.0**
Hepatic vein damage							25.0	**1.2**
Splenomegaly							8.3	**4.0**

Figure 3.15. A) Exophthalmia and corneal emphysema and **B)**, exophthalmia and corneal haemorrhaging, in mulloway following simulated capture from depth. Arrows indicate clusters of gas bubbles or haemorrhaging in each image, respectively.

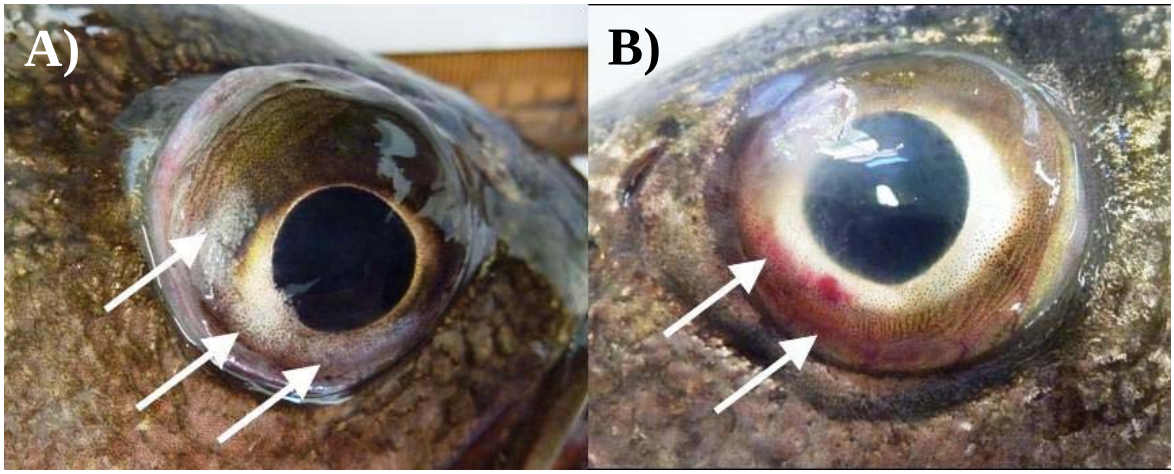


Figure 3.16. Internal haemorrhaging in mulloway following simulated capture from depth. Arrows indicate haemorrhage **A)** in viscera, and **B)** beneath the mesentery covering the swimbladder.

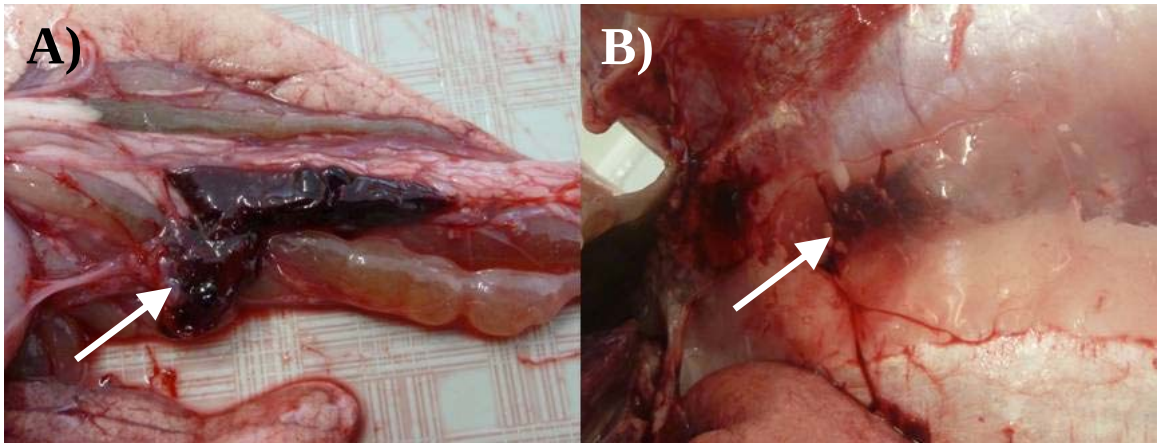


Figure 3.17. A) Stomach eversion into pharyngeal cavity in mulloway following simulated capture from depth, and **B)** dissected visceral mass showing everted stomach and displaced organs.

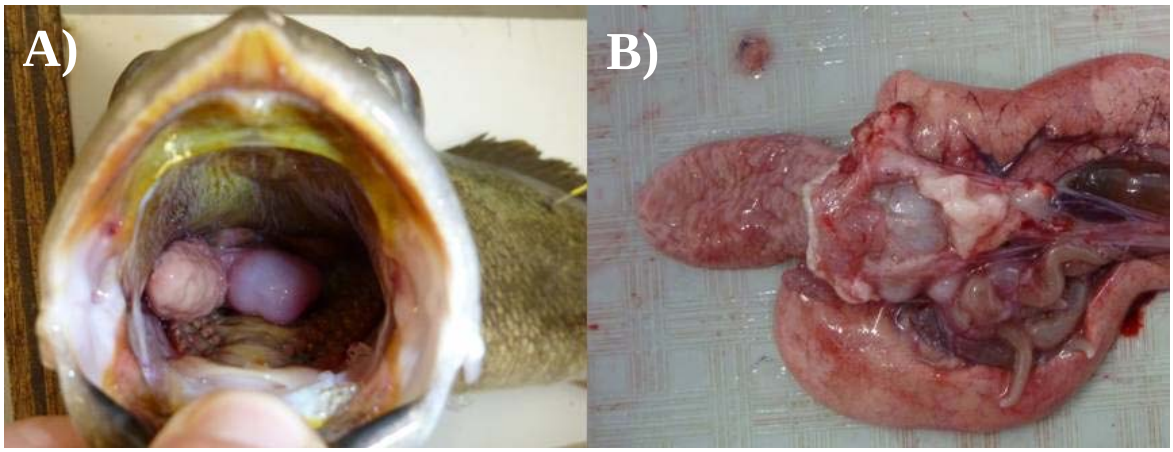


Figure 3.18. Dissected visceral mass of mulloway showing **A)** normal condition of liver with lobes connected by thick bridge of tissue (circled), and **B)** condition of liver following simulated capture from depth with bridge of tissue connecting lobes significantly torn and compressed (circled).

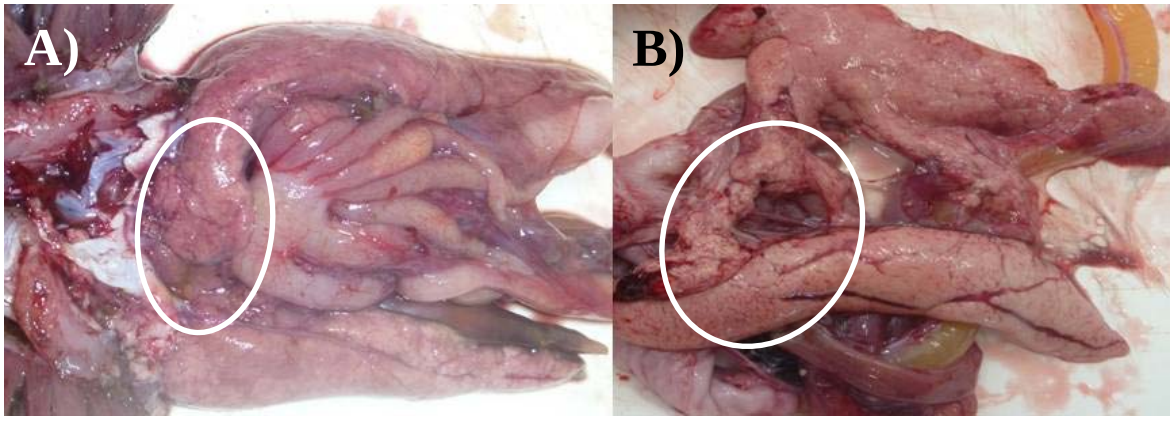


Figure 3.19. Closeup of dissected visceral mass of mulloway showing **A)** normal position of hepatic veins (arrows), and **B)** completely severed hepatic veins following simulated capture from depth (arrows indicate direction of tearing).

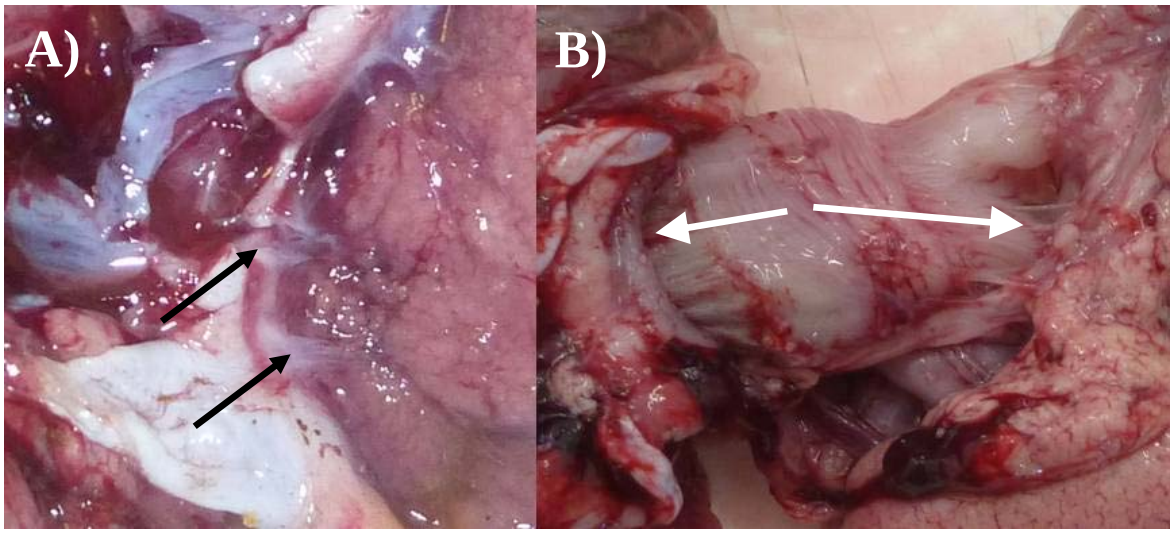
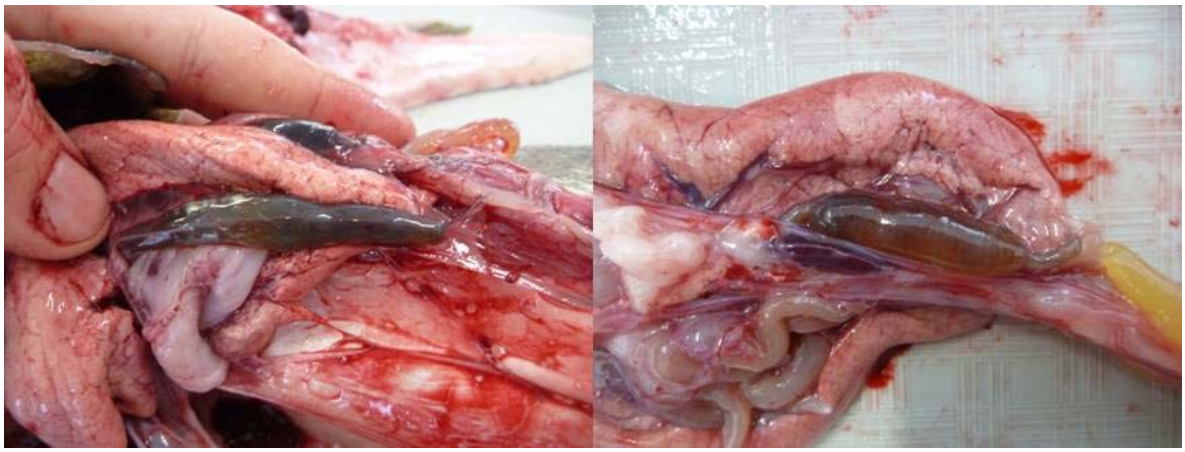


Figure 3.20. Enlarged spleen (splenomegaly) in mulloway following simulated capture from depth.



A high proportion (58%) of fish subjected to simulated C&R from 30 and 50 m had perforated swimbladders (Fig. 3.21) or had swimbladders with scar tissue (Fig. 3.22) indicative of previous perforation. Approximately 70% of fish did not have any gas in their swimbladders (Fig. 3.23), which compromised the fishes' ability to maintain neutral buoyancy in the water column and resulted in the fish swimming constantly in their pen.

Figure 3.21. Perforated swimbladders of mulloway following simulated capture from depth, **A)** perforation of swimbladder (circled) and mesentery (arrows), and **B)** perforation of swimbladder only (gas trapped inside mesentery).

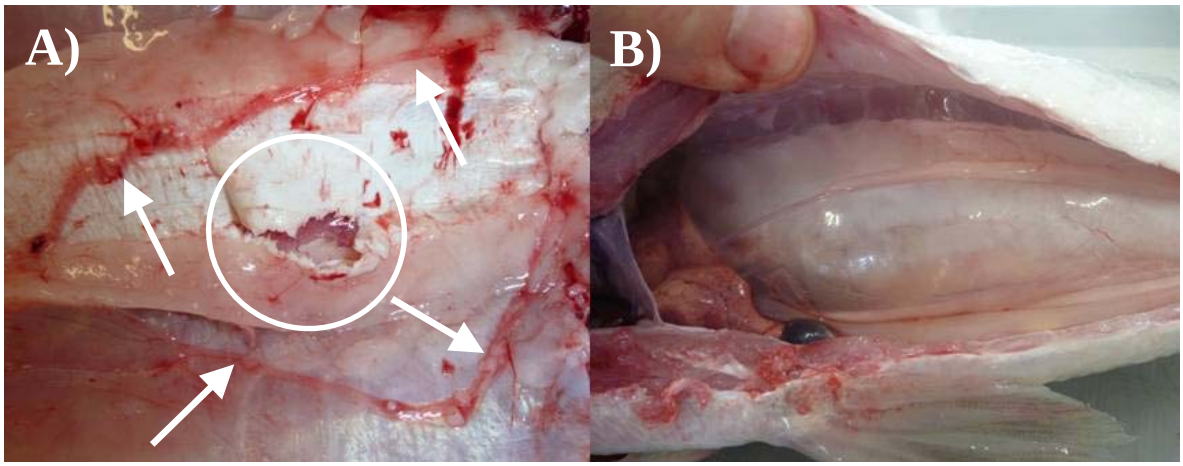


Figure 3.22. Deflated mulloway swimbladder with healed perforation scars (arrows): **A)** external view, and **B)** internal view.

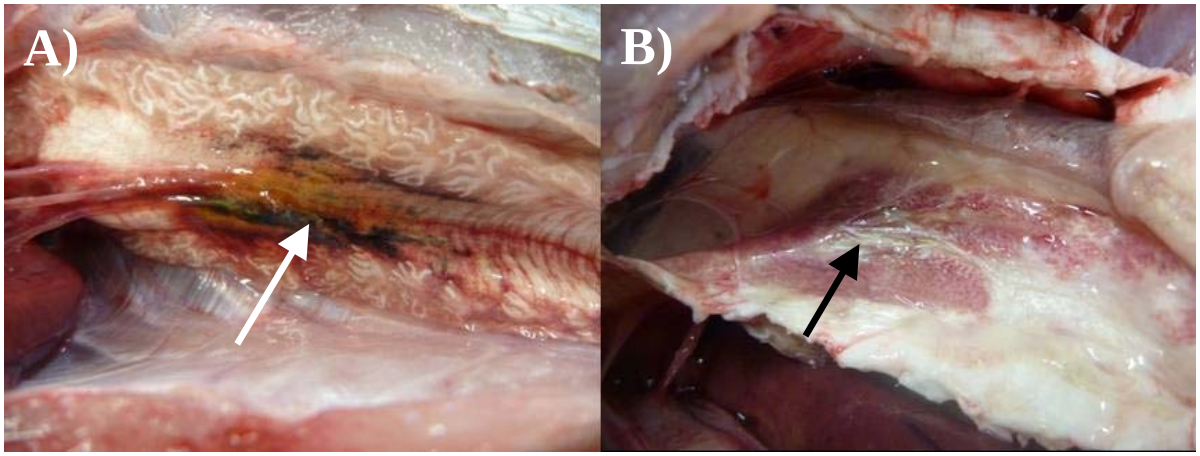
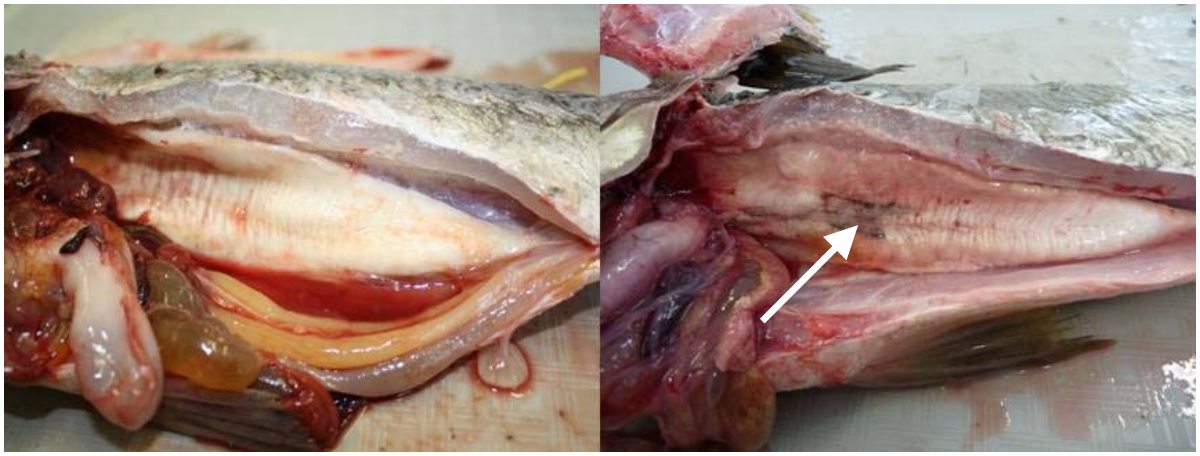


Figure 3.23. Deflated mulloway swimbladders containing no gas following simulated capture from depth. Note healed perforation scar (arrow).



3.3.1.5. Delayed mortality

This behaviour caused the fish to slowly lose condition resulting in eventual delayed mortality an average of 64 days after simulated capture and release from both 30 (range: 28-135 days) and 50 m (5-219 days) (Figs 3.14B and 3.24, Table 3.6).

Figure 3.24. Cumulative frequency of mulloway mortalities in experiment 1 for each depth treatment.

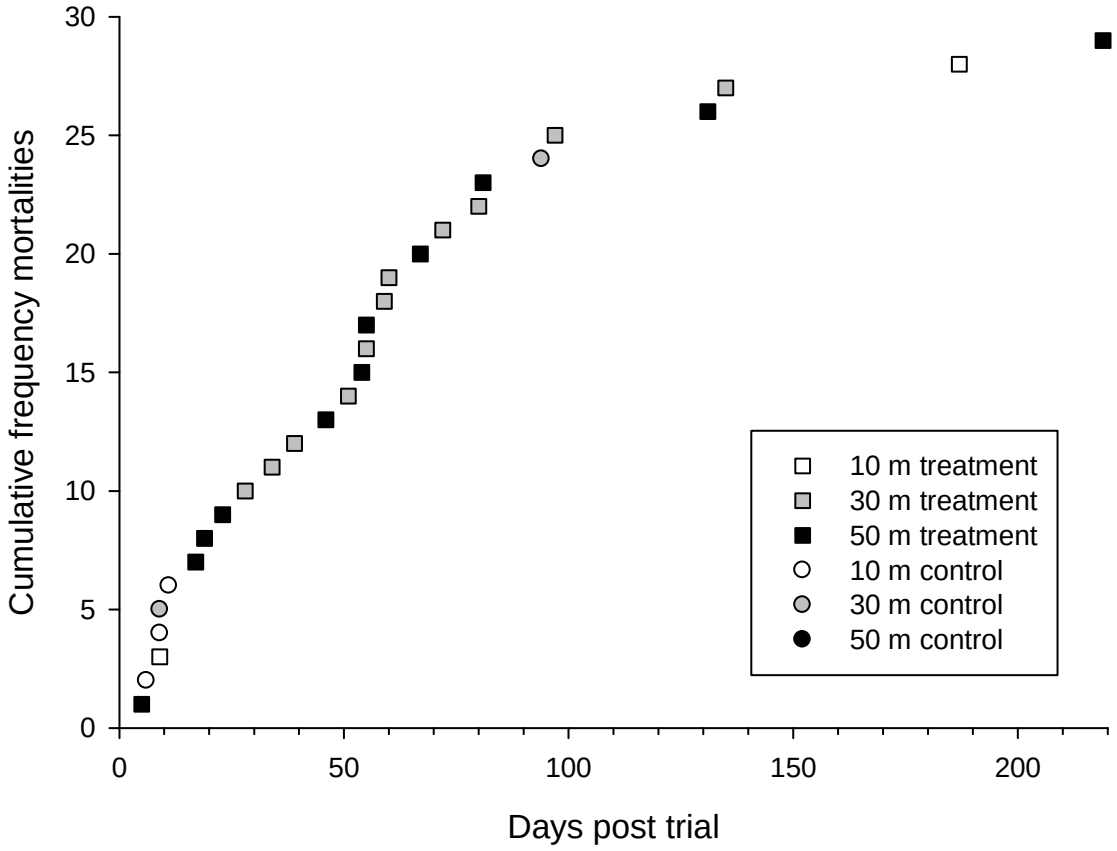


Table 3.6. Mortality of mulloway by chamber and run during experiment 1. There were four mulloway in each chamber for each run.

Run	Chamber 1	No. deaths	Days	Chamber 2	No. deaths	Days
1	30T	3	28, 34, 72	10T	0	
2	50C	0		50T	1	67
3	30T	2	51, 135	50T	2	23, 219
4	10T	0		50T	2	5, 54
5	30T	1	80	30C	1	9
6	10C	3	6, 9, 11	10T	1	9
7	30T	3	55, 59, 60	50T	2	46, 46
8	10T	0		10C	0	
9	50T	2	17, 55	50C	0	
10	10T	1	187	30T	1	39
11	30C	1	94	30T	1	97
12	50T	3	19, 81, 131	10T	0	

Most control fish (4 out of 5) died soon after their removal from the chambers (within 11 days): three fish acclimated to 10 m equivalent depth all died between 6 and 11 days after removal and two fish acclimated to 30 m equivalent depth died after 9 and 94 days respectively (Fig. 3.24). No fish acclimated to 50 m equivalent depth died.

Delayed mortality of treatment fish acclimated to 30 and 50 m were almost identical to one another and occurred at a rate of approximately one fish every 4 days up to 81 days after removal from the chambers. After 81 days, the rate of mortality for both these groups slowed considerably. In contrast, only two fish died after simulated capture and release from 10 m, one after 9 days and the other after 187 days (Fig. 3.24).

Likelihood ratio tests for each of the terms from the full model indicate significant treatment by depth interactions, but no evidence of chamber, run or run by chamber effects (Table 3.7). Overall, this result indicates that mortality in control fish occurred significantly sooner than for treatment fish and that mortality in treatment fish simulated to C&R from 30 and 50 m occurred significantly later (and more often) than treatment fish simulated to C&R from 10 m.

Table 3.7. Results of likelihood ratio tests for each of the terms in the Cox random effects model for the first mulloway chamber experiment. LogL is log-likelihood, LRT is likelihood ratio test, df is degrees of freedom.

	LogL	LRT statistic	df	*P*-value
Full model (1)	-120.69			
(1) - Chamber	-121.67	1.96	1	0.162
(1) - Treatment × Depth	-127.42	13.47	2	0.001
(1) - Treatment * Depth	-130.24	19.10	5	0.002
(1) - Run	-120.69	0.00	1	1.000
(1) - Run × Chamber	-120.68	0.00	1	1.000

3.3.2. Experiment 2

3.3.2.1. In situ observations

As recorded for Experiment 1, video footage showed that slow swimming and resting on the bottom were the most common behaviours exhibited by fish whilst the internal chamber pressure was steady or being slowly adjusted (Fig. 3.4).

Both treatment groups ('repressurize' and 'leave at surface') of fish acclimated to 10 m equivalent depth developed a firm abdomen during simulated capture and swam around the chamber with their heads angled down to overcome their increased buoyancy (Fig 3.5). 'Repressurized' fish returned to normal chamber behaviour once returned to acclimation pressure after 10 mins, but fish 'left at the surface' continued to swim head-down until they had resorbed enough swimbladder gas to become neutrally buoyant again. Normal chamber behaviour resumed in this group after ~24 h. A single particularly buoyant individual floated upside down against the roof of the chamber for an entire day following simulated capture, before resuming normal behaviour the next day.

The reaction of fish to simulated capture when acclimated to 30 m for both treatment groups ('repressurize' and 'leave at surface') were much more severe than from 10 m equivalent depth. As the pressure progressively decreased, the fish became extremely agitated swimming haphazardly around the tank with heads angled downwards (Fig. 3.5) in an attempt to overcome their excessive buoyancy and remain near the bottom of the chamber. All fish from both treatment groups displayed extreme distension of the abdomen in addition to flaring of the gill covers (Fig. 3.6) and head shaking (Fig. 6). In many fish, this was followed by the eversion of the stomach into the buccal cavity and out the mouth (Fig. 3.7) as pressure approached 1 bar. Once at surface pressure, bubbles were observed exiting the body cavity of many fish via the pharyngo-cleithral membrane (Fig. 3.8).

For fish acclimated to 30 m and repressurized after a 10 min surface interval (the 'repressurize' group), there were no fish visible to the camera after ~5 mins as all were floating upside down against the roof of the chamber. Upon repressurization, the buoyancy of these fish decreased and they slowly descended to the bottom of the chamber; three (of the eight) fish in this treatment had ceased ventilating at this point (<10 mins) and the remainder rested on the bottom ventilating rapidly.

In the 'leave at surface' group depressurized from 30 m, once again all fish became exhausted and floated upside down against the roof of the chamber after ~ 5 mins. However, all eight fish had ceased ventilating completely within 18 mins of simulated capture and floated motionless on the surface with severely distended abdomens (Fig. 3.9).

3.3.2.2. Barotrauma symptoms

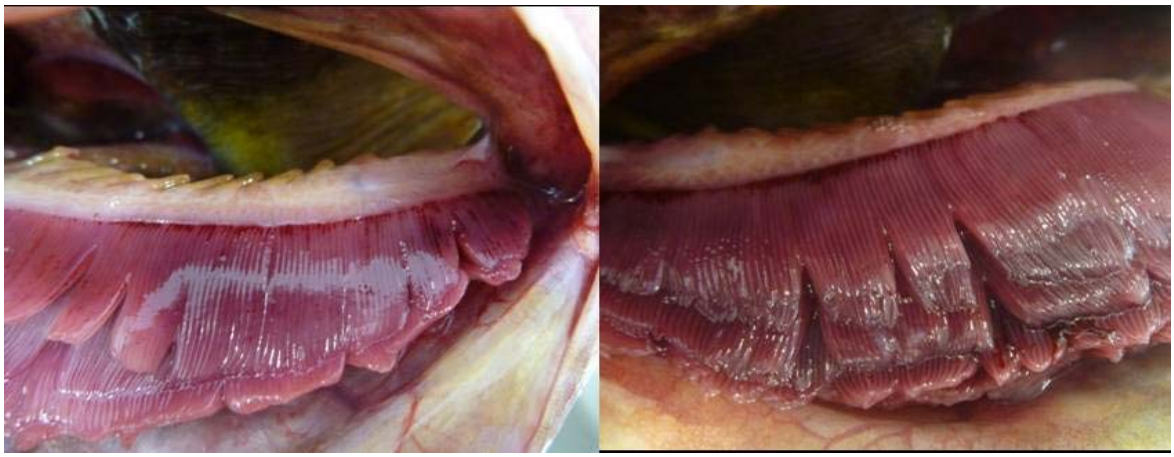
In contrast to the results of Experiment 1 (above), some mortality had occurred immediately after simulated capture or by the time the fish were removed from the chambers at the cessation of trials (Table 3.8). One control fish died from each of the two acclimation depths. Three out of eight (38%) fish acclimated to 30 m depth from the 'repressurize' group died before they could be repressurized to depth. All (100%) fish depressurized from 30 m and left at surface pressure died in the chamber as a result of this treatment. There was no mortality for treatment fish acclimated to 10 m, regardless whether repressurized or left at the surface. The most common symptom in treatment fish

was a distended abdomen and occurred in 44% of fish which were repressurized from depth and 50% of fish which were left at the surface, including all fish acclimated to 30 m. Bloodshot cloacas occurred only in fish acclimated to 30 m: in 38% of fish repressurized and in 88% of fish left at the surface. Similarly, stomach eversion occurred in 38% of fish repressurized from 30 m and in all fish left at the surface. Thirty eight and 50% of fish respectively became negatively buoyant following acclimation to 30 m and either being repressurized or being left at surface pressure. Haemorrhaging from the gills (Fig. 3.25) occurred only in fish which had been depressurized from 30 m simulated depth and left at the surface.

Table 3.8. Percent occurrence of external symptoms of barotrauma observed in mulloway during experiment 1 following removal from the chambers for each depth and treatment. *n* is sample size.

	Controls			**Repressurize**			**Leave at surface**		
Simulated Depth (m)	**10**	**30**	**overall**	**10**	**30**	**overall**	**10**	**30**	**overall**
n	*8*	*8*	***16***	*8*	*8*	***16***	*8*	*8*	***16***
Symptom									
Mortality	12.5	12.5	**12.5**		37.5	**18.8**		100.0	**50.0**
Exophthalmia					14.3	**7.2**		62.5	**31.3**
Corneal haemorrhage									
Corneal emphysema					12.5	**6.3**			
Skin haemorrhage									
Fin haemorrhage									
Gill haemorrhage								25.0	**12.5**
Bloodshot cloaca					37.5	**12.5**		87.5	**43.8**
Distended abdomen				50.0	37.5	**43.8**		100.0	**50.0**
Stomach eversion					37.5	**12.5**		100.0	**50.0**
Rippled skin									
Negatively buoyant					37.5	**12.5**		50.0	**25.0**

Figure 3.25. Haemorrhaging of gill filament blood vessels in mulloway following simulated capture from depth. Note blood in between gill filaments and accumulated along edge of gill arch.



3.3.2.3. Mortality

Using the quasi-binomial GLM to analyse death rates, there was a significant effect of depth and a significant interaction between treatment and depth (Table 3.9). No mortalities occurred for fish subjected to simulated C&R from 10 m regardless whether they were left at surface pressure, or repressurized to 10 m equivalent depth after 10 min (Fig. 3.26A). However, mortality was significantly higher (Table 3.9) for fish subjected to simulated C&R from 30 m: 87.5 ± 7.2 % for fish kept at surface pressure for 10 min and 100% for fish left at surface pressure (Fig. 3.26A).

Once again, the unexpected deaths of 38% of 10 m control fish and 25% of 30 m control fish (Table 3.10) impaired the ability of the model to find a statistically significant effect of treatment on mortality. Nonetheless, obvious trends were clearly evident: overall more fish died as a result of simulated capture (with or without release) from depth ('repressurize': 43.8 ± 12.2 %, 'leave at surface': 50.0 ± 13.6 %) compared with controls (31.3 ± 5.1 %). There was no statistical evidence of non-independence of outcomes between fish; the dispersion component was estimated to be 0.28, which suggested under-dispersion likely due to the small sample sizes involved. If the deviance tests are re-performed but with the conservative assumption that the dispersion parameter is 1, there are still significant interactions between depth and treatment (Table 3.9). If the treatment parameter is further broken down into the two contrasts with depth (see 3.2.2.2), only the control *versus* treatment contrast, and its interaction with depth, is significant. The 'leave at surface" *versus* 'repressurize' contrast was not significant indicating that there was no difference between the effects of either treatment on mortality.

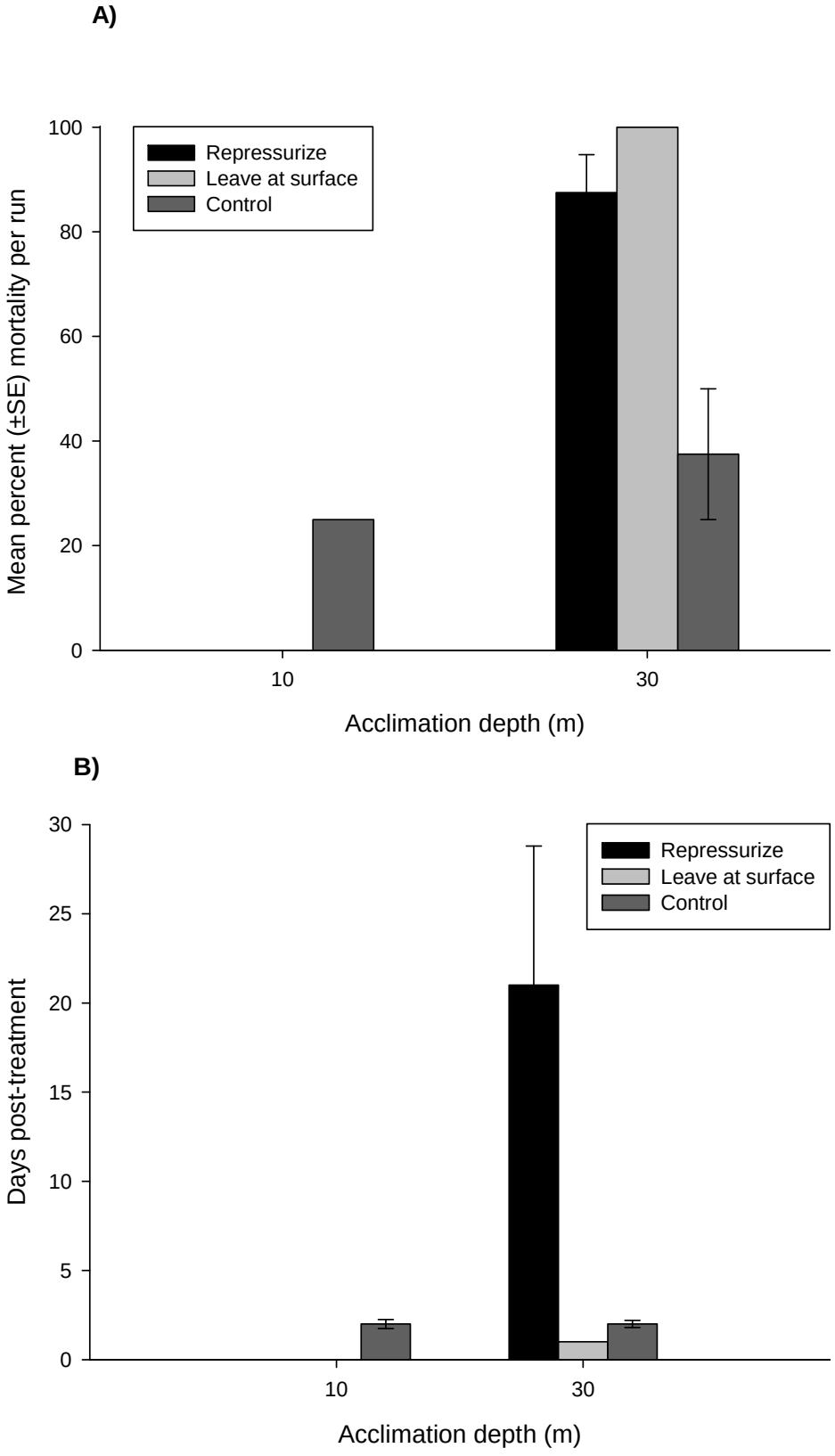
Table 3.9. Analysis of deviance table for the quasi-binomial GLM relating to the probability of a fish dying model for the second mulloway chamber experiment. T is treatment, C is control, S is the 'leave at surface' treatment, R is the 'repressurized' treatment and df is degrees of freedom. The estimated overdispersion parameter was 0.28. * indicates additional F-statistic and *P*-value calculated using the conservative overdispersion parameter of 1 (see text for explanation).

	df	Deviance	Residual df	Residual Deviance	F (F*)	*P*-value (*P**)
			11	41.70		
Depth	1	24.44	10	17.20	87.50 (24.40)	<0.0001 (0.003)
Treatment	2	2.26	8	15.00	4.04 (1.13)	0.077 (0.382)
T *v* C	1	1.99	9	15.23	7.14 (1.99)	0.037 (0.208)
S *v* R	1	0.27	8	14.96	0.95 (0.264)	0.368 (0.625)
Depth × Treatment	2	12.89	6	2.07	23.10 (6.44)	0.0015 (0.032)
Depth × T *v* C	1	12.89	7	2.07	46.10 (12.89)	0.0005 (0.012)
Depth × S *v* R	1	0	6	2.07	0 (0)	1 (1)

Table 3.10. Mortality of mulloway by chamber and run during experiment 2. There were four mulloway in each chamber for each run.

Run	Chamber 1	No. deaths	Days	Chamber 2	No. deaths	Days
1	30R	3	0, 39, 55	10R	0	
2	10C	1	1	30C	2	1, 2
3	10R	0		30S	4	0, 0, 0, 0
4	30R	4	0, 0, 20, 32	10S	0	
5	10S	0		30S	4	0, 0, 0, 0
6	10C	1	2	30C	1	2

Figure 3.26. Results of the second hyperbaric chamber experiment on mulloway where fish were acclimated to 10 or 30 m simulated depth and depressurized to surface pressure, before being either repressurized to acclimation pressure after 10 mins (■), or left at surface pressure (■): **A)** mean percent mortality (± SE), **B)** the mean number of days post-treatment (± SE) mortality occurred. Control bars (■).



3.3.2.4. Delayed mortality

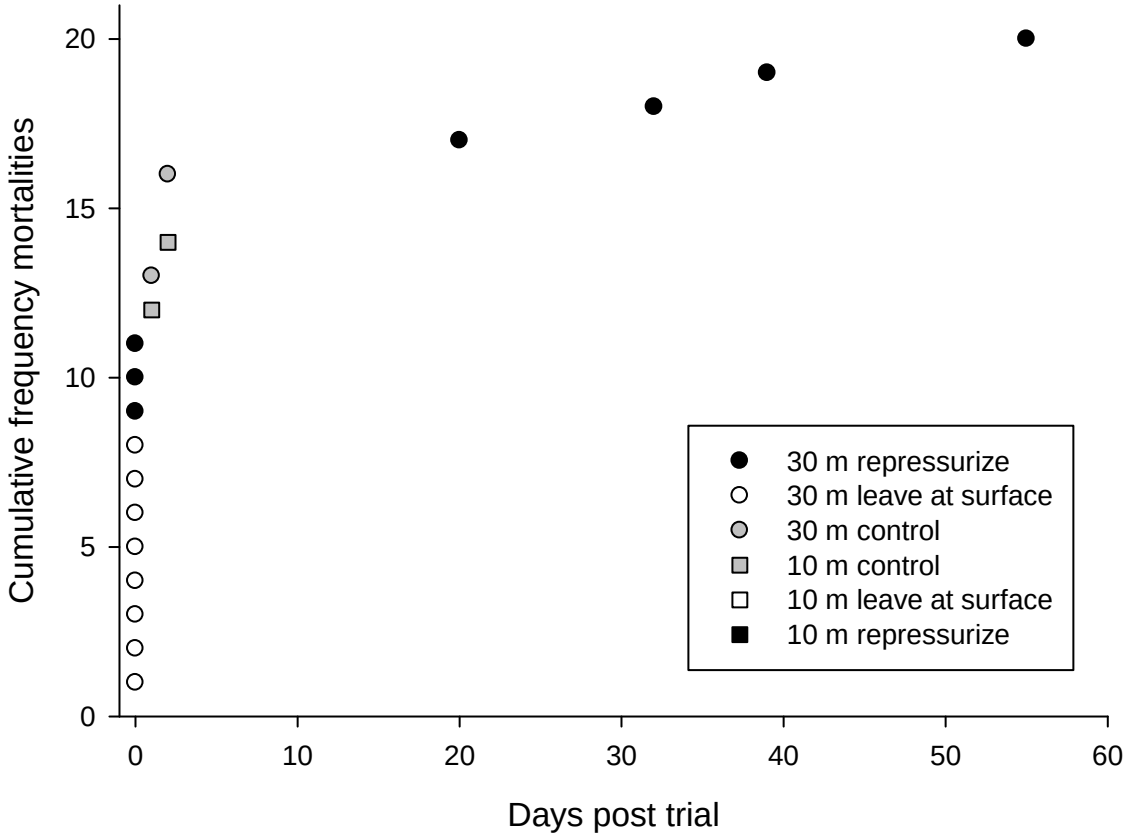
The semi-parametric Cox proportional hazards random effects model showed a significant effect of depth (because no fish acclimated to 10 m died) and a treatment by depth interaction, but no evidence of an overall treatment effect (Table 3.11). This result indicates that mortality in fish that were acclimated to 30 m depth and repressurized occurred significantly later than those left at surface pressure.

Death occurred almost immediately (within ~15 mins) for all fish left at surface pressure after simulated capture from to 30 m acclimated depth (Fig. 3.27). However, overall mortality for fish which were subject to simulated C&R from 30 m acclimated depth after spending 10 min at surface pressure was delayed by an average of 21 ± 8 days (range: 0-55 days) compared with fish left at surface pressure (Fig. 3.27). Three of these fish died before they could be repressurized (i.e. <10 min) and the remaining four fish died over the ensuing 55 days at the rate of approximately one fish every 9 days. There were no mortalities for fish after simulated capture from 10 m acclimated depth regardless whether they were repressurized to depth or left at surface pressure. The control fish which died (n=5) all died within 2 days of their removal from the chambers: two fish acclimated to pressure equivalent to 10 m depth and three fish acclimated to 30 m (Fig. 3.27).

Table 3.11. Analysis of deviance table for the semi-parametric Cox proportional hazards random effects model for the second mulloway chamber experiment. df is degrees of freedom.

	df	**Deviance**	**Residual Deviance**	***P*-value**
Null model		125.98		
Depth	1	115.35	10.63	0.001
Treatment	2	110.18	5.17	0.075
Depth × Treatment	2	97.84	12.34	0.002

Figure 3.27. Cumulative frequency of mulloway mortalities in experiment 2 for each depth and treatment.



3.3.2.5. Post-mortems

Post-mortems revealed that all treatment fish that died had multiple injuries consistent with rapid decompression (Table 3.12). Forty percent of control fish which died suffered haemorrhaging to the skin and fins. There were no mortalities of treatment fish acclimated to 10 m (either repressurized or left at surface pressure).

Table 3.12. Percent occurrence of injuries revealed by post-mortems performed on mulloway mortalities during experiment 2. *n* is the number of mortalities out of the original sample size for each depth and treatment. PCM is pharyngo-cleithral membrane.

	Controls			Repressurize			Leave at surface		
Simulated Depth (m)	**10**	**30**	**overall**	**10**	**30**	**overall**	**10**	**30**	**overall**
n	*3/8*	*2/8*	***5/16***	*0/8*	*7/8*	***7/16***	*0/8*	*8/8*	***8/16***
Symptom									
Exophthalmia					14.3	**7.2**		62.5	**31.3**
Corneal haemorrhage					14.3	**7.2**		12.5	**6.3**
Corneal emphysema									
Skin haemorrhage	33.3	50.0	**40.0**		28.6	**14.3**		75.0	**37.5**
Fin haemorrhage	33.3	50.0	**40.0**		66.7	**33.4**		12.5	**6.3**
Gill haemorrhage					42.9	**21.5**		100.0	**50.0**
PCM emphysema					28.6	**14.3**		100.0	**50.0**
PCM perforation					14.3	**7.2**			
Bloodshot cloaca					14.3	**7.2**		87.5	**43.8**
Distended abdomen					42.9	**21.5**		100.0	**50.0**
Swimbladder empty					71.4	**35.7**		75.0	**37.5**
Swimbladder hyperextension					14.3	**7.2**		37.5	**18.8**
Swimbladder perforation					28.6	**14.3**		100.0	**50.0**
Swimbladder scar tissue					57.1	**28.6**			
Viscera displacement					42.9	**21.5**		100.0	**50.0**
Viscera haemorrhage					14.3	**7.2**		37.5	**18.8**
Torn mesentery					42.9	**21.5**		75.0	**37.5**
Stomach eversion					42.9	**21.5**		100.0	**50.0**
Liver trauma					71.4	**35.7**		100.0	**50.0**
Hepatic vein damage					57.1	**28.6**		100.0	**50.0**
Splenomegaly					28.6	**14.3**		100.0	**50.0**

For fish which were repressurized after simulated capture from 30 m, every barotrauma symptom listed occurred in at least one fish which subsequently died. The most common injuries in this group were swimbladder perforation or scar tissue indicative of earlier perforation (86%), followed by liver trauma (71%), hepatic vein damage (57%), haemorrhaging from the gills, a distended abdomen and viscera displacement (all 43%). In addition, 71% of fish which died contained no swimbladder gas.

Every fish which was left at surface pressure following depressurization from pressure equivalent to 30 m depth died. All fish (100%) suffered haemorrhaging from the gills, formation of gas bubbles in tissue posterior to the gills (Fig. 3.28), swimbladder perforation, a distended abdomen, viscera displacement, severe stomach eversion (Fig. 3.29), liver trauma, hepatic vein damage and an enlarged spleen. In addition, 88% had a bloodshot cloaca, 75% torn internal mesentery, 63% had exophthalmia, 38% internal haemorrhaging and swimbladder hyperinflation and 13% corneal haemorrhaging. Seventy-five percent of fish contained no swimbladder gas.

Figure 3.28. A) Gas bubble formation in, and **B)** perforation of, the pharyngo-cleithral membrane in mulloway following simulated capture from depth. Arrows indicate clusters of gas bubbles or perforation in each image, respectively.

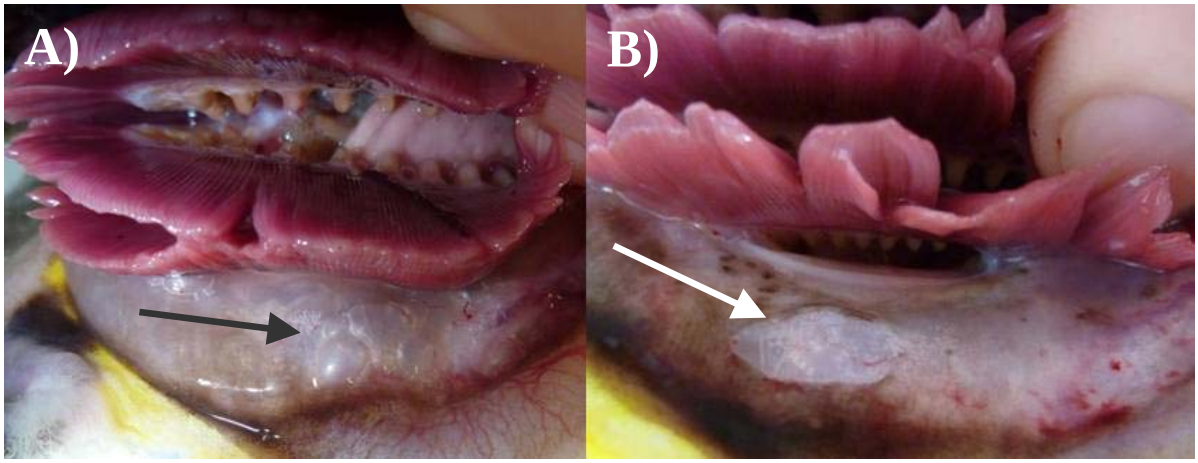
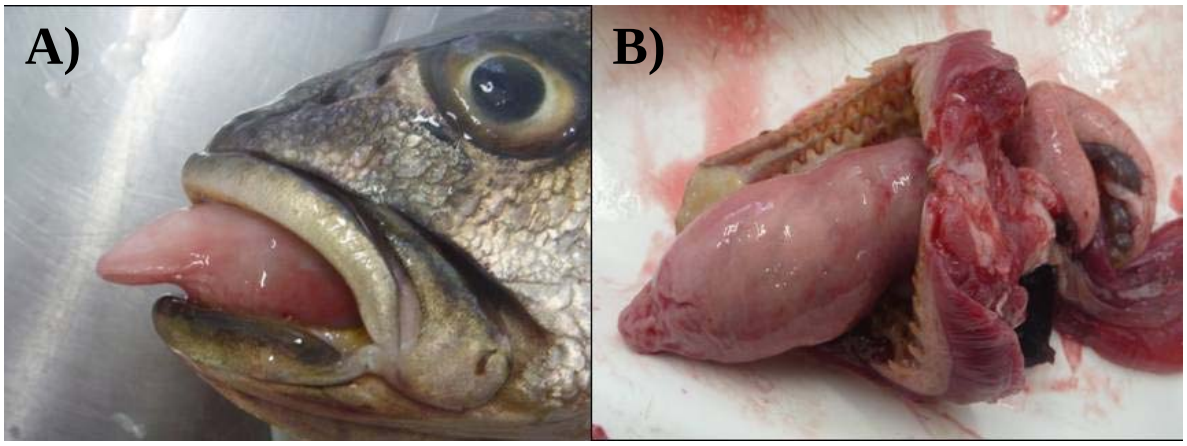


Figure 3.29. A) Severe stomach eversion and protrusion from the mouth in mulloway following simulated capture from depth, and **B)** dissected visceral mass showing everted stomach and displaced organs. Note than one lobe of the liver has been forced into the everted stomach.



3.4. Discussion

3.4.1. Mortality

The hyperbaric chamber experiments simulating the pressure changes experienced by mulloway during capture in this study have shown capture depth to be a significant factor in determining whether a fish survives following capture and release. In experiment 1, approximately half of the fish subjected to simulated C&R when acclimated to pressure equivalent to 30 and 50 m water depth subsequently died, compared with just 12.5% of fish acclimated to 10 m. Similarly, no mortalities occurred in experiment 2 when fish were acclimated to 10 m (and either left at surface pressure, or repressurized after 10 min), but almost all fish died in the same treatments when fish were acclimated to 30 m. Capture

depth has also been shown to be the critical factor affecting mortality in almost every study of the effects of barotrauma in fish with increasing mortality occurring with increasing depth (e.g. Gitschlag & Renaud, 1994; Wilson & Burns, 1996; Collins et al., 1999; McGovern et al., 2005; St. John & Syers, 2005). In addition to capture depth, these experiments have shown that the length of time a fish is held at the surface (surface duration or interval) to be an important determinant of post-release mortality in mulloway. In experiment 1, mortality of fish acclimated to 30 m and repressurized after a surface interval of 2 mins was 46%. In contrast, fish in experiment 2 acclimated to the same depth (30 m) but repressurized after a surface interval of 10 min suffered much higher (88%) mortality. Even when held in water at the surface, Burns et al. (2002) similarly found that mortality of reef fishes captured from 40 m ranged from 20% when held at the surface for 3 min to 100% when held at the surface for 18 min. Koenig (2001) also found a significant relationship between depth-related mortality and surface interval for various Mexican reef fishes. Jarvis & Lowe (2008) found the most significant predictor of short-term survival in rockfishes (*Sebastes* spp.) was surface holding time, with short-term survival increasing with decreasing surface holding time. Fish held at the surface for long periods of time may experience thermal stress which may have a detrimental synergistic effect when combined with reduced blood flow as a result of intravascular bubble formation following decompression, especially in warmer surface waters where oxygen demand is higher and oxygen concentration is lower (Feathers & Knable, 1983). Survival of mulloway after barotrauma therefore depends on both capture depth and surface interval.

3.4.2. In-chamber behaviour & symptoms

Observations of fish behaviour within the chambers also varied substantially with depth. During depressurization (simulated capture) in both experiments, fish acclimated to a pressure equivalent of 10 m water depth displayed distended abdomens as the expanding swimbladdder progressively occupied the available space within the body cavity. These fish became increasingly buoyant as a result of the reduced pressure. This in turn caused the fish to swim with a head-down attitude in order to remain near the bottom of the chamber. This behaviour has been previously reported for a number of other species when subjected to barotrauma inducing pressure changes in laboratory pressure chambers (Pribyl et al. 2012, Pflugrath et al., 2012). Despite this excessive buoyancy, even fish in experiment 2 which were decompressed from 10 m equivalent pressure and left at surface pressure regained normal buoyancy <24 h after decompression. This timeframe is well within the gas absorption rates calculated in Chapter 2. Excess gas in swimbladders of Western Australian dhufish *Glaucosoma hebraicum* has also been reported to diffuse in ~24 h (St. John & Syers, 2005). Fish acclimated to greater depths (i.e. 30 and 50 m) exhibited much more extreme behaviour in addition to that of the 10 m-acclimated fish. In these fish, acclimation to greater depths resulted in expansion of swimbladder gases into much larger volumes during depressurization, which post-mortems of mortalities revealed to have caused perforation of the swimbladder and release of gas directly into the body cavity. For these fish, the presence of such a large amount of gas in the gut cavity appeared to cause the fish extreme discomfort and resulted in rapid and haphazard swimming behaviour, gill flaring and head shaking. Pressure exerted on the alimentary tract eventually resulted in eversion of the stomach through the mouth. During decompression from 35 m equivalent depth in similar hyperbaric pressure chambers, rockfishes exhibited everted stomachs and exophthalmia, but an accurate count of the number of fish with each symptom could not be made because the fish were moving so rapidly during the surface

interval (Pribyl et al. 2012), just as occurred for mulloway in the present study. Rupture of the swimbladder and resultant sudden release of gas into the abdominal cavity has been shown to induce eversion and prolapse of the stomach in another Australian sciaenid, the black jewfish *Protonibea diacanthus* (Phelan, 2008). When the body tissues could no longer constrain the increasing volume of gas, it then escaped to the exterior through rupturing the body wall in the region between the pharynx and oesophagus via the pharyngo-cleithral membrane. A number of physoclistous fish species have been observed releasing gas bubbles when being brought to the surface (Pearcy, 1992; Nichol and Chilton, 2006; Hannah et al., 2008), suggesting terminal rupture and release of swimbladder gas to the exterior. Gas escaping from the gut cavity through the body wall in this region has also been reported to occur in several species of rockfish (Pearcy 1992, Hannah et al. 2008, Pribyl et al. 2009) and red emperor *Lutjanus sebae* (Brown et al. 2010).

Upon removal from the chamber, the number of fish in experiment 1 exhibiting a distended abdomen decreased with depth from 33% when acclimated to 10 m, to 21% at 30 m, to just 4% at 50 m. This occurred inversely with the numbers of fish which were negatively buoyant when anaesthetised (i.e. had lost swimbladder gas from the body cavity)- no fish acclimated to 10 m depth were negatively buoyant, but 17 and 29% were from 30 and 50 m acclimated depth, respectively. All rockfishes were also observed to be negatively buoyant due to swimbladder gas loss after being subjected to simulated C&R from 35 m depth in hyperbaric chambers (Pribyl et al., 2012). This suggests that the expansion of gas when depressurised from water deeper than 30 m in mulloway is great enough to cause at least some individuals to lose gas through body wall perforation and the numbers of fish to which this occurs increases with increasing depth. Swimbladder hyperinflation (resulting in abdominal bloating) were similarly recorded to occur in more black jewfish caught from 10-15 m deep water (92%) than from 15-20 m (16%) (Phelan, 2008). Clearly, expansion of swimbladder gases in mulloway acclimated to 10 m is not sufficient to cause rupture of the body wall to occur (see also Chapter 2), the result being a distended abdomen. The escape of swimbladder gas has also been suggested to reduce some of the internal injuries that can arise when contained swimbladder gases expand (Rummer & Bennett 2005, Hannah et al. 2008). Similarly, Brown et al. (2010) found that red emperor caught from greater depths which had vented gas in this way appeared to suffer less extreme barotrauma symptoms than fish from shallower depths which did not vent gas. In contrast to these studies, all barotrauma symptoms and injuries (with the exception of abdominal bloating) presented here increased in frequency and severity with increasing depth.

A similar pattern occurred in experiment 2, but in addition many other symptoms of barotrauma (corneal emphysema, exophthalmia, haemorrhaging from the gills, bloodshot cloaca and stomach eversion) occurred only in fish acclimated to 30 m compared with fish acclimated to 10 m. Corneal haemorrhaging, exophthalmia and stomach eversion also increased in frequency when black jewfish were caught from increasingly deep water (Phelan, 2008). These symptoms also occurred in far more 30 m-acclimated fish which were left at surface pressure following simulated capture than those that were repressurized. Exophthalmia, bloodshot cloacas, bloated abdomens and stomach eversion occurred in 63-100% of mulloway left at surface pressure, but in only 14-38% of repressurized fish suggesting that these symptoms (and immediate mortality) are mitigated slightly by repressurization to depth. Progressive increases in the incidence of swim-bladder over-inflation, gut eversion and exophthalmia with increasing capture depth have also been reported in Western Australian dhufish (St. John & Syers, 2005), tautog *Tautoga*

onitis (Lucy & Arendt, 2002), red snapper (Patterson et al., 2001) and rockfishes (Hannah & Matteson 2007, Hannah et al. 2008).

The prevalence and severity of external barotrauma injuries in mulloway increased with depth of capture and internal barotrauma injuries followed the same trend. Post-mortem examinations revealed that 100% of fish acclimated to 30 and 50 m which subsequently died in experiment 1 had suffered swimbladder perforation as a result of their simulated capture and ~70% of these fish did not contain any swimbladder gas. In contrast, the swimbladders of the few fish acclimated to 10 m which died were all fully inflated and showed no evidence of previous perforation. Similarly, swimbladder perforations were observed in only 3% of individuals of the closely related black jewfish caught from 10-15 m, but increased to 90% of fish caught from 15-20 m (Phelan, 2008). All other barotrauma injuries identified in mulloway by post-mortems (corneal haemorrhaging, perforation of the body wall, viscera haemorrhage, torn mesentery, stomach eversion, liver trauma, hepatic vein damage and splenomegaly) in both experiments occurred only in fish acclimated to 30 or 50 m. Positive relationships between the frequency and severity of internal traumas in red snapper also increased with increasing decompression depth and were significant from depths as shallow as 10 m in field studies (Gitschlag & Renaud, 1994; Dorf, 2003; Rummer & Bennett, 2005; Diamond & Campbell, 2009). Curiously, the incidence of internal barotrauma injuries like liver tissue trauma and hepatic vein damage decreased in frequency from 10-15 m to 15-20 m in black jewfish and was considered to be related to the increasing incidence of swimbladder rupture with increasing depth (Phelan, 2008). As with external symptoms, many internal barotrauma symptoms (gill haemorrhage, pharyngo-cleithral membrane emphysema, viscera displacement and haemorrhage, torn mesentery, liver trauma, hepatic vein damage and splenomegaly) occurred in more (75-100%) mulloway left at surface pressure than those that were repressurized (14-43%), again suggesting that these symptoms (and immediate mortality) are mitigated slightly by repressurization to depth.

Compared with the taxonomically, morphologically and ecologically similar black jewfish, which was considered to be highly susceptible to barotrauma (Phelan, 2008), mulloway in the present study appears to be more resilient. Both black jewfish landed from less than 10 m of water, and mulloway acclimated to 10 m in chambers, showed few signs of barotrauma (swimbladder hyperinflation/abdominal distension). The low levels of mortality seen for mulloway here concur well with the conclusions of Phelan, (2008) that all black jewfish landed from water <10 m deep should survive if handled and released properly. However, the mortality of mulloway acclimated to greater depths (30 and 50 m) of between 46 and 50% was similar to that estimated for black jewfish caught from just 10-15 m deep water (48%), and much lower than estimated 100% mortality from 15-20 m deep water, where post-release survival was considered unlikely due to the likely effect and severity of injuries sustained during capture (Phelan, 2008). Severe injuries considered likely to be fatal or life-threatening in black jewfish caught from >10 m water depth in the wild were liver trauma, hepatic vein damage, viscera displacement and splenomegaly (Phelan, 2008) and occurred in similar proportion of fish as did the same injuries in mulloway acclimated to >10 m water pressure in the chambers.

3.4.3. Delayed mortality

This series of experiments on mulloway have also highlighted the importance of understanding the timescale over which mortality caused by C&R from deep water occurs in this species. In experiment 1, where all fish were repressurized after a 2 min surface interval, there was no immediate mortality whatsoever and all mortality was delayed (by an average of 64 days after simulated C&R). The first mortality occurred 5 days after simulated C&R and continued at the rate of approximately one fish every 4 days up to 81 days after removal from the chambers. After 81 days, even though the rate of mortality for both these groups slowed considerably, mortality continued to occur up to 219 days later. In contrast, in experiment 2, where all fish were repressurized after a much longer (10 min) surface interval or left at surface pressure, most mortality was immediate. Diamond & Campbell (2009) similarly found that immediate mortality of red snapper was 17%, while 64% of the fish which survived the discard process later died. Factors predicting immediate mortality were related to the environmental conditions of capture, such as depth and thermal stress, while delayed mortality was related to the condition of the fish, including injury, barotrauma, and behavioural impairment (Diamond & Campbell, 2009). Delayed mortality in fish with stomach eversion may be a result of internal organ torsion associated with the occurrence of stomach eversion and (or) internal organ damage resulting from the overinflated swimbladder crushing organs (Keniry et al., 1996; Rummer & Bennett, 2005; Jarvis & Lowe, 2008).

Much of the consistent delayed mortality which occurred up to 81 days post-treatment in experiment 1 was likely due to loss of condition resulting in increased susceptibility to infections and parasites. This loss of condition occurred as a result of the constant swimming behaviour exhibited by fish which had lost their swimbladder gas through the body wall and therefore swam continuously to generate hydrodynamic lift in order maintain position in the water column to compensate for their lack of buoyancy usually provided by the inflated swimbladder. It has been shown that the more the swimbladder volume deviates from its optimal volume at a given depth, the higher the compensatory swimming speed must be (Strand et al., 2005), indicating that a swimbladder containing no, or little gas, as occurred in this study, would require substantial compensatory swimming behaviour and consequent energy expenditure. This mechanism has been previously suggested to also lead to delayed mortality in various reef fish species via progressive loss of condition as a result of an inability to meet the energetic demands required to regulate their position in the water column by actively swimming if they have damaged swimbladders (Burns et al., 2002). In the wild, many such fish would swim away apparently healthy only to succumb to disease, predation or starvation potentially long after C&R. Burns et al. (2002) suggested for a water-column species, like vermilion snapper *Rhomboplites aurorubens*, predation on released individuals would be high because of their inability to maintain position in the water column. Therefore, even though physoclistous swimbladders can heal (Burns et al., 2002), they would be susceptible to epibenthic predators until their bladders healed sufficiently for them to return to their normal habitat a few metres above the bottom. Long term behavioural impairment, such as a decreased ability to catch food and to avoid predators has previously been suggested to have the potential to cause delayed mortality due to the physical trauma and physiological imbalance associated barotrauma in walleye pollock *Theragra chalcogramma* caught in bottom trawls (Ryer, 2002; Ryer et al., 2004). Stress associated with barotrauma produced stress levels in individual red snapper which resulted in them being less capable than their counterparts in responding to and escaping from predators (Campbell et al., 2010). Decreased performance with increasing stress level has also been demonstrated for juvenile sablefish *Anoplopoma fimbria* and walleye pollock and has been associated with

elevated predation mortality in the laboratory (Ryer, 2002; Ryer et al., 2004). Results from these experiments showed delayed mortality can be much higher than immediate mortality in mulloway, making it likely that surface observations of a mulloway's ability to submerge in the wild may substantially underestimate discard mortality in this species.

4. SNAPPER CHAMBER EXPERIMENTS

4.1. Introduction

Snapper *Pagrus auratus* (Bloch & Schneider, 1801) are distributed throughout the Indo-Pacific region (MacDonald, 1982). In Australia, snapper are a sub-tropical and temperate species distributed from approximately Hinchinbrook Island in Queensland, around the south of the continent, to approximately Barrow Island in Western Australia (Wakefield, 2006). Similar to mulloway, juvenile snapper are found almost exclusively in estuarine and inshore waters, while adults are mainly inshore and generally found in waters less than 100 m deep. Snapper can grow to more than 1.2 m in length (MacDonald, 1982) and in Australian waters have been reported to live for more than 40 years (Norriss & Crisafulli, 2010). Sexual maturity in NSW varies with latitude, being at approximately 22 cm fork length (FL) and 1.7 years in northern NSW and at approximately 27 cm FL and 3 years in southern NSW (Stewart et al., 2010).

In NSW snapper are assessed as being "growth overfished" (Rowling et al., 2010), meaning that on average snapper are being harvested at sizes which are too small to optimize yield. In addition, the snapper fishery in NSW appears to be based on too few, young, age classes to ensure resilience. In NSW it is estimated that approximately half the total harvest is taken by recreational fishers. Current management arrangements for snapper in NSW include a minimum legal length of 30 cm total length (TL) and a recreational bag limit of ten fish per person.

Substantial numbers of snapper are released following capture by recreational fishers. The most recent estimate is that approximately 78% of all snapper captured by recreational fishers in NSW are subsequently released (Henry and Lyle, 2003). There is considerable pressure in NSW, mainly from the recreational fishing lobby, to both increase the minimum legal length (MLL) and also to consider a slot-limit for snapper. Any such changes to the regulations would see an increase in the proportion of snapper needing to be released following capture. It is therefore vital that research be done into whether snapper survive following capture and subsequent release.

Some work has been done investigating the survival of snapper following capture by line fishing and subsequent release, however these studies have largely ignored the effect of capture depth. Two short-term confinement studies on small juvenile snapper report post-release mortality rates of between 8 and 33% (Broadhurst et al., 2005; Grixti et al., 2010). Butcher et al. (2012) attempted to simulate capture of snapper from depths to 20 m by keeping them in cages at those depths and hauling them to the surface. They observed no mortalities after 3 days. In contrast, Stewart (2008) reported capture depth to be a significant factor in determining the survival of commercially trap-caught snapper. Mortality increased from 0% in <21 m water depth, to ~2% in <30 m water depth, 39% between 30 and 44 m and 55% between 45 and 59 m. Given the observed rapid increase in mortality in trap-caught snapper at depths >~30 m (Stewart, 2008), it is imperative that survival of line-caught snapper from depths >30 m be examined. Butcher et al. (2012) reported symptoms of barotrauma in all recreationally captured snapper from waters >20 m

suggesting that barotrauma may have negative effects on all snapper captured from water depths of >20 m.

4.2. Materials & methods

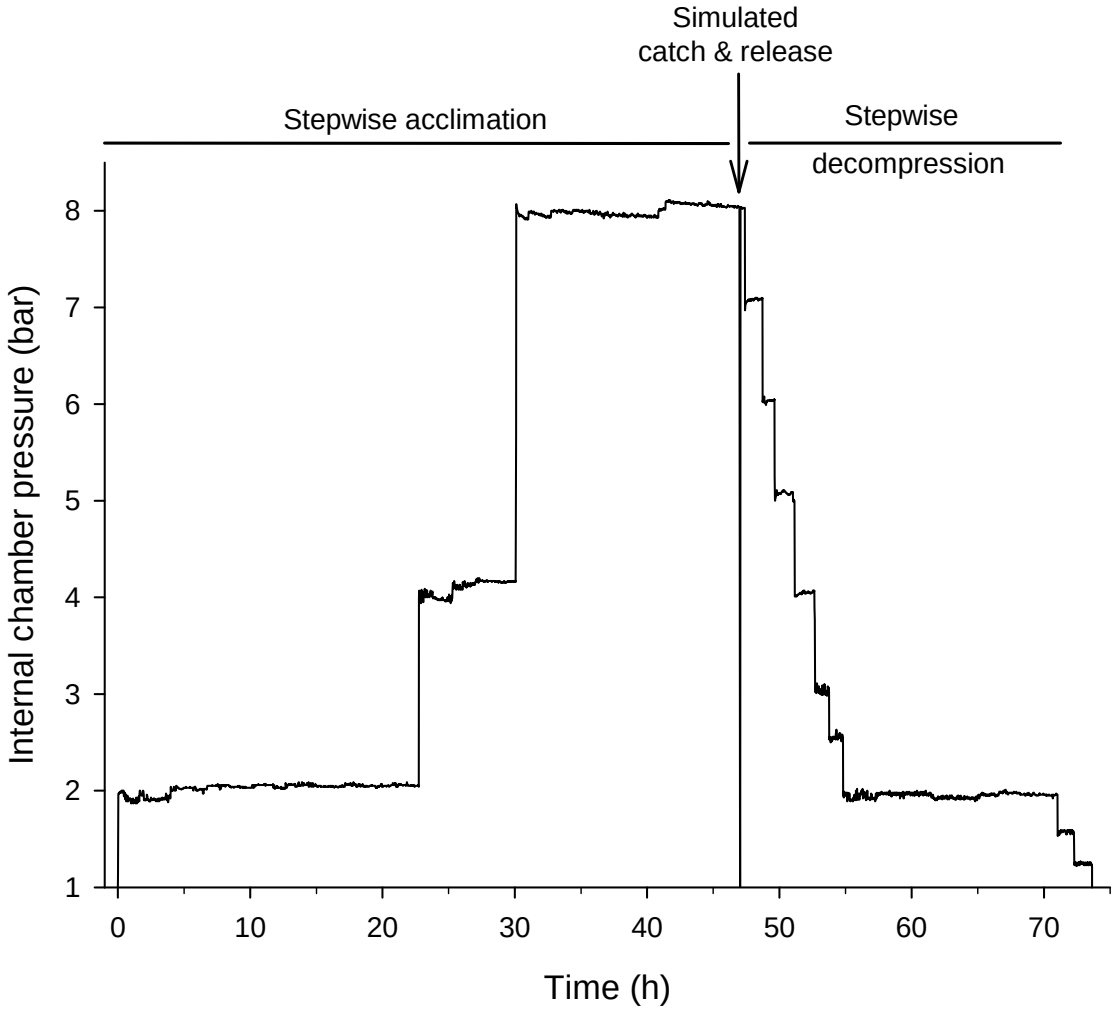
Snapper for use in chamber trials were caught using baited hook and line from shallow water (<10 m deep) in Port Hacking (34º04'24"S, 151º07'43"E) between 19 January and 19 March, 2010. All animals were transferred to the Cronulla Fisheries Research Centre Aquarium Facility and housed in a circular 35,000 L recirculated seawater tank. Prior to use in experiments, fish were transferred into circular 5,000 L tanks, a few at a time and tagged with numbered t-bar tags to allow individual fish to be indentified. All experiments were done using the custom-built pressure chambers described above between June and October 2011 at water temperatures of 16 to 19 ºC.

4.2.1. Experiment 1

The first experiment was a pilot study where we simulated capture and release and subsequent return to acclimation depth following a short surface interval of fish from the maximum water depth the chambers were capable of simulating (70 m) and monitored their short- and long-term survival. This was done in order to ascertain whether it was useful to carry out subsequent experiments on snapper which examined the effect of capture depth on survival after simulated C&R.

Following introduction of the experimental fish, the chambers were completely filled, closed and pressurized to an equivalent of 10 m water depth (2.02 bar) approximately 10 min later. According to our estimated swimbladder gas secretion rate for snapper (0.027 ml/kg/min), stepwise changes to the pressure within the chambers were done so that all fish were fully acclimated to experimental depths. The pressure was changed to equivalent 30 m water depth (4.04 bar) after 24 h for fish being acclimated to 30 m depth, and to 70 m (8.08 bar) after a further 7 h. An example of the internal chamber pressure changes which occurred during a trial is given in Fig. 4.1.

Figure 4.1. An example of a typical hyperbaric chamber depth profile for snapper during experiment 1.



Once acclimated to depth (after a total of 48 h), treatment fish were subjected to simulated capture by reducing the pressure in the chambers to surface pressure (1.01 bar) at the rate of approximately 1 m/s. The chamber remained at this pressure for 2 mins before being repressurized to acclimation depth at the rate of approximately 1 m/s to simulate the release of the fish and subsequent return to capture depth. Control fish were acclimated to depth as for treatment fish, but were not subjected to this simulated capture and release.

According to our estimated swimbladder gas resorption rate for snapper (0.309 ml/kg/min), stepwise depressurizations based on Boyle's Law were done so that all fish became fully acclimated to each new depth before further depressurization occurred. This ensured that fish could be brought back to surface pressure without causing further barotrauma. This depressurization took a total of 27 h from 70 m equivalent depth (8.08 bar), including 16 h at 10 m equivalent depth (2.02 bar).

Once the chambers had been returned to surface pressure, the fish were removed as described above and their tag number, FL (to the nearest mm) and barotrauma symptoms recorded. The fish were then transferred into a circular 5,000 L fibreglass tank for short term monitoring before being released into a separate large mesh pen within the

recirculated seawater pond. When a fish died, the date of mortality and tag number was recorded and an autopsy was performed to look for internal barotrauma-related injuries which may have contributed to death.

4.2.1.1. Sampling Design

As in the mulloway experiments, the two chambers were used simultaneously and the pressure in each chamber adjusted independently. The pilot study consisted of four runs containing six randomly assigned treatment runs and two control runs, constrained so that one control run and three treatment runs were carried out in each chamber (Table 4.1). Two fish were used in each chamber each run, so there were a total of 12 treatment fish and 4 control fish (Table 4.1).

Table 4.1. Experimental design for snapper in experiment 1. Simulated acclimation depth for all runs was 70 m, C = control and T = treatment.

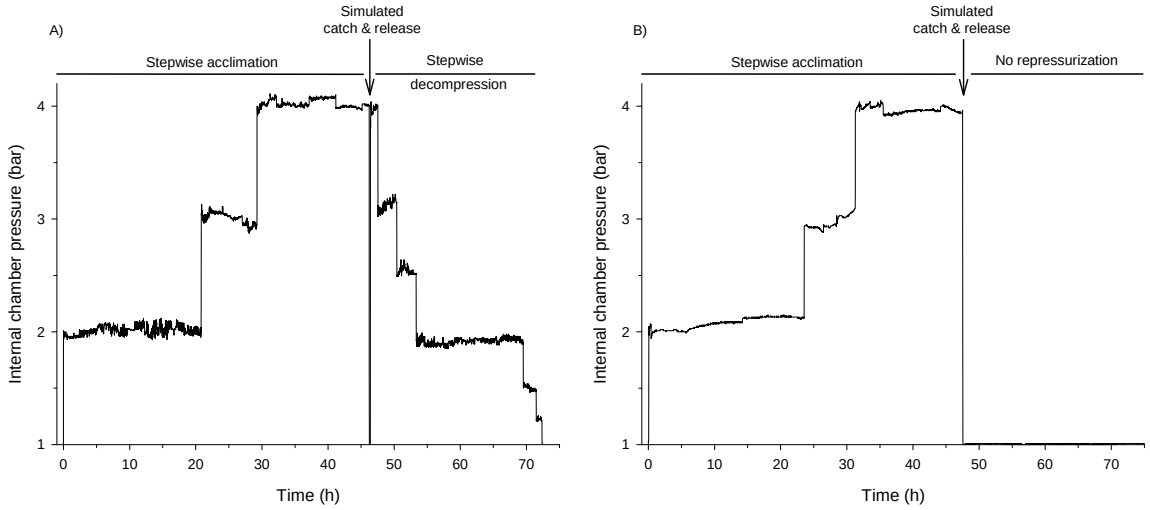
Run	Chamber 1	Chamber 2
1	C	T
2	T	T
3	T	C
4	T	T

4.2.2. Experiment 2

As there were no mortalities of either treatment or control fish in experiment 1 (see Results), in the second experiment we examined the effect of surface duration on mortality. There were two treatments: “repressurized” - where fish were returned to acclimation depth following a long surface interval, and “leave at surface” - where fish were kept at surface pressure for the remainder of the trial (to simulate fish unable to return to depth). Capture was simulated from only one experimental depth (30 m) and short- and long-term survival was monitored. Pressure equivalent to 30 m depth was chosen as the experimental depth as field observations showed barotrauma injuries in snapper caught from 30 m to be significant.

Following introduction of the experimental fish, the chambers were completely filled, closed and pressurized to an equivalent of 10 m water depth (2.02 bar) approximately 10 min later. According to our estimated swimbladder gas secretion rate for snapper (0.027 ml/kg/min), stepwise changes to the pressure within the chambers were done so that all fish were fully acclimated to experimental depths. The pressure was changed to equivalent 20 m water depth (3.03 bar) after 23 h and 30 m water depth (4.04 bar) after 31 h to fully acclimate the fish to 30 m depth. Examples of the internal chamber pressure changes which occurred during a trial is given in Fig. 4.2.

Figure 4.2. An example of a typical hyperbaric chamber depth profile for snapper during experiment 2 for: **A)** "repressurized" and **B)** "leave at surface" treatments.



Once acclimated (after a total of 47 h), both groups of treatment fish were subjected to simulated capture by reducing the pressure in the chambers to surface pressure (1.01 bar) at the rate of approximately 1 m/s. For the "repressurized" treatment, the chamber remained at this pressure for 10 mins before being repressurized to acclimation depth at the rate of approximately 1 m/s to simulate the release of the fish and subsequent return to capture depth. The "leave at surface" group were left at surface pressure for the remainder of the trial. Control fish were acclimated to depth as for treatment fish, but were not subjected to this simulated capture and release. A video camera mounted on the outside of the chamber recorded the behaviour of the fish during experimental treatments through the lower observation port (Fig. 4.3).

According to our estimated swimbladder gas resorption rate for snapper (0.309 ml/kg/min.), stepwise depressurizations based on Boyle's Law were done so that all "repressurized" treatment and control fish became fully acclimated to each new depth before further depressurization occurred, including 16 h at 10 m equivalent depth. This ensured that fish could be brought back to surface pressure without causing further barotrauma. This depressurization took 27 h from 30 m equivalent depth (4.04 bar).

Once the chambers had been returned to surface pressure, the fish were removed as described above and their tag number, FL (to the nearest mm) and barotrauma symptoms recorded. The fish were then transferred into a circular 5,000 L fibreglass tank for short term monitoring before being released into a separate large mesh pen within the recirculated seawater pond. When a fish died, the date of mortality and tag number was recorded and an autopsy was performed to look for barotrauma-related injuries which may have contributed to death.

4.2.2.1. Sampling Design

Two chambers were used simultaneously with separate borehole pumps and diaphragm valves so that the pressure in each chamber could be adjusted independently. The

experiment consisted of a "repressurize" treatment (R), a "leave at surface" treatment (S) and controls (C). Two blocks of five runs were used with four replicate runs for each of the two treatments and two control runs in each block. The order of treatment and control runs to chambers was allocated by a process of constrained/structured/blocked randomisation in order to ameliorate the impact of any systematic variation between runs and chambers (Table 4.2). The randomisation was also constrained to ensure that each of the treatments and controls were used the same number of times in each chamber. Four fish were used in each chamber each run, so there were a total of 32 fish for each of the treatments and 16 control fish (Table 4.2).

Table 4.2. Experimental design for snapper in experiment 2. Simulated acclimation depth for all runs was 30 m, C = control, R = "repressurize" and S = "leave at surface.

Block	Run	Chamber 1	Chamber 2
	1	R	S
	2	S	R
1	3	R	C
	4	C	S
	5	S	R
	6	R	C
	7	S	R
2	8	C	S
	9	S	R
	10	R	S

4.2.2.2. Statistical Analyses

To examine effects of treatment, the chamber was treated as the experimental unit, to allow for possible non-independence in the outcomes for individual fish in the same chamber at the same time. The data analysed consisted of counts of the number of fish which died (out of four) in each run in each chamber. The probability of a fish dying was modelled as a function of treatment (R, S or C) on an underlying logistic scale using a generalized linear model (GLM). To allow for additional variation in the death rates between runs and chambers, above that expected from binomial distributed data with treatment effects only, an overdispersion component was fitted using the quasi-binomial family with the 'glm' function in 'R' (R Development Core Team, 2010). This overdispersion component allows for lack of independence between fish in the same chamber which could result in such additional variation between runs and chambers.

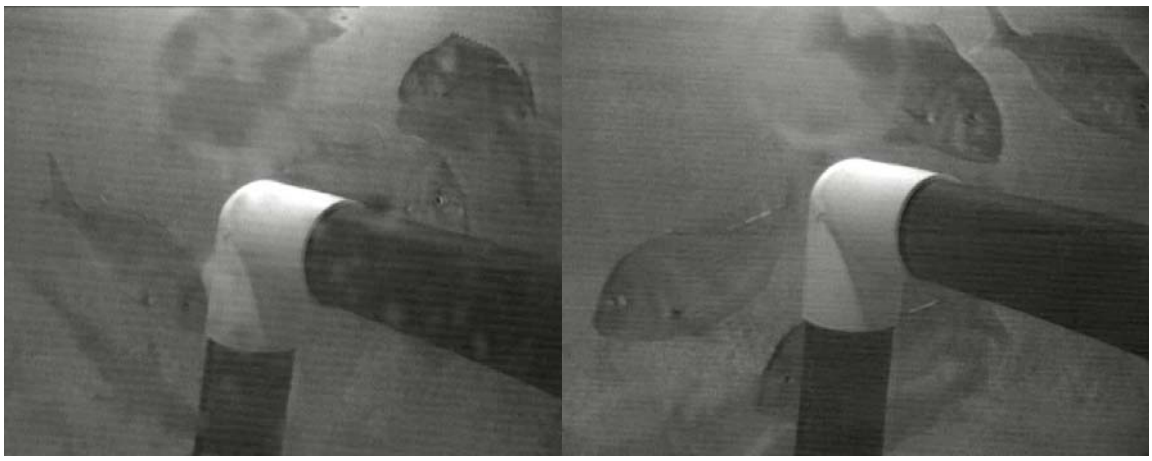
4.3. Results

4.3.1. Experiment 1

4.3.1.1. In situ observations

Video footage recorded during experimental treatments revealed that the most common behaviour exhibited by fish whilst in the chambers was slow swimming (Fig. 4.3) and resting on the bottom. This was consistent over periods when the pressure was steady as well as during stepwise pressurization to, and depressurization from, acclimation depth for both treatment and control fish.

Figure 4.3. Two stills from video footage captured within the chamber of snapper exhibiting normal swimming behaviour.



During simulated capture and release, however, fish became increasingly agitated as the pressure progressively decreased as they became excessively buoyant and haphazardly rushed around the tank attempting to remain near the bottom (Fig. 4.4). During decompression and once at surface pressure, all treatment fish displayed a distended abdomen (Fig. 4.5). Head shaking and gill flaring followed by stomach eversion into the buccal cavity (Fig. 4.6) preventing the fish from closing their mouths was observed in some individuals. Bloodshot cloacas (Fig. 4.7) were also observed. Clusters of bubbles were also recorded exiting the body cavity of many treatment fish via the tissue around the cloaca (Fig. 4.8), largely relieving the excess buoyancy of these fish, which subsequently swam slowly around the chamber or rested on the bottom. Fish which did not expel bubbles in this way remained excessively buoyant and continued swimming with a head down attitude until they eventually floated, exhausted, upside down against the roof of the chamber (Fig. 4.9). Upon repressurization to acclimation depth the buoyancy of these fish decreased and they slowly descended to the bottom of the chamber (Fig. 4.10); many rested on the bottom ventilating quickly (Fig. 4.11) whilst others attempted to maintain neutral buoyancy in the chamber by swimming constantly with a head-up attitude (Fig. 4.12).

Figure 4.4. Two examples of snapper attempting to overcome excess buoyancy by swimming downwards towards the bottom of the chamber taken from video footage captured during simulated catch (depressurization).

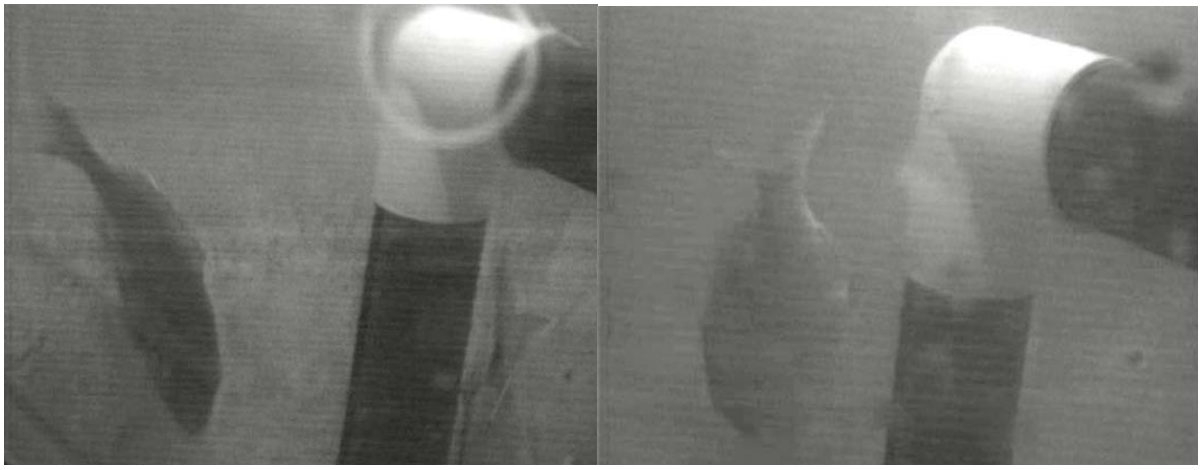


Figure 4.5. Two examples of snapper exhibiting distended abdomens taken from video footage captured following simulated capture (depressurization).

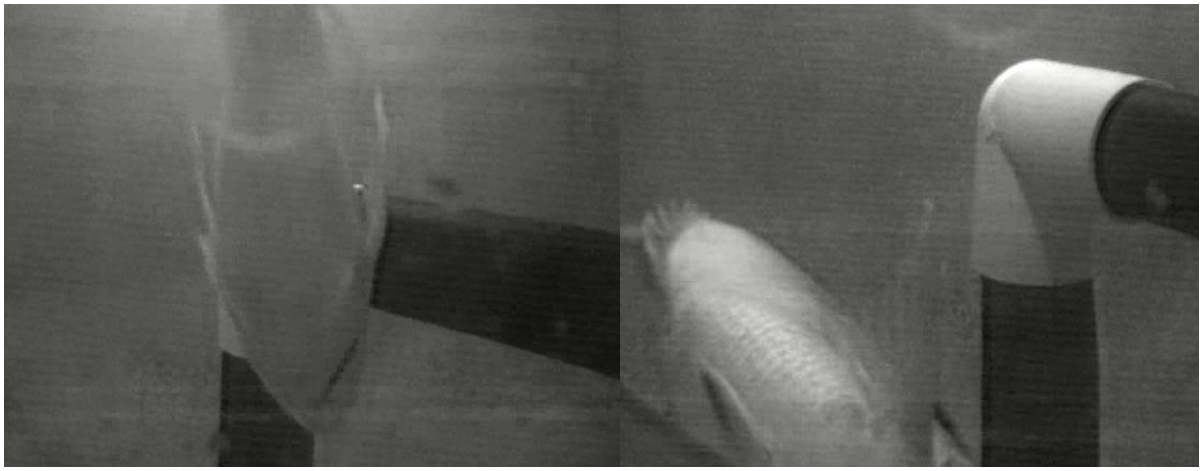


Figure 4.6. A sequence of stills from video footage captured within the chamber during simulated catch (depressurization) of snapper showing gill flaring and head shaking followed by stomach eversion (circled).

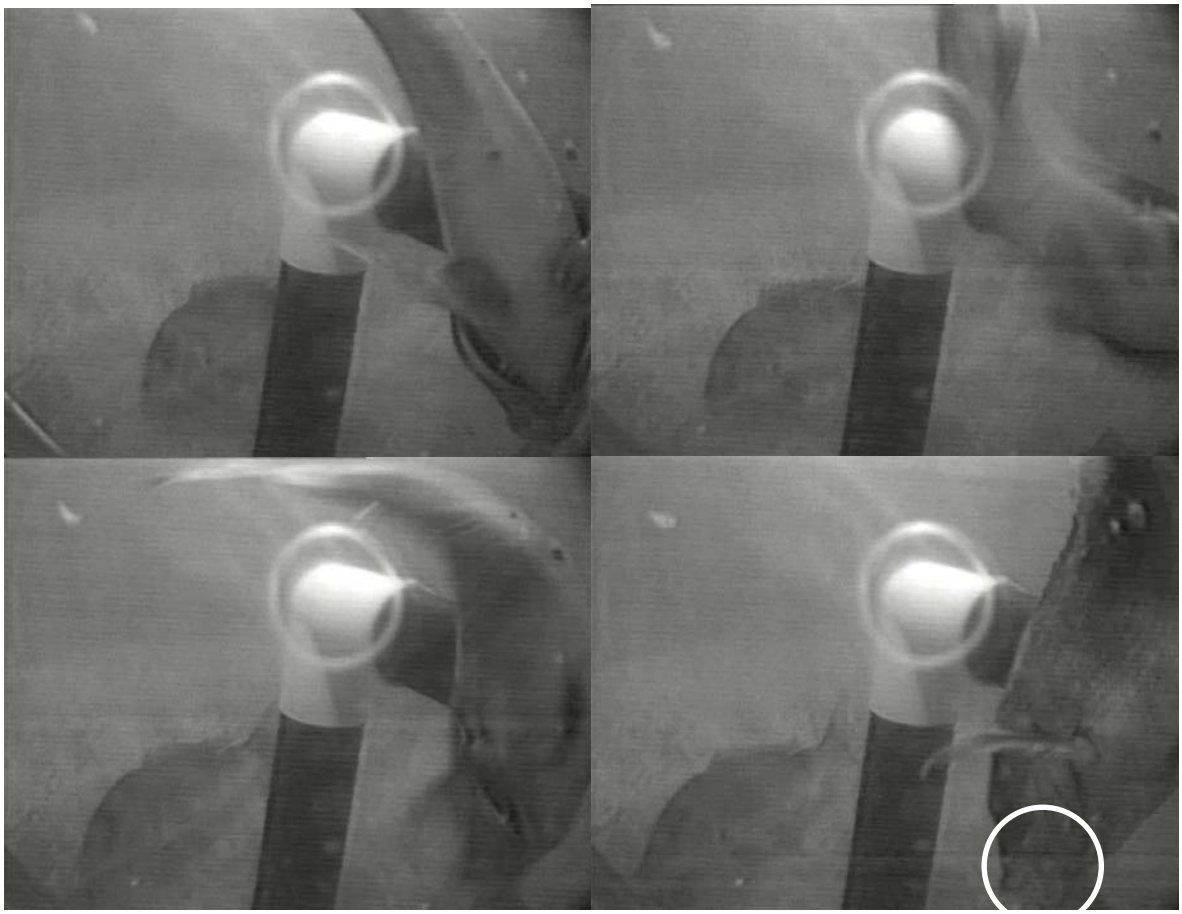


Figure 4.7. Two examples of bloodshot cloacas in snapper from video footage captured within the chamber during simulated catch (depressurization).

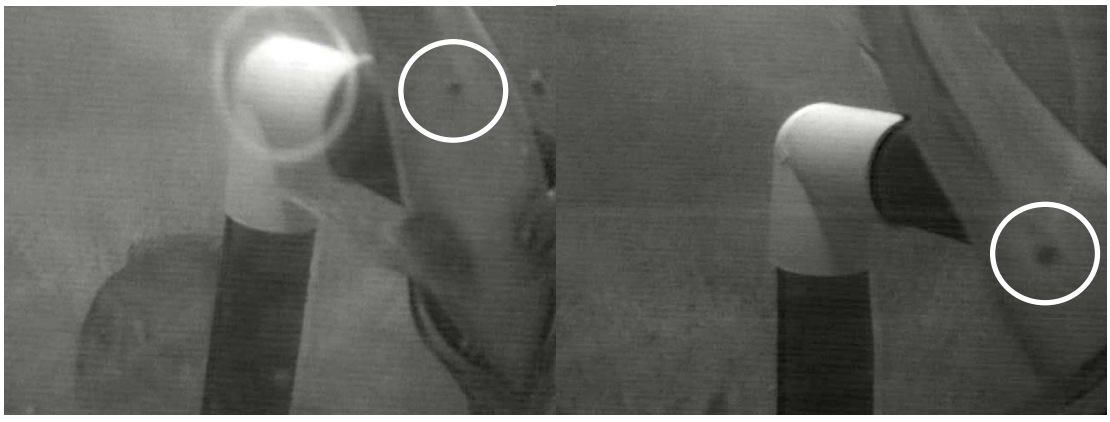


Figure 4.8. A sequence of stills from video footage captured within the chamber during simulated catch (depressurization) showing a snapper with an everted stomach exhibiting head shaking and gill flaring followed by release of gas bubbles (circled) from tissue surrounding the cloaca.

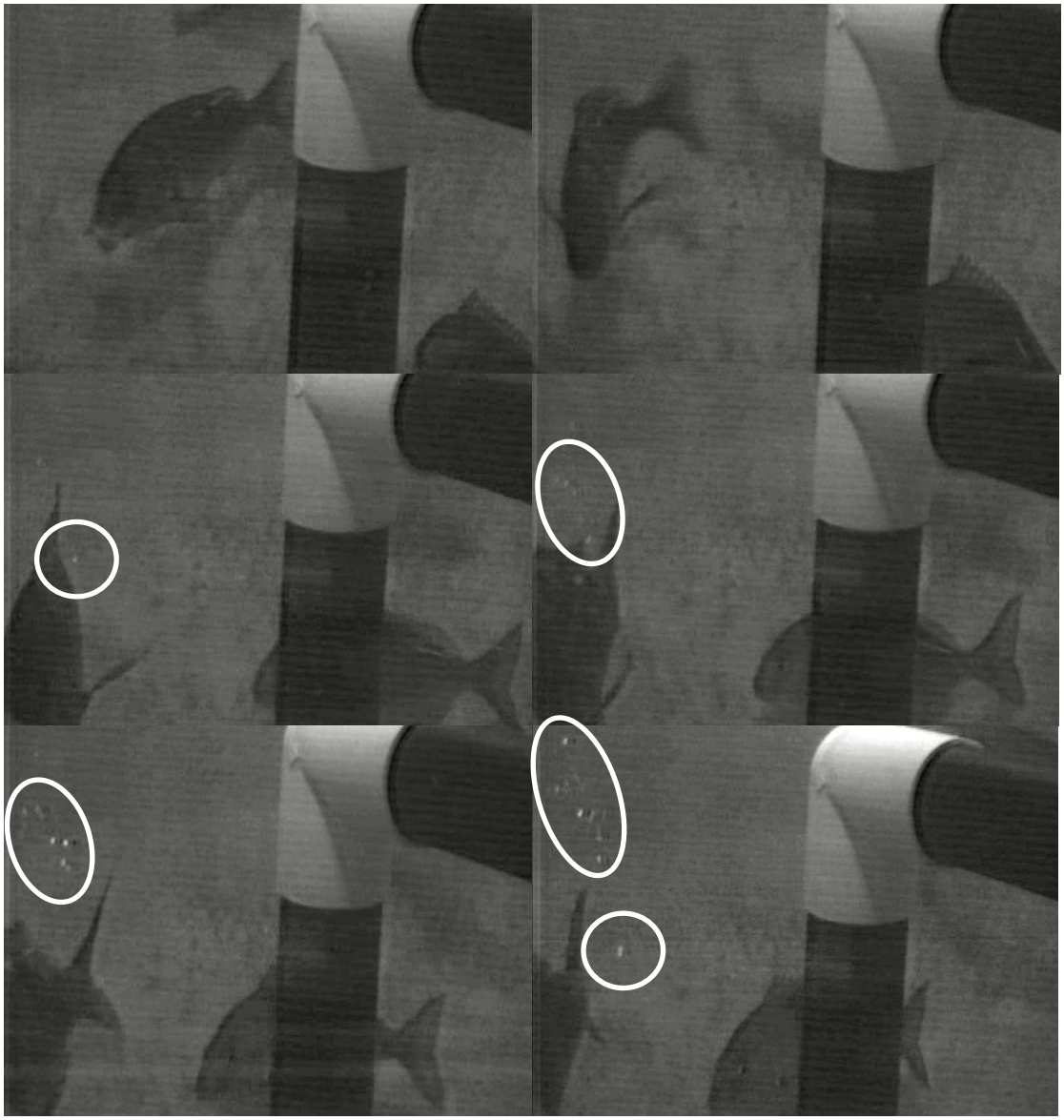


Figure 4.9. Stills taken from video footage captured within the chamber during simulated catch (depressurization) showing excessively buoyant snapper floating upside down towards the roof of the chamber.

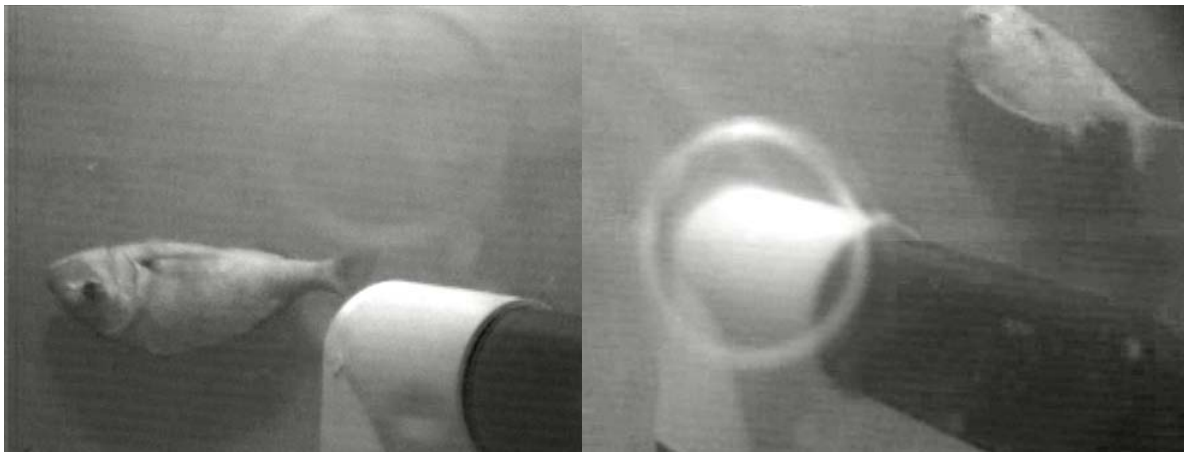


Figure 4.10. Stills taken from video footage captured within the chamber following simulated release (repressurization to 30 m equivalent depth) of snapper after 10 mins at surface pressure showing slow descent to the bottom of the chamber and cessation of ventilation.

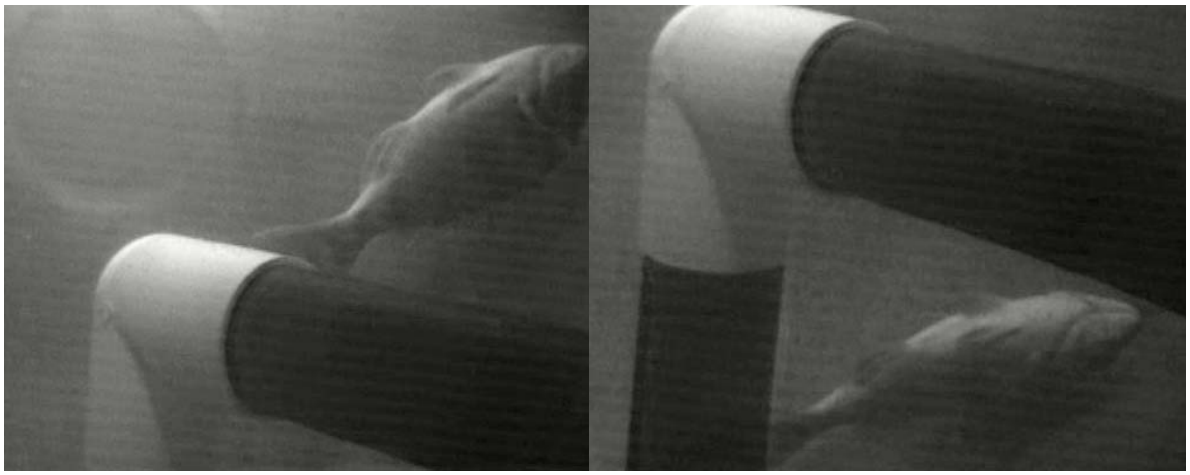


Figure 4.11. Stills taken from video footage captured within the chamber following simulated release (repressurization to 30 m equivalent depth) of snapper after 10 mins at surface pressure showing fish resting on the bottom of the chamber (the fish in the right hand image had ceased ventilation entirely).

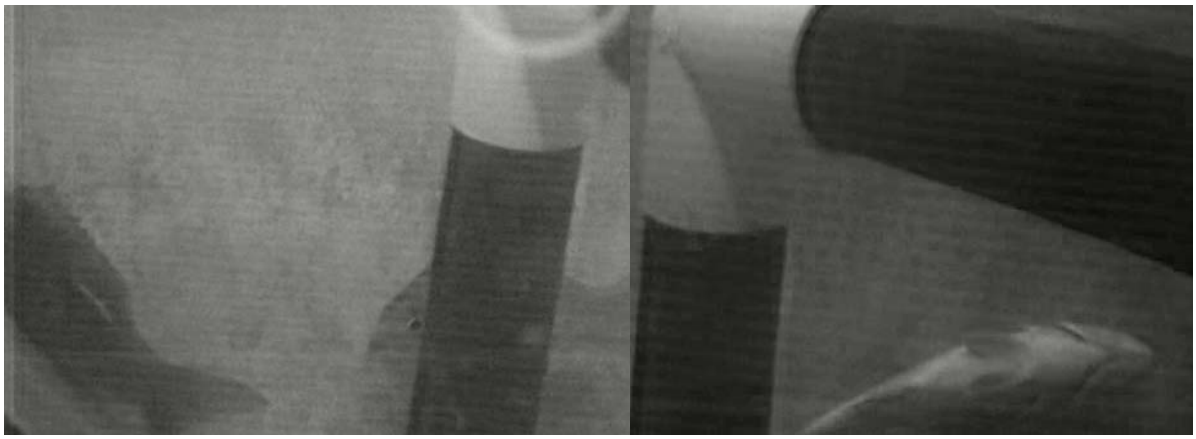


Figure 4.12. Two examples of snapper attempting to maintain neutral buoyancy by swimming upwards towards the top of the chamber taken from video footage captured following simulated release (repressurization).

4.3.1.2. Barotrauma symptoms

Upon removal from the chamber at the cessation of the trials, the only symptom exhibited by both control and treatment fish were distended abdomens (Fig. 4.5, Table 4.3).

Table 4.3. Percent occurrence of external symptoms of barotrauma observed in snapper during experiment 1 following removal from the chambers for each treatment. *n* is sample size.

	Controls	**Treatments**
Simulated Depth (m)	**70**	**70**
n	*4*	*12*
Symptom		
Mortality		
Exophthalmia		
Corneal haemorrhage		
Corneal emphysema		
Skin haemorrhage		
Fin haemorrhage		
Gill haemorrhage		
Bloodshot cloaca		
Distended abdomen	50.0	41.7
Stomach eversion		
Rippled skin		
Negatively buoyant		

4.3.1.3. Mortality

There were no mortalities of either control or treatment fish.

4.3.2. Experiment 2

4.3.2.1. In situ observations

As recorded for Experiment 1, video footage showed that slow swimming and resting on the bottom were the most common behaviour exhibited by fish whilst the internal chamber pressure was steady or being slowly adjusted. Fish behaviour was also similar to that reported in Experiment 1 during simulated capture from pressure equivalent to 30 m water depth in both treatment ('repressurize' and 'leave at surface') groups (i.e. excessive buoyancy, firm abdomen, head shaking, gill flaring, stomach eversion, bloodshot cloacas, erratic head-down swimming, expulsion of bubbles from the tissue surrounding the cloaca).

Upon return to acclimation depth after 10 mins at surface pressure, 'repressurized' fish which had expelled bubbles swam slowly around the chamber with a head-up attitude in order to remain in midwater. Those fish which had not expelled bubbles returned to normal chamber behaviour. Fish which had become exhausted by constantly swimming head down that were floating upside down against the roof of the chamber slowly descended to the bottom of the chamber.

For fish 'left at the surface' pressure, individuals which had not expelled bubbles continued swimming head-down until they had resorbed enough swimbladder gas to become neutrally buoyant again or they became so exhausted as to float upside down against the roof of the chamber. Five fish died as a result of this treatment, cessation of ventilation occurring whilst floating upside down at the surface within 10 mins of simulated capture (depressurization). Individuals that had expelled bubbles exhibited normal behaviour. For all surviving members of this treatment group, normal chamber behaviour was observed the next day.

4.3.2.2. Barotrauma symptoms

In contrast to the results of the pilot study (Experiment 1, above), some mortality occurred immediately after simulated capture with 16% of fish (5/32) depressurized from 30 m and left at surface pressure dying (Table 4.4). All control and 'repressurized' group fish were alive upon removal from the chambers. The most common symptom in both control and treatment fish was a distended abdomen (Fig. 4.13) and occurred in 56% of control fish, 100% of fish which were repressurized after 10 mins and 47% of fish which were left at surface pressure. Bloodshot cloacas (Fig. 4.14) occurred only in treatment fish: in 6% of fish repressurized and in 9% of fish left at the surface. The remaining symptoms occurred only in fish depressurized and left at the surface: 3% (one fish) had an everted stomach (Fig. 4.15), 6% exhibited haemorrhaging from the gills and 34% were negatively buoyant upon removal from the chambers.

Figure 4.13. A) Lateral and **B)**, ventral views of abdominal distension in snapper following simulated capture from depth. Arrows indicate areas of maximum swelling.

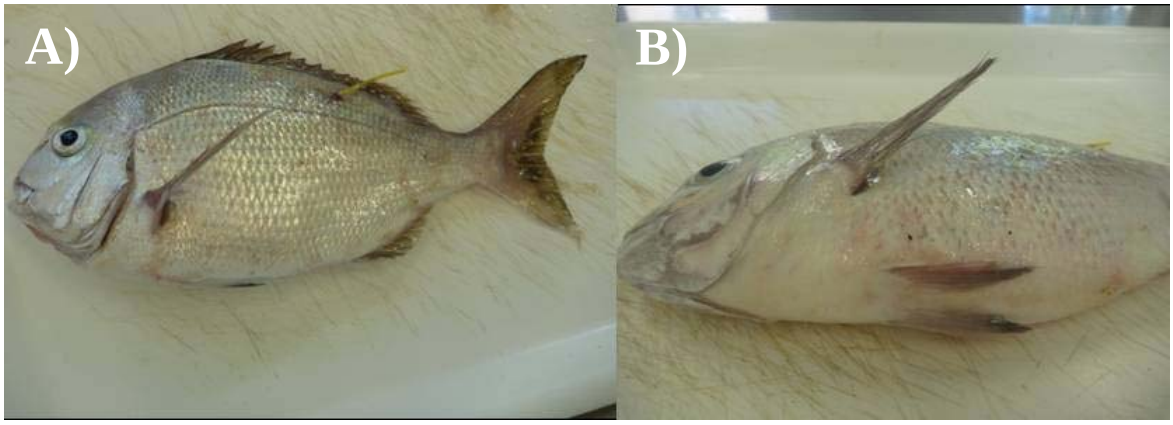


Figure 4.14. Bloodshot cloacas of snapper following simulated capture from depth, **A)** after death, and **B)** immediately following removal from the chamber.

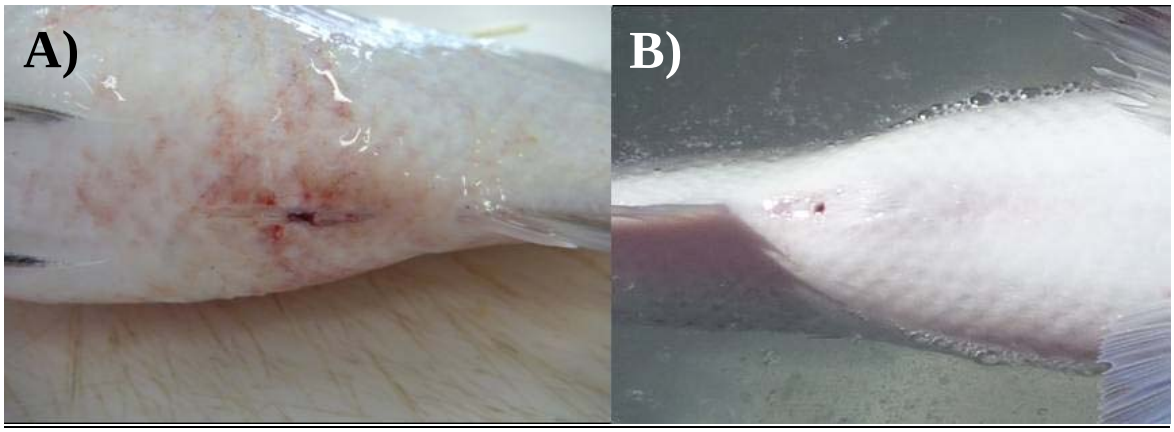


Figure 4.15. A) Stomach eversion into pharyngeal cavity (arrow) in snapper following simulated capture from depth, and **B)** dissected visceral mass showing 'inside out' stomach and bunched organs.

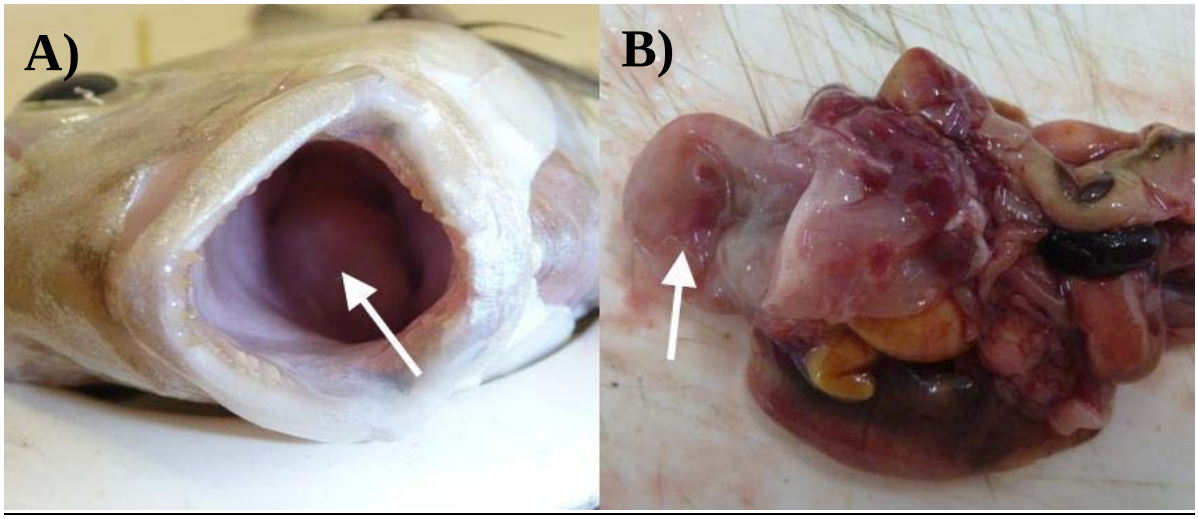


Table 4.4. Percent occurrence of external symptoms of barotrauma observed in snapper during experiment 2 following removal from the chambers for each treatment. *n* is sample size.

	Controls	**Repressurize**	**Leave at surface**
Simulated Depth (m)	**30**	**30**	**30**
n	*16*	*32*	*32*
Symptom			
Mortality			15.6
Exophthalmia			
Corneal haemorrhage			
Corneal emphysema			
Skin haemorrhage			
Fin haemorrhage			
Gill haemorrhage			6.3
Bloodshot cloaca		6.3	9.4
Distended abdomen	56.3	100.0	46.9
Stomach eversion			3.1
Rippled skin			
Negatively buoyant			34.4

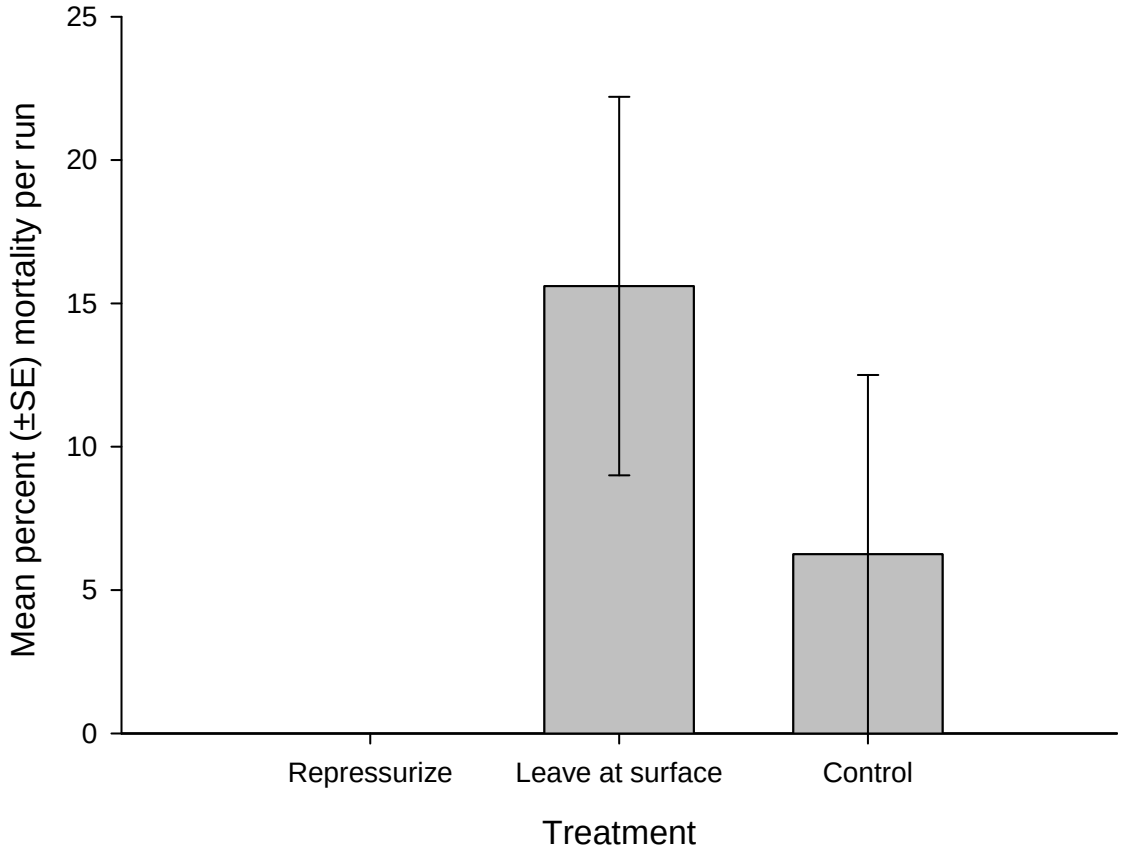
4.3.2.3. Mortality

No mortalities occurred for fish subjected to simulated capture followed by repressurization to acclimation depth after 10 mins at surface pressure. Some mortality did however occur for fish which were not repressurized to depth ('leave at surface': 15.6 ± 6.6 %) as well as a single control fish which died 7 d after removal from the chamber (Table 4.4, Fig. 4.16). As a result of the death of this control fish, the quasi-binomial GLM analysis failed to find a significant effect of treatment (Table 4.5) even though 83% of mortality occurred in the 'leave at surface' group.

Table 4.5. Analysis of deviance table for the quasi-binomial GLM relating to the probability of a fish dying model for the second snapper chamber experiment. The estimated overdispersion was 1.15. df is degrees of freedom.

	df	**Deviance**	**Residual df**	**Residual Deviance**	**F**	***P*-value**
			19	19.08		
Treatment	2	2.13	17	16.95	0.924	0.416

Figure 4.16. Mean percent mortality (± SE) of snapper where fish were acclimated to 30 m simulated depth and depressurized to surface pressure, before being either repressurized to acclimation pressure after 10 min, or left at surface pressure.



Death occurred almost immediately (within ~15 mins) for the five fish left at surface pressure after simulated capture from to 30 m acclimated depth (Fig. 4.17). The single control mortality died seven days after removal from the chamber. There were no further mortalities of treatment or control fish after this time.

The four fish which died under the surface (S) treatment occurred in four separate replicates. In fact, there was only one replicate where two fish died together in a simultaneous chamber (Table 4.6: run 9, chamber 1). This fact by itself suggests that there is little evidence of non-independence in the outcomes between fish.

Figure 4.17. Cumulative frequency of snapper mortalities in experiment 2 for each treatment.

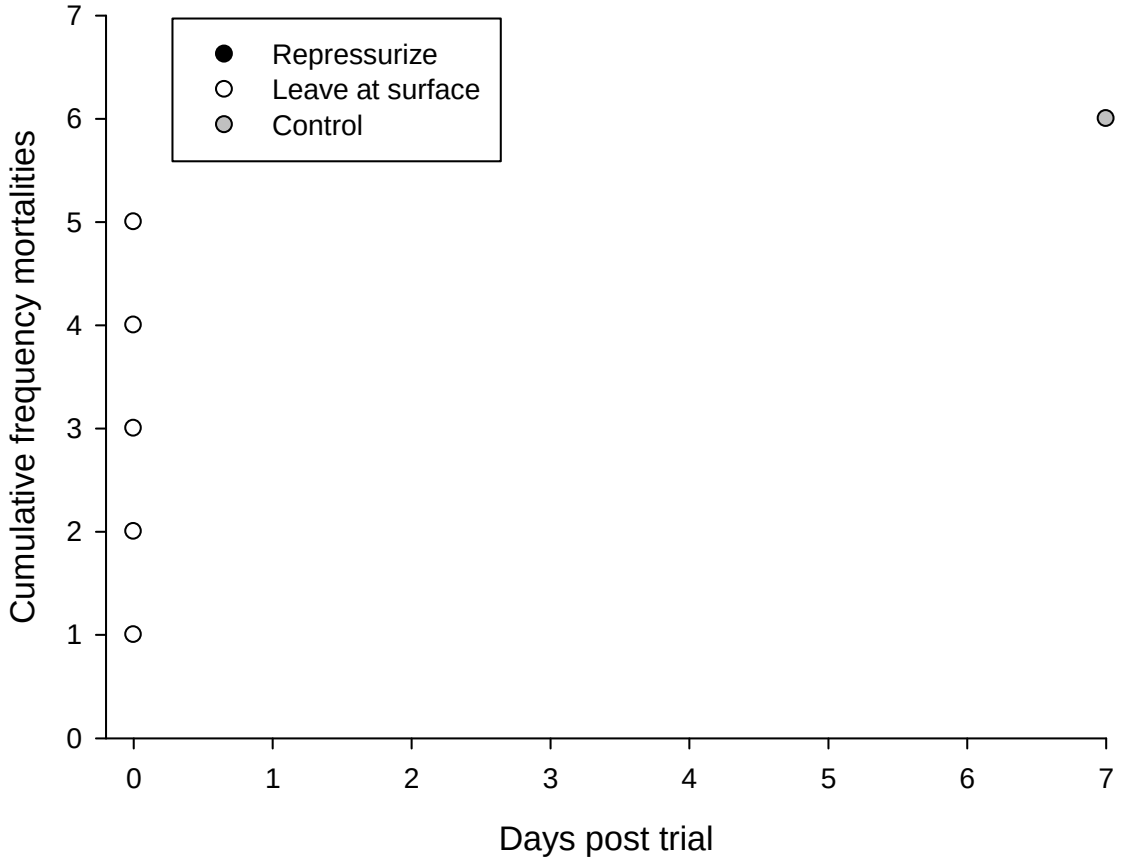


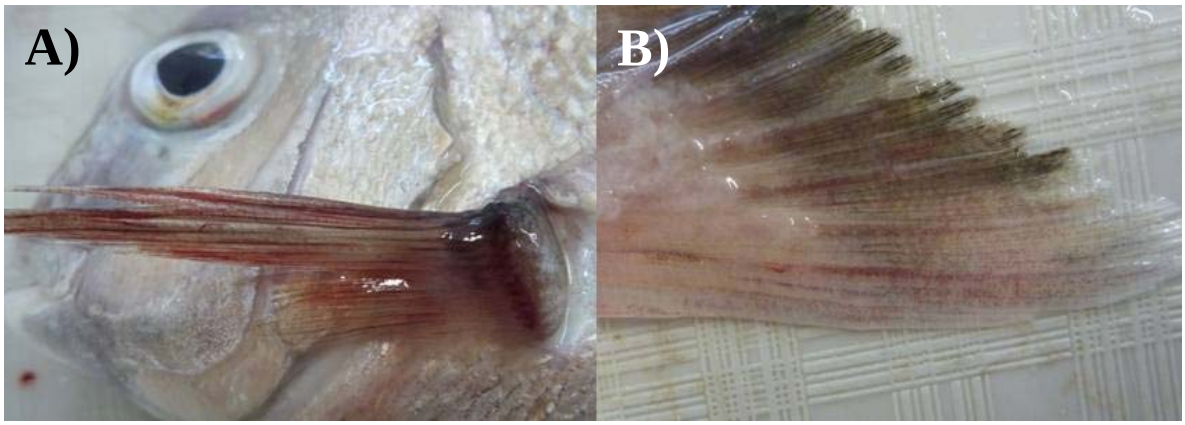
Table 4.6. Mortality of snapper by chamber and run during experiment 2. Simulated acclimation depth for all runs was 30 m, C = control, R = "repressurize" and S = "leave at surface".

Run	Chamber 1	No. deaths	Days	Chamber 2	No. deaths	Days
1	R	0		S	1	0
2	S	0		R	0	
3	R	0		C	1	7
4	C	0		S	1	0
5	S	0		R	0	
6	R	0		C	0	
7	S	0		R	0	
8	C	0		S	1	0
9	S	2	0, 0	R	0	
10	R	0		S	0	

4.3.2.4. Post-mortems

Post-mortems revealed that all treatment fish that died had multiple injuries consistent with rapid decompression (Table 4.7). The only symptoms possessed by the single control fish which died was haemorrhaging on the fins and skin (Fig. 4.18).

Figure 4.18. Haemorrhaging of **A)**, the pectoral fin and **B)**, the caudal fin, in snapper following simulated capture from depth.



All fish which died as a result of being left at surface pressure following depressurization from pressure equivalent to 30 m depth suffered a distended abdomen and swimbladder perforation (Figs. 4.19 and 4.20). Other barotrauma injuries observed included corneal haemorrhage (20%) (Fig. 4.21), pharyngo-cleithral membrane emphysema (20%) (Fig. 4.22), bloodshot cloaca (40%), viscera displacement (20%) or haemorrhage (40%) (Fig. 4.23), stomach eversion (40%), liver trauma, hepatic vein damage and an enlarged spleen (all 20%).

Figure 4.19. Perforated swimbladders of snapper following simulated capture from depth, **A)** perforation of swimbladder only (gas trapped inside mesentery), and **B)** perforation of swimbladder (circled) and mesentery (arrow).

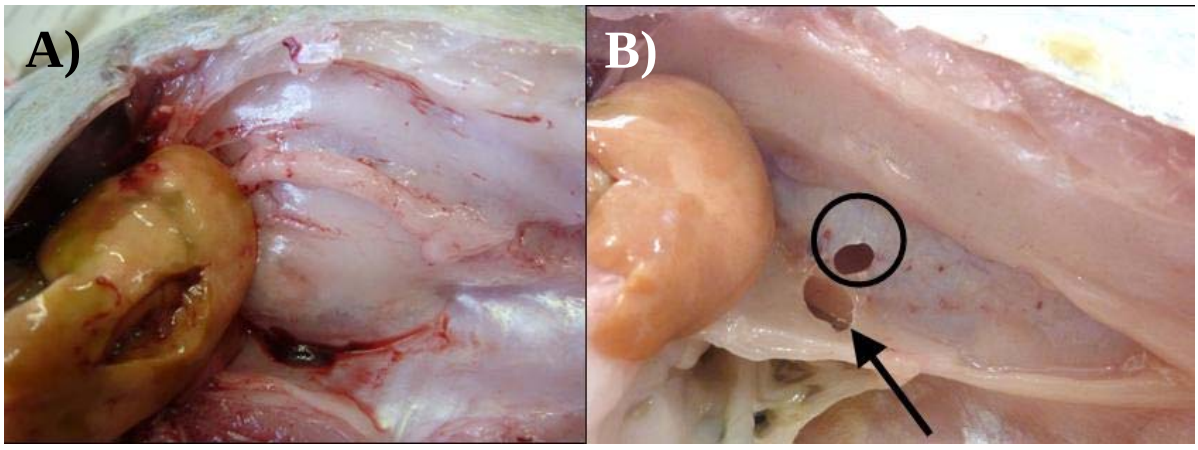


Figure 4.20. Perforated swimbladders of snapper following simulated capture from depth.

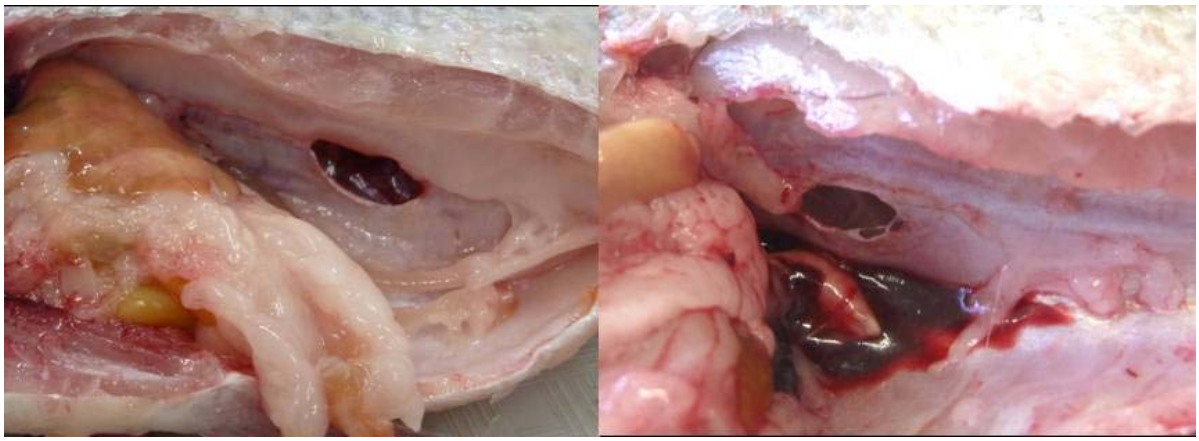


Figure 4.21. A) Normal eye condition and **B)**, corneal haemorrhaging, in snapper following simulated capture from depth. Arrow indicates haemorrhaging.

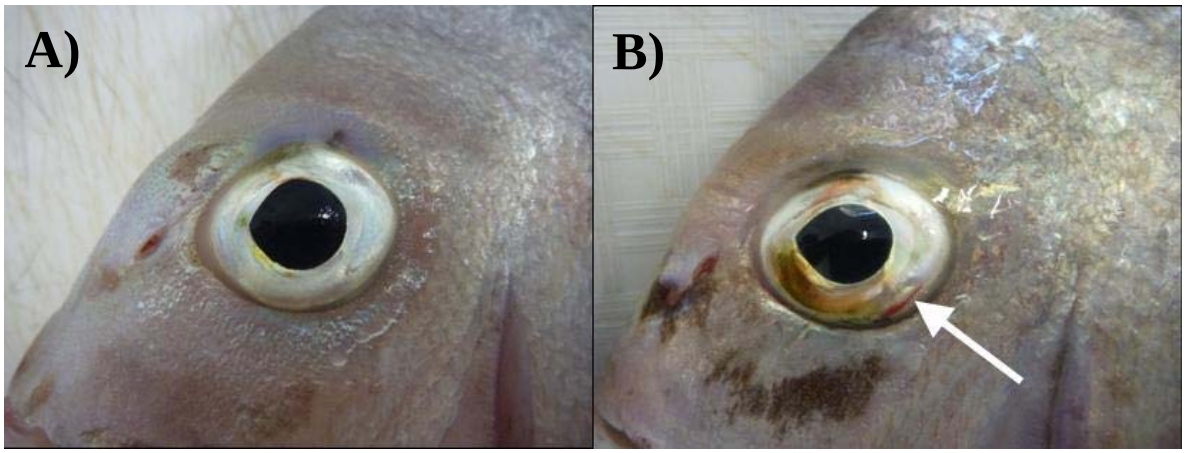


Figure 4.22. A) Pharyngo-cleithral membrane emphysema in mulloway following simulated capture from depth, and **B)** close-up. Arrows indicate clusters of gas bubbles.

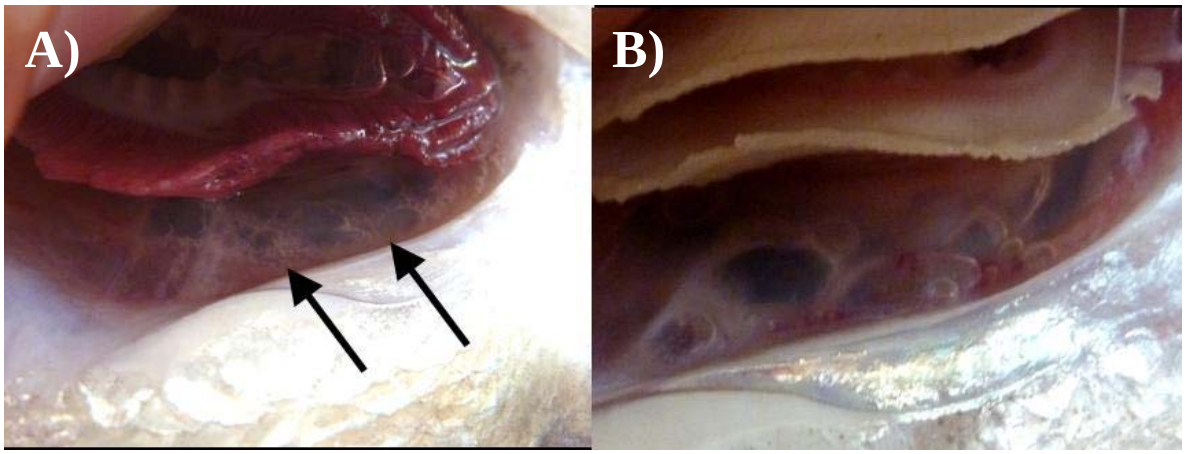


Figure 4.23. Internal haemorrhaging in mulloway following simulated capture from depth. Arrows indicate haemorrhage **A)** around the heart, and **B)** beneath the mesentery covering the swimbladder.

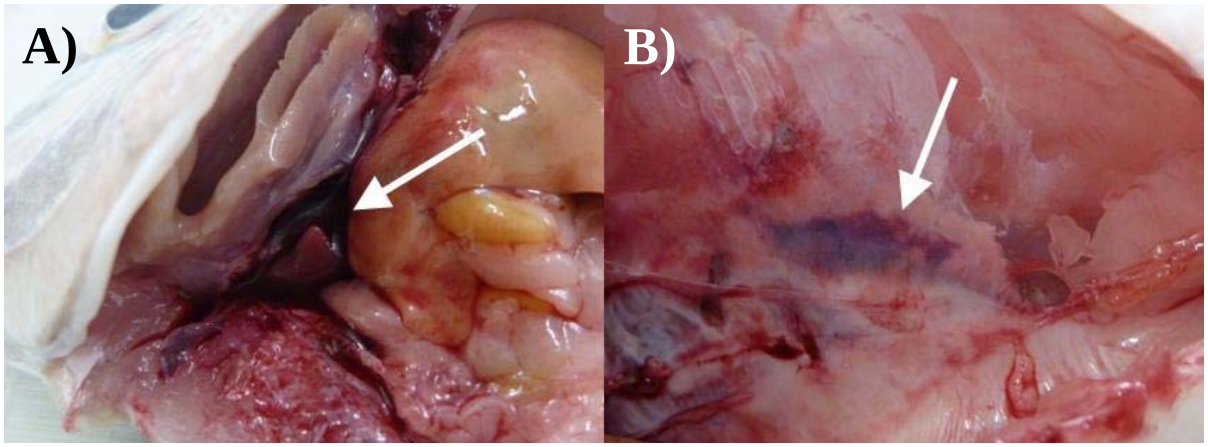


Table 4.7. Percent occurrence of injuries revealed by post-mortems performed on snapper mortalities during experiment 2. *n* is the number of mortalities out of the original sample size for each treatment. PCM is pharyngo-cleithral membrane.

	Controls	**Repressurize**	**Leave at surface**
Simulated Depth (m)	**30**	**30**	**30**
n	*1/16*	*0/32*	*5/32*
Symptom			
Exophthalmia			
Corneal haemorrhage			20.0
Corneal emphysema			
Skin haemorrhage	100.0		20.0
Fin haemorrhage	100.0		60.0
Gill haemorrhage			
PCM emphysema			20.0
PCM perforation			
Bloodshot cloaca			40.0
Distended abdomen			100.0
Swimbladder empty			
Swimbladder hyperextension			
Swimbladder perforation			100.0
Swimbladder scar tissue			
Viscera displacement			20.0
Viscera haemorrhage			40.0
Torn mesentery			
Stomach eversion			40.0
Liver trauma			20.0
Hepatic vein damage			20.0
Splenomegaly			20.0

4.4. Discussion

4.4.1. Mortality

The hyperbaric chamber experiments simulating the pressure changes experienced by snapper during capture in this study have shown it to be a relatively robust species to the effects of barotrauma. In pilot experiments, despite using the greatest depths the chambers were capable of simulating (70 m), which were also some of the highest pressures yet experimentally simulated, there were no mortalities recorded. It was thus concluded from this one result alone, that in contrast to the conclusions of much of the literature concerning post-release survival of fish caught from deep water (e.g. Gitschlag & Renaud, 1994; Wilson & Burns, 1996; Collins et al., 1999; McGovern et al., 2005; St. John & Syers, 2005), that mortality of snapper due solely to the effects of barotrauma is not positively related to depth of capture if the fish can quickly (~ 2 min) repressurize. Even in experiment 2 when snapper were depressurized and left at surface pressure, very few fish (5 out of 32) subsequently died.

4.4.2. In-chamber behaviour & symptoms

Observations of fish behaviour within the chambers did not appear to vary substantially with depth. During depressurization (simulated capture) in both experiments, fish displayed distended abdomens as the expanding swimbladdder progressively occupied the available space within the body cavity in response to reduced ambient pressure. Post-mortems of mortalities revealed this expansion to eventually cause the perforation of the swimbladder and release of gas directly into the body cavity, as a result of which the fish became increasingly buoyant. This in turn caused the fish to swim with a head-down attitude in order to remain near the bottom of the chamber. Despite this excessive buoyancy, even fish in experiment 2 that were decompressed from 30 m simulated depth and left at surface pressure regained normal buoyancy <24 h after decompression. This timeframe is well within the gas absorption rates calculated in Chapter 2 for snapper. Excess gas in swimbladders of Western Australian dhufish *Glaucosoma hebraicum* (St. John & Syers, 2005) and mulloway (Chapter 3) has also been reported to diffuse in ~24 h. The presence of such a large amount of gas in the gut cavity also appeared to cause the fish extreme discomfort and resulted in rapid and haphazard swimming behaviour, gill flaring and head shaking. In many cases, pressure exerted on the alimentary tract resulted in eversion of the stomach into the pharyngeal or buccal cavity. Rupture of the swimbladder and resultant sudden release of gas into the abdominal cavity has been shown to induce eversion and prolapse of the stomach in various species including mulloway (Chapter 3), black jewfish *rotonibea diacanthus* (Phelan, 2008), and rockfishes *Sebastes* spp. (Hannah et al., 2008). When the body tissues could no longer constrain the increasing volume of gas, it then escaped to the exterior through rupturing the body wall in the region around the cloaca. A number of physoclistous fish species have been observed releasing gas bubbles when being brought to the surface (Pearcy, 1992; Nichol & Chilton, 2006; Hannah et al., 2008; Brown et al., 2010, Chapter 3), suggesting terminal rupture and release of swimbladder gas to the exterior. To our knowledge, this is the first time gas escapement from the gut cavity through the body wall in the region around the cloaca has been reported, the previously reported route for swimbladder gas escapement being the region

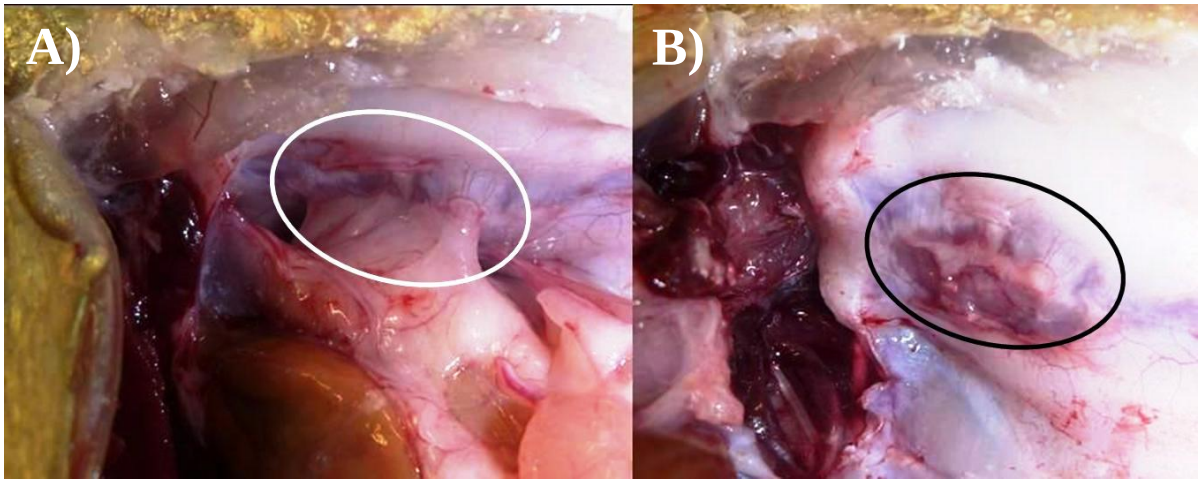
between the pharynx and oesophagus (Pearcy, 1992; Hannah et al., 2008; Brown et al., 2011; Chapter 3).

Upon removal from the chambers, the only externally visible symptom of barotrauma in experiment 1 was a distended abdomen which was recorded in just under half of treatment fish. Post-mortems of mortalities in experiment 2 (30 m) and the free vertical range (FVR) estimated for snapper in Chapter 2 show that all snapper would have suffered a perforated swimbladder when decompressed from 70 m simulated depth. That less than half the fish exhibited abdominal bloating suggests that some, or all, the excess swimbladder gas must have exited the fish's body at some stage during simulated capture. A similar proportion of fish were observed to possess a distended abdomen in experiment 2 when left at surface pressure, but 34% of fish were also recorded to be negatively buoyant providing additional confirmation that these fish had suffered body wall perforation. However, all snapper which were repressurized after simulated capture from 30 m exhibited a bloated abdomen suggesting that the body tissues can contain the gas from escaping to the exterior as long as repressurization (return to depth) occurs within 10 min. Therefore, expansion of swimbladder gases in snapper will be great enough to cause body wall rupture if caught from deep enough water or caught from shallower water, but not returned to depth. Even though body wall perforation may have helped to relieve some barotrauma symptoms (i.e. excessive buoyancy and abdominal bloating) as suggested for black jewfish (Phelan, 2008) and red emperor *Lutjanus sebae* (Brown et al., 2011), it may affect post-release survival via the potential for internal infection and peritonitis resulting from water entering the body cavity (Hannah et al., 2008).

Just as mortality was higher in snapper which were not repressurized after simulated capture than those that were, so too were the prevalence and severity of internal barotrauma injuries. Calculations of FVR made in Chapter 2 and post-mortem examinations revealed that all fish in both experiments would have suffered swimbladder perforation as a result of their simulated capture. Some species have the capacity to repair damaged swimbladders remarkably quickly. For example, red grouper *Epinephelus morio* and red snapper *Lutjanus campechanus* are known to be able to seal large perforations in the swimbladder in <4 days (Burns & Restrepo, 2002), and Pacific cod *Gadus macrocephalus* within a period of 2-4 days (Nichol & Chilton, 2006). Parker et al. (2006) found that 75% of rockfish swimbladders which had ruptured during experimental decompression had at least partially healed and were holding gas by 21 days post-treatment. Staged post-mortems done on several fish which were unintentionally decompressed from pressure equivalent to 30 m water depth due to a power outage in the current study showed that swimbladder healing in snapper likely occurs at a similar rate to that of these species. Parts of the mesentery within the body cavity of snapper appeared to form a plug of connective tissue which effectively sealed over the perforation sufficient for the swimbladder to hold gas in ~7 days (Fig. 4.24). All other internal injuries were recorded only in fish which were left at surface pressure following simulated capture as no fish which were repressurized subsequently died. With the exception of swimbladder perforation and abdominal distension all other injuries (corneal haemorrhage, pharyngo-cleithral membrane emphysema, bloodshot cloaca, viscera displacement, viscera haemorrhage, stomach eversion, liver trauma, hepatic vein damage and splenomegaly) were recorded in only some (20-60%) of the mortalities suggesting that none of these were the direct cause of death.

In comparison with other fish species, snapper appears to be remarkably robust to the effects of barotrauma and less susceptible than many other fish species studied such as Western Australian dhufish (St. John & Syers, 2005), red snapper *Lutjanus campechanus* (Jarvis & Lowe, 2008), black jewfish, (Phelan, 2008), and mulloway (Chapter 3). Even compared with other sparid fishes (e.g. Booth & Buxton, 1997; Götz et al., 2007; Rudershausen et al., 2007), the levels of mortality due to barotrauma for snapper in the present study are relatively low. The morphologically and ecologically similar panga *Pterogymnus laniarius* from the Agulhas Bank off southern African is reported to suffer heavy mortalities resulting from severe barotrauma as a result of its capture from depths in excess of 60 m (Booth & Buxton, 1997). Similarly, the red porgy *Pagrus pagrus*, was estimated to suffer ~40% mortality from 25-55 m deep water off North Carolina in two separate studies (Guccione, 2005; Rudershausen et al., 2007). The occurrence of barotrauma symptoms recorded in these experiments was also substantially lower than that reported for other sparid fishes. Gastric distension (stomach eversion) in red porgy was recorded in 16% of wild-caught individuals from 25-50 m deep water (Rudershausen et al., 2007) compared with just 3% of snapper acclimated to 30 m depth. However, the occurrence of bleeding from the gills was similar in the present study (6%) compared with 5% for red porgy.

Figure 4.24. Swimbladder healing in snapper following perforation: **A)** circled is the plug of connective tissue formed by mesentery within the body cavity, and **B)** after the plug has been dissected away from the swimbladder showing a complete seal.



4.4.3. Delayed mortality

In contrast to the results for mulloway presented in the Chapter 3,where significant delayed mortality occurred, all snapper mortalities of treatment fish occurred immediately after (<15 min) simulated capture. Studies on the congeneric red porgy (Rudershausen et al., 2007) have shown delayed mortality to be much higher (26%) than immediate mortality (14%) when caught from comparable depths (25-55 m). In contrast, Burns et al. (2004) found immediate mortality to be much higher (71%) than delayed mortality (21%) for red snapper, but found all mortality to be immediate in red grouper in hyperbaric chamber experiments. As discussed above, all barotrauma-related injuries (corneal haemorrhage, pharyngo-cleithral membrane emphysema, bloodshot cloaca, viscera displacement, viscera haemorrhage, stomach eversion, liver trauma, hepatic vein damage and splenomegaly)

were recorded in only some (20-60%) of the snapper mortalities indicating that none of these were the direct cause of their immediate death. Barotrauma occurs not only from the physical effects of rapid and/or extensive reduction in barometric pressure on visible gases in the swimbladder, but also dissolved gases forming cryptic gas bubbles in the bloodstream and tissue cells (Feathers & Knable, 1983; Kieffer, 2000; Stephens et al., 2002). This gas bubble formation can cause gas embolism, haemorrhaging and clotting as well as other haematological changes (Kulshrestha & Mandal, 1982). It is therefore probable that the sudden death observed in snapper was caused by events such as arterial embolism or haemorrhaging which can cause vascular occlusion (blockage of blood vessels). When vascular occlusion occurs in the brain, the result may be ischemic cardiovascular accident (or "stroke"); when occurring in the heart may result in myocardial infarction (or "heart attack"). Both events are capable of causing almost immediate death as observed in snapper which were not recompressed after simulated capture from depth. Both Gitschlag & Renaud (1994) and Rummer & Bennett (2005) suggested arterial embolism as a potential cause of death in some red snapper caught from deep water. Similarly, Beyer et al. (1976) reported that some bubbles present in the blood could be tolerated by coho salmon *Onchorhynchus kisutch*, whereas large gas bubbles in vital areas such as the heart were lethal. The only tissue-level injury found to be directly attributable to decompression in rockfishes was emphysema in the heart ventricle, concentrated in the compact myocardium (Pribyl et al., 2012). Longbottom (2000) also observed lesions in the heart ventricle of snapper after capture from depths ranging between 10 and 35 m.

Interestingly, no mortalities were recorded in snapper which were recompressed after 10 minutes surface interval following simulated capture from depth, even though at least some fish must have developed emboli and/or haemorrhaging in the bloodstream and tissues that caused death in snapper that were not recompressed. For rockfish, emboli in blood vessels disappeared once fish were recompressed because the excess gas was forced back into solution (Pribyl et al., 2012).

5. DEPTH PROFILES

5.1. Introduction

Recompression (or repressurization) is a technique used by some recreational fishers to reduce discard mortality in physoclist fish species that suffer from barotrauma. Recompression involves any method that will help fish overcome the main effect of barotrauma, hyperinflation or rupture of the swimbladder and associated excessive buoyancy, to submerge to a depth where they can swim back down on their own. If a fish can resubmerge close to its original capture depth, the expanded gases will be compressed again, relieving the fish of its excessive buoyancy. The two most popular techniques for reducing excess buoyancy and allowing the fish to return to depth are: (i) *Venting*- a hypodermic needle or similar is inserted through the body wall into the swimbladder or body cavity (Fig. 5.6) to release the excess gases trapped inside, and; (ii) *Release Weight*- a barbless hook combined with a heavy lead weight (tethered to the surface) is inserted through the lip of the fish (Fig. 5.5) allowing the fish to be lowered back to a chosen depth when the hook is jerked free and the fish released (Bartholomew & Bohnsack, 2005). Other devices used by recreational fishers to recompress physoclist fish also include weighted cages (Theberge & Parker, 2005). Recent studies have shown that recompression using these techniques provide equivocal results regarding post-release survival: some have shown improvements to survival (e.g. Collins et al., 1999; Wilson & Burns 1996; Keniry et al. 1996; Hannah & Matteson 2007; Jarvis & Lowe 2008, Pribyl et al. 2012), others have been shown to make no difference to survival (e.g. Bruesewitz et al. 1993; Gitschlag & Renaud, 1994; St. John & Syers, 2005), and still others have been shown to actually increase post-release mortality (e.g. Gotshall, 1964; Render & Wilson, 1996; Burns et al., 2002).

Similarly, recent research in Australia has shown that these techniques are successful in improving the survival of some species, but not others (Brown et al., 2008; Sumpton et al. 2008; 2010). For example, tag/recapture studies in both Western Australia (WA) and Queensland have shown that neither treatment method improves recapture rates in many species such as WA dhufish *Glaucosoma hebraicum* (St. John & Syers 2005) or red emperor *Lutjanus sebae* (Brown et al. 2010), and for coral trout *Plectropomus maculatus*, saddletail snapper *Lutjanus malabaricus* and spangled emperor *Lethrinus nebulosus*, venting was found to result in lower chances of survival (Brown et al. 2010). A recent international review on venting fish used a meta-analysis of 17 published and unpublished studies and found that all available information provided virtually no support for the practice of venting as a means of increasing survival of captured and released fish and concluded that the practice should be discouraged by fishery management agencies (Wilde, 2009). No information exists on the effectiveness of barotrauma treatments for NSW species.

The first step in examining the effectiveness of barotrauma treatments is to understand normal fish behaviour after release. Surprisingly, this has only been attempted on fish released into cages after capture, thereby spatially restricting their movement and potentially affecting recovery (Hannah et al., 2012). Previous studies on snapper have released fish into small cages which have then been lowered back to capture depth (e.g.

Stewart, 2008; Lenanton et al., 2009); a technique that is recognized as affecting fish behaviour and may produce erroneous results. An alternative method recently used has involved release fish into 'bathy-cages' or 'socks' that encompass more of the water column, thereby allowing fish to better orientate vertically after release (Brown et al., 2010; Roach et al., 2011, Butcher et al., 2012). Whilst such increased vertical choice is clearly important for assessing the survival and recovery of many species (Nichol & Chilton, 2006), the use of 'bathy-cages' or 'socks' is still a method that confines the fish thus spatially restricting their movement and therefore potentially affecting their behaviour and recovery. The vertical distribution of fish within these structures is also restricted by the vertical extent of the structure itself and in many cases fish released into the 'sock' were caught from water depths which exceeded the maximum vertical range of the 'sock' by some distance (e.g. Brown et al., 2011; Butcher et al., 2012). As a result, knowledge of the post-release behaviour of barotrauma-affected fish following release is limited, particularly in terms of the depths they occupy following release (depth profiles). For example, barotrauma-affected fish may 'prefer' to recover swimming in mid-water, before making their way to the bottom where they will be negatively buoyant. In this situation, sending fish to the seafloor when attached to a heavy release weight may not be best practice.

There is therefore a clear need to understand the depth-related behaviour of released fish and the influence of different release treatments on this behaviour if the usefulness of these mitigation techniques are to be assessed in NSW. In terms of welfare, both the use of release weights and especially venting are potentially more contentious than releasing fish untreated (Butcher et al., 2012). Here, we therefore present a series of field trials on snapper and mulloway where externally-attached depth-sensitive acoustic transmitters were used to examine the natural post-release behaviour and depth profiles of fish angled from deep water following release using different mitigation techniques.

5.2. Materials & methods

Snapper and mulloway for use in post-release depth profile trials were caught using standard recreational angling techniques (rod and line) and tackle (soft plastic lures, Lucanus™ jigs or dead baits- prawns, squid, fish). Snapper were caught from various locations within the Port Hacking estuary (Dolans Bay - 34º03'55"S 151º06'19"E, South West Arm - 34º04'52"S 151º06'21"E) and offshore (Jibbon Head - 34º04'46"S 151°10'40"E, Bate Bay - 34º03'20"S 151°10'48"E), as well as from various locations within the Port Stephens-Great Lakes Marine Park (Cabbage Tree Island – 32º41'18"S 152°13'32"E, Big Seal Rock - 32º27'46"S 152º33'11"E, Little Seal Rock - 32º28'27"S 152º32'49"E, Broughton Island - 32º37'18"S 152º20'21"E). Mulloway were caught from various locations within the Georges River estuary between the Captain Cook Bridge (34º00'29"S 151º07'39"E) and Alfords Point (33º58'29"S 151º01'13"E).

When a fish was hooked, the water depth and time was recorded. When the fish was landed, the time was again recorded allowing calculation of the time elapsed from hooking to landing. Once on board, the fish were measured (FL- fork length and TL- total length for snapper, TL for mulloway) to the nearest 0.1 cm and information was recorded on barotrauma symptoms observed, hooking conditions and capture method. A small acoustic transmitter containing a pressure sensor (Fig. 5.1; V9P, Vemco, Nova Scotia, Canada) was then externally attached to the fish using a t-bar tag inserted just under the skin of the fish

ventral to the dorsal fin rays (Fig. 5.2). This transmitter was tethered to a rod and reel spooled with fine braided fishing line (PowerPro™; 10 lb test, 0.15 mm dia.) onboard the vessel (Fig. 5.3).

Each fish was the subject to one of three randomly chosen treatments for release: i) untreated - returned to the water with no treatment (Fig. 5.4), ii) vented - an 11 gauge hypodermic needle was used to puncture the body cavity of the fish thus releasing any excess gas resulting from overexpansion and rupture of the swimbladder before returning the fish to the water (Fig. 5.6), or iii) release weight - a custom made release weight (consisting of a barbless hook and 500 g lead weight; Sunset Sinker Supplies, Neerabup, WA) was inserted through the lip of the fish (Fig. 5.5) and the fish lowered back to the bottom via a second rod and reel before the weight was jerked free releasing the fish at depth. The time of release was also recorded to allow calculation of the time elapsed between landing and release for each fish.

Figure 5.1. V9P acoustic transmitter for recording post-release depth profile data for snapper and mulloway. Uppermost is the t-bar tag modified by attaching a nylon monofilament loop to the end. Below is the transmitter with snap swivel attached to one end for connection to the t-bar tag and nylon monofilament attached to the other end for tethering to a vessel on the surface.

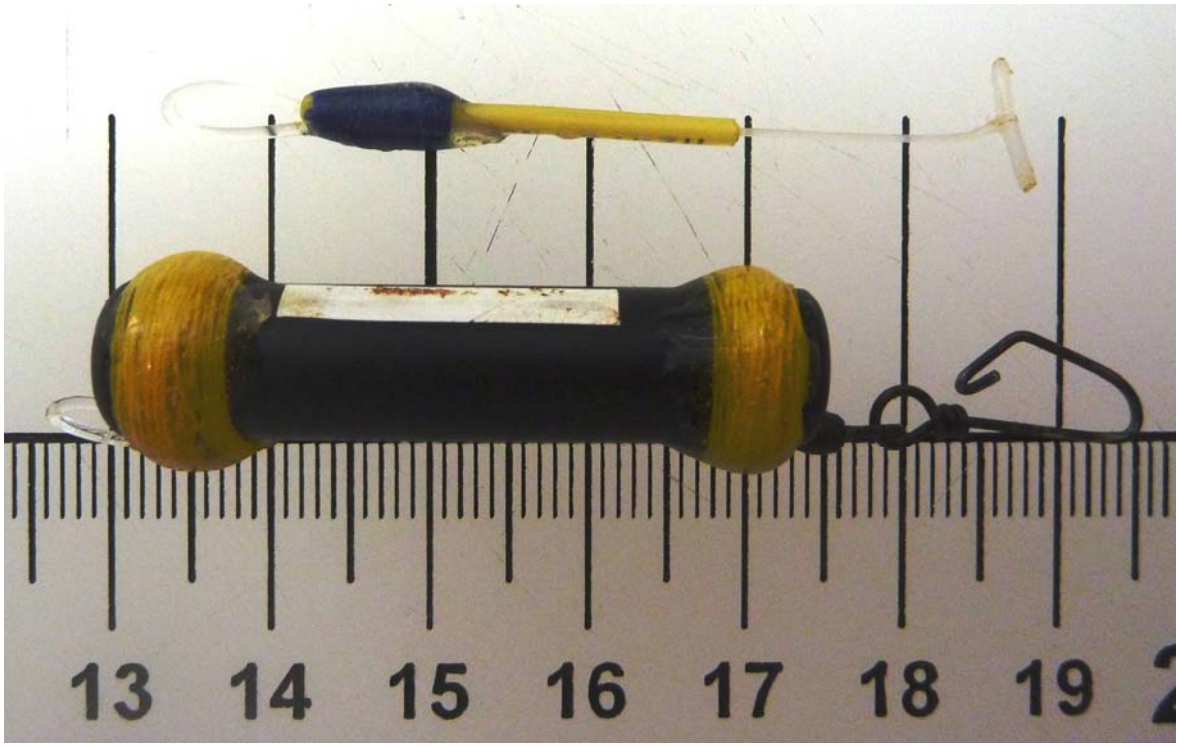


Figure 5.2. Example of external attachment of V9P acoustic transmitter for recording depth profile data for mulloway.

Figure 5.3. Acoustic tracking onboard the research vessel. The fish is tethered via thin braided line to the surface, but allowed to swim unimpeded.

Figure 5.4. An example of an "untreated" mulloway prior to release.

Figure 5.5. A snapper fitted with a release weight prior to release.

Figure 5.6. Venting a snapper prior to release. Note the modified t-bar tag already in the fish to which the acoustic tag will be attached.

Once released, the initial post-release behaviour of each fish was recorded; whether the fish swam away quickly, swam away slowly, floated or sank. The V9P transmitter attached to each fish emitted an acoustic signal relaying depth information approximately once per second. This signal was received by a VH165 omni-directional hydrophone connected to an onboard VR100 mobile telemetry receiver unit (Vemco, Nova Scotia, Canada) (Fig. 5.3). Each fish was followed with minimum tension on the tether line and depth data recorded for ~10 mins after release, before a sharp jerk on the tether line detached the transmitter from the fish by pulling the t-bar tag out from under the skin. The transmitter was then retrieved for use in the next trial.

5.2.1. Data analysis

5.2.1.1. Barotrauma symptoms

Information on the presence of the following external barotrauma symptoms were recorded for each fish captured: mortality, exophthalmia, corneal haemorrhage, corneal emphysema, skin haemorrhage, fin haemorrhage, gill haemorrhage, bloodshot cloaca, distended abdomen, stomach eversion, anal prolapse, bubbles escaping from the body cavity, rippled skin and excessive buoyancy. The percentage of fish with each of these symptoms was then calculated and stratified by capture depth (10-20 m, 20-40 m and >40 m).

5.2.1.2. Post-release behaviour

The percentage of fish which exhibited each type of initial post-release behaviour (i.e. whether the fish swam away quickly, swam away slowly, floated or sank) was calculated stratified by capture depth and for each post-release treatment (i.e. untreated, vented or release weight).

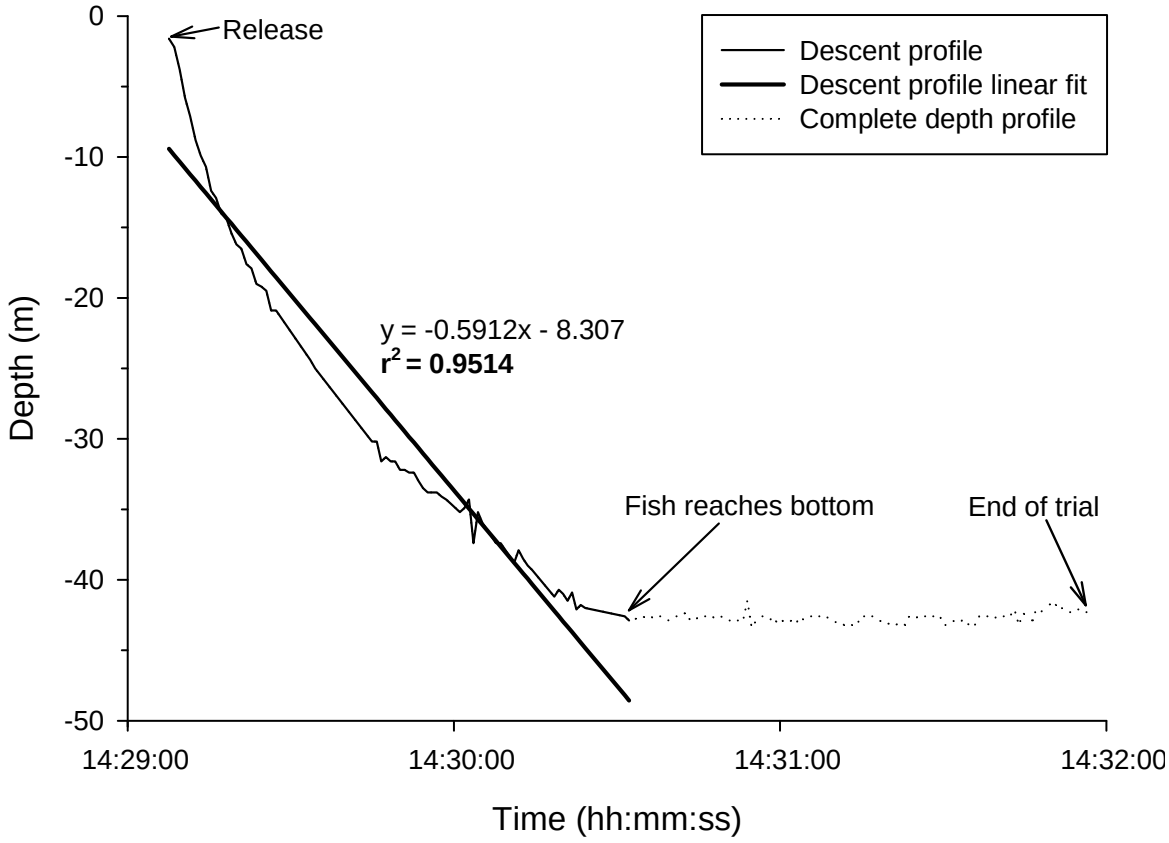
5.2.1.3. Depth profiles

For each trial, recorded depth data were downloaded from the VR100 to a PC and plotted against time after release in order to visually represent the depth of each fish over time following release (depth profile).

5.2.1.4. Descent rates and variability

Using the descending part of the depth profile performed by each fish in each trial, a descent rate was calculated by dividing the time each fish took to reach the bottom by the water depth recorded at release (m/min). A mean descent rate for each release treatment could then be calculated and compared between release treatments using a single factor analysis of variance (ANOVA). The variability in overall descent rate was estimated by fitting a linear curve to the descending part of the depth profile and calculating an r^2 value (Fig. 5.7). This value represents the variability in descent rate. A high r^2 value therefore indicates that the data was well fitted by the curve and corresponds to a relatively constant descent. Conversely, a low r^2 value indicates a poor curve fit to the data and corresponds to a much more variable descent. A mean descent profile for each release treatment could then be calculated and compared between release treatments using a single factor ANOVA.

Figure 5.7. An example depth profile of a snapper captured and released in 42 m of water.



5.3. Results

5.3.1. Snapper

The post-release behaviour and depth profiles of a total of 57 snapper were recorded after capture from a range of water depths from 10.0 to 54.0 m (Table 5.1). Due to the logistics involved in catching snapper from deep water, we did twice as many trials in 10-20 and 20-40 m deep water (*n*=24 and 21, respectively) than water depths >40 m (*n*=12). The mean size of snapper used was 36.2 ± 2.1 cm FL with the smallest fish caught 14.2 cm FL and the largest 68.6 cm FL. On average, larger fish were caught and released from 20-40 m deep water (47.3 ± 2.7 cm FL) than from 10-20 or >40 m deep water (28.1 ± 3.4 and 32.8 ± 2.4 cm FL, respectively) (Table 5.1). This was also reflected by the longer average fight times seen in fish captured from 20-40 m (2.7 ± 0.3 min), which was more twice as long as those for fish caught from 10-20 or >40 m deep water (1.3 ± 0.3 and 1.3 ± 0.1 min, respectively). The mean period the fish were kept at the surface prior to release was 3.9 ± 0.4 min, but ranged from 0.5 min up to 15.0 min.

Table 5.1. Summary of information (depth range, fork length- FL, fight duration & surface interval) for snapper captured for analysis of post-release behaviour and depth profiles.

Depth range (m)	***n***	**Mean FL (cm) ± SE (range)**	**Mean fight duration (min) ± SE (range)**	**Mean surface interval (min) ± SE (range)**
10-20	*24*	28.1 ± 3.4 (14.2 – 61.5)	1.3 ± 0.3 (0.2 – 4.0)	3.7 ± 0.6 (0.5 – 12.0)
20-40	*21*	47.3 ± 2.7 (19.0 – 68.6)	2.7 ± 0.3 (1.0 – 5.0)	3.1 ± 0.7 (0.5 – 13.0)
>40	*12*	32.8 ± 2.4 (20.5 – 49.1)	1.3 ± 0.1 (1.0 – 2.0)	5.6 ± 1.1 (2.0 – 15.0)
Overall	*57*	36.2 ± 2.1 (14.2 – 68.6)	1.8 ± 0.2 (0.2 – 5.0)	3.9 ± 0.4 (0.5 – 15.0)

5.3.1.1. Barotrauma symptoms

The prevalence of externally observed barotrauma symptoms increased with depth (Table 5.2, Fig. 5.8). The percentage of fish with bloodshot cloacas (Fig. 5.9) increased from 33% when caught from 10-20 m depth, to 76% in 20-40 m to almost all fish (92%) captured from >40 m. Distended abdomens (Fig. 5.10) were much more prevalent (90% of fish caught overall), but also increased with depth from 79% in 10-20 m to 95% in 20-40 m and 100% in >40 m. The prevalence of fish with everted stomachs (Fig. 5.11) was low (4%) when captured from 10-20 m deep water, but much higher in fish captured from >20 m (38 & 58% from 20-40 & >40 m, respectively). Excess buoyancy (Fig. 5.12) when held onboard prior to release was observed in only 4% of fish caught from 10-20 m deep water, but increased to almost half (48%) of fish caught from 20-40 m and 92% of fish caught from >40 m. Bubbles escaping from the body cavity (Fig. 5.13) on approach to the surface from depth was only observed in fish captured from water >20 m deep (14 and 42% of fish from 20-40 and >40 m, respectively). Similarly, anal prolapse (Fig. 5.14) was only observed in fish caught from >40 m deep water (8%).

Table 5.2. Percent occurrence of external barotrauma symptoms observed in field-caught snapper angled from 10-20, 20-40 and >40 m depths. *n* is sample size.

Capture depth range (m)	**10-20**	**20-40**	**>40**	**Overall**
n	*24*	*21*	*12*	***57***
Symptom				
Mortality				
Exophthalmia				
Corneal haemorrhage				
Corneal emphysema				
Skin haemorrhage				
Fin haemorrhage				
Gill haemorrhage				
Bloodshot cloaca	33.3	76.2	91.9	**61.4**
Distended abdomen	79.2	95.2	100.0	**89.5**
Stomach eversion	4.2	38.1	58.3	**28.1**
Anal prolapse			8.3	**1.8**
Bubbles from body cavity		14.3	41.7	**14.0**
Rippled skin				
Excessively buoyant	4.2	47.6	91.7	**38.6**

Figure 5.8. Percent occurrence of external symptoms of barotrauma observed in field-caught snapper angled from 10-20, 20-40 and >40 m depths.

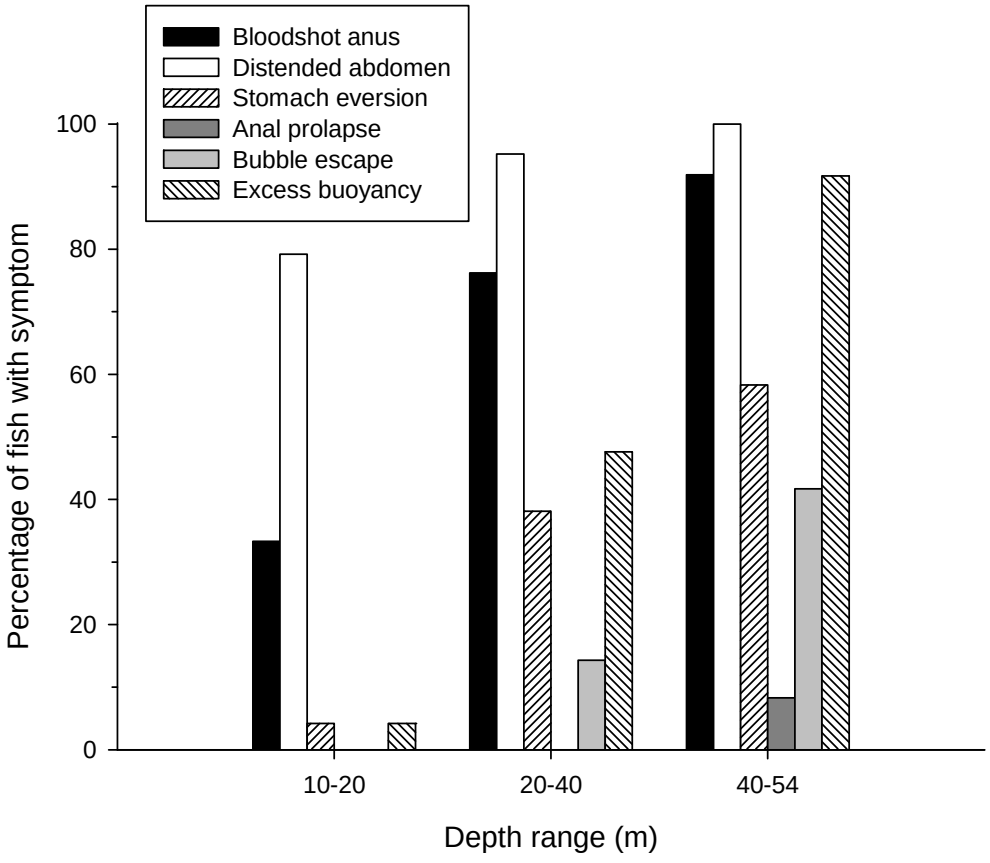


Figure 5.9. Field-caught snapper showing bloodshot cloacas and distended abdomens.

Figure 5.10. Field-caught snapper showing distended abdomens.

Figure 5.11. Field-caught snapper showing everted stomachs.

Figure 5.12. Field-caught snapper showing excessive buoyancy.

Figure 5.13. Field-caught snapper showing gas bubbles escaping through a perforation in the everted stomach that the fish has punctured with its teeth.

Figure 5.14. Field-caught snapper showing anal prolapse.

5.3.1.2. Release treatments

The large sample size for the untreated releases (*n*=32), the shallower mean release depth (23.4 ± 2.8 m) and smaller average sizes of fish (29.6 ± 2.8 cm FL) was a result of trials which were carried out in estuarine (Port Hacking) and nearshore waters on juvenile snapper which, due to their small sizes, presented logistical difficulties in releasing them after being either vented or using a release weight. As a result, most of these small fish were released untreated (Table 5.3). Nonetheless, all three treatments were done in comparable depth ranges and on fish of comparable sizes (Table 5.3). In addition, after the first series of trials, it became clear that the only release treatment which affected the fish's ability to successfully return to depth was to release the fish untreated (Table 5.3) and as a result, was used more often when releasing fish than the vented (*n*=14) or release weight (*n*=11) treatments.

5.3.1.3. Post-release behaviour

The most common behaviour exhibited by snapper upon release was to swim towards the bottom and occurred in 98% of trials (Table 5.3). Most (65%) of this behaviour was characterised as being 'fast swimming' with the remaining 33% swimming away more slowly. A single untreated fish caught from 25.4 m floated upside down on the surface for ~8 min before being retrieved (Fig. 5.15). The speed at which the fish swum away varied with both depth of capture and release treatment (Tables 5.3 and 5.4). Almost all fish (92%) captured from 20-40 m swam away quickly compared to only 52 and 60% of fish captured from 10-20 and >40 m, respectively. This behaviour also occurred in 63% of untreated trials and in 71% of vented trials (Tables 5.4 and 6.4, Fig. 5.16). In the remainder of untreated (34%) and vented (29%) trials, released fish were observed to swim off more

slowly towards the bottom. Almost all (98%) snapper, regardless of treatment or depth of capture, were able to successfully return to depth. Due to the nature of the release weight treatment whereby the fish are tethered and physically lowered to the bottom before release, post-release behaviour was not recorded. No fish were observed to sink.

Table 5.3. Summary of information (depth & fork length- FL) and the percentage of fish that exhibited each type of behaviour for snapper following release after the three experimental treatments. * not including trials where fish were released using a release weight. *n* is sample size.

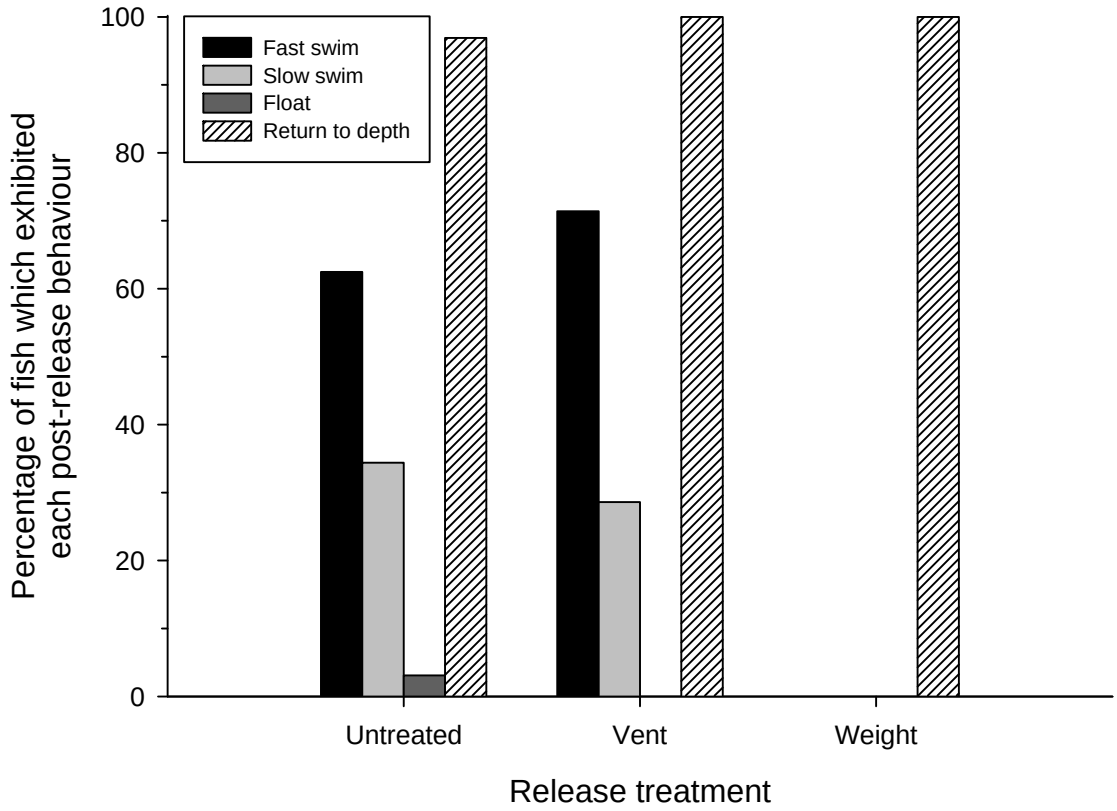
Treatment	*n*	Mean depth (m) ± SE (range)	Mean FL (cm) ± SE (range)	Behaviour at release (%)			Overall
				Swim fast	Swim slow	Float	
Untreated	*32*	23.4 ± 2.8 (10.0 – 49.9)	29.6 ± 2.8 (14.2 – 68.6)	62.5	34.4	3.1	96.9
Vented	*14*	34.3 ± 3.6 (14.0 – 54.0)	41.0 ± 3.6 (20.5 – 61.5)	71.4	28.6	0.0	100.0
Weight	*11*	31.4 ± 2.5 (16.0 – 41.0)	49.2 ± 2.9 (32.5 – 61.0)	-	-	-	100.0
Overall	***57***	**27.6 ± 1.6 (10.0 – 54.0)**	**36.2 ± 2.2 (14.2 – 68.6)**	**65.2***	**32.6***	**2.2***	**98.3**

Table 5.4. Percentage of fish that exhibited each type of behaviour for snapper captured from different 10-20, 20-40 and >40 m depth. * not including trials where fish were released using a release weight. *n* is sample size.

Depth range (m)	*n*	Behaviour at release (%)		
		Swim fast	Swim slow	Float
10-20	*23**	52.2	47.8	0.0
20-40	*13**	92.3	0.0	7.8
>40	*10**	60.0	40.0	0.0
Overall	***46****	**65.2**	**32.6**	**2.2**

Figure 5.15. Field-caught acoustically tagged snapper floating upside down at the surface after an untreated release.

Figure 5.16. Post-release behaviour of snapper following application of the three experimental barotrauma mitigation treatments (untreated, vented or release weight).



5.3.1.4. Depth profiles

Typical example depth profiles for each release treatment are presented in Figure 5.17. The profiles for each release treatment showed substantial variation between trials in terms of both descent rates and variability in descent rates (Table 5.5). Some patterns however occurred consistently between treatments. The depth profiles for untreated fish (Fig. 5.17A) showed the descent of fish to occur at a relatively constant rate from release until the fish reached the bottom. Upon arrival at the bottom, all fish remained in close proximity to the seafloor for the remainder of the trial. Fish which were vented prior to release (Fig. 5.17B) descended slightly faster overall than untreated fish, but their descent was far more variable often with multiple increases and decreases in descent speed and reflected by the lower overall r^2 than for untreated fish (Table 5.5). For example, the depth profile in Figure 5.17B shows an initial rapid descent from the surface down to ~30 m depth followed by a progressive reduction in descent rate from ~30 m depth until the fish reached the bottom at ~40 m with descent through the first 30 m of the water column taking almost as long as the final 10 m. Fish which were released with a release weight (Fig. 5.17C) showed consistent and extremely fast descent rates as the fish were towed to the bottom by the mass of the release weight. Several trials where release weights were used to return the fish to the bottom showed that the water depth that the fish were occupying to decrease slightly after initial arrival on the bottom (Fig. 5.17C).

5.3.1.5. Descent rates

Mean descent rates were similar for untreated (14.3 ± 1.8 m/min) and vented (16.2 ± 2.6 m/min) treatments where the fish descend using their own propulsion (Table 5.5). Once again, due to the nature of the release weight treatment whereby the fish are attached to a 500 g lead weight and physically towed to the bottom, average descent rate was significantly higher (69.7 ± 11.2 m/min) than for 'do nothing' and vented treatments (ANOVA; $F_{2,55}$=39.1, p<0.001). The r^2 value calculated from the descending part of the depth profile for each trial showed the release weight treatment to have the most consistent mean rate of descent (0.99 ± 0.00) with the least variability (0.96-0.99) (Table 5.5). For the untreated release, the consistency of descent profiles was significantly lower (mean 0.95 ± 0.01, ANOVA; $F_{2,55}$=7.2, p<0.01) with a much greater variability (0.80-0.99), reflecting the greater variability in descent profiles in this group; some fish descended at a relatively constant rate (e.g. Fig. 5.17A), whilst others descended at variable speeds throughout their overall descent to the bottom. Vented fish had even less consistency in descent rate, having the lowest mean r^2 value (0.92 ± 0.02) and similar variability in descent profiles (0.80-0.99).

Figure 5.17. Typical examples of depth profiles of snapper following experimental release using three methods: **A)** untreated, **B)** vented, and **C)** release weight.

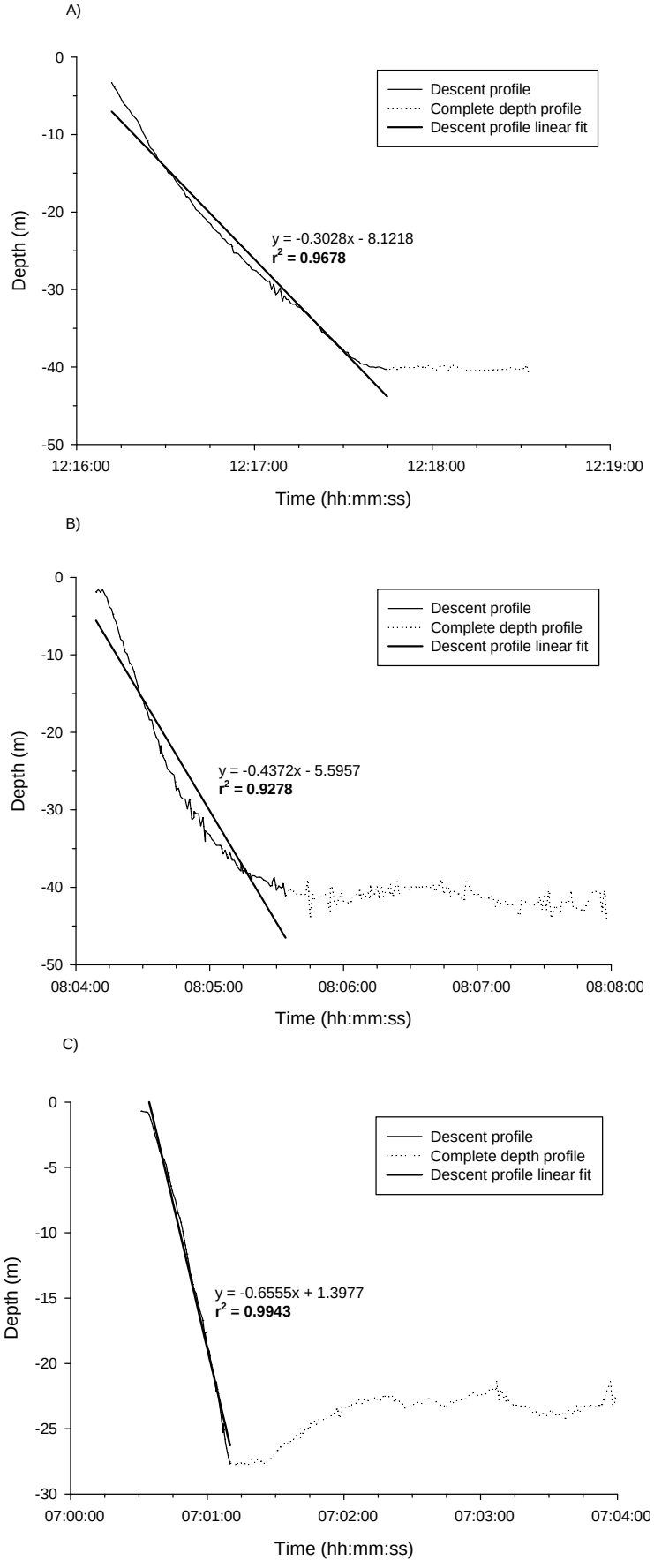


Table 5.5. Mean descent rates and associated descent variability for snapper following different release methods (untreated, vented or release weight). *- not including the fish which floated. *n* is sample size.

Release Treatment	*n*	Descent rate (m/min) ± SE (range)	Descent variability (r^2) ± SE (range)
Untreated	*31**	14.3 ± 1.8 (4.0 – 43.6)	0.95 ± 0.01 (0.80 – 0.99)
Vented	*14*	16.2 ± 2.6 (5.8 – 33.1)	0.92 ± 0.02 (0.80 – 0.99)
Weight	*11*	69.7 ± 11.2 (37.0 – 135.8)	0.99 ± 0.00 (0.96 – 0.99)

5.3.2. Mulloway

Due to difficulties involved in catching mulloway from deep water, the post-release behaviour and depth profiles of only 5 individuals were recorded after capture from water depths of between 7.5 and 12.0 m. The mean size of mulloway used was 58.1 ± 1.2 cm TL (range 55.2 – 62.1 cm). Because of the relatively shallow depth of capture and size of fish, mean fight time was short (1.5 ± 0.4 min). Four out of five fish were hooked in the mouth using soft plastic lures and the single fish caught using dead bait (squid) was hooked in the throat. The mean period the fish were kept at the surface prior to release was 13.6 ± 5.7 min, but ranged from 5.0 min up to a maximum of 35.0 min.

5.3.2.1. Barotrauma symptoms

Mulloway displayed several barotrauma symptoms, but no single symptom was present in all five fish caught. A bloodshot cloaca or slight anal prolapse was observed in each of one out of five fish, whilst a firm abdomen and excessive buoyancy was recorded in three out of five fish each. No other barotrauma symptoms were observed.

Figure 5.18. Field-caught mulloway showing bloodshot cloacas.

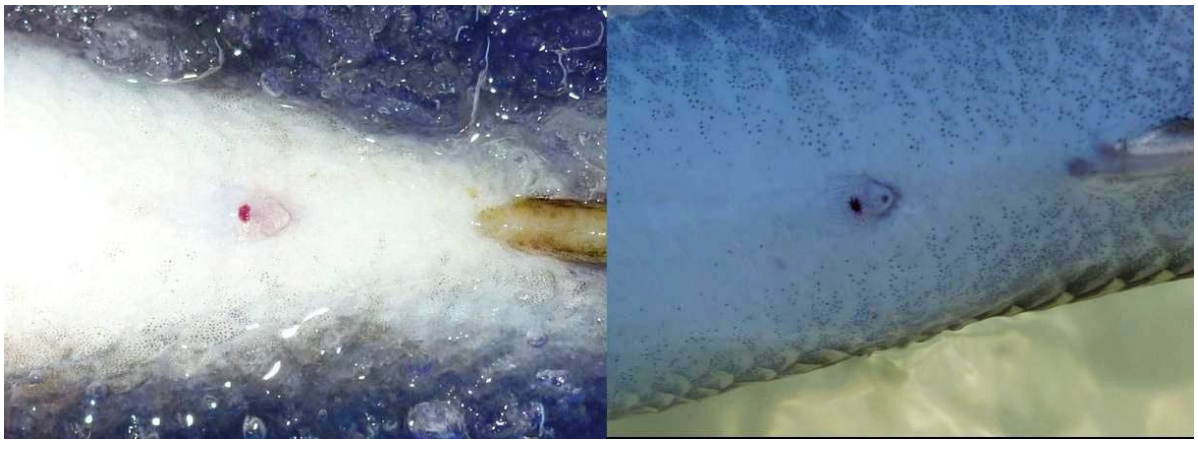


Figure 5.19. Field-caught mulloway showing distended abdomen and excessive buoyancy.

5.3.2.2. Release treatments

Because of the small sample size and relatively shallow capture depth, all five fish were released without any mitigation measures (i.e. untreated).

5.3.2.3. Post-release behaviour

Upon release, all five fish immediately began swimming towards the bottom. Four out of five fish exhibited 'slow swimming' behaviour after release with the remaining fish swimming away more quickly. All mulloway successfully returned to the bottom.

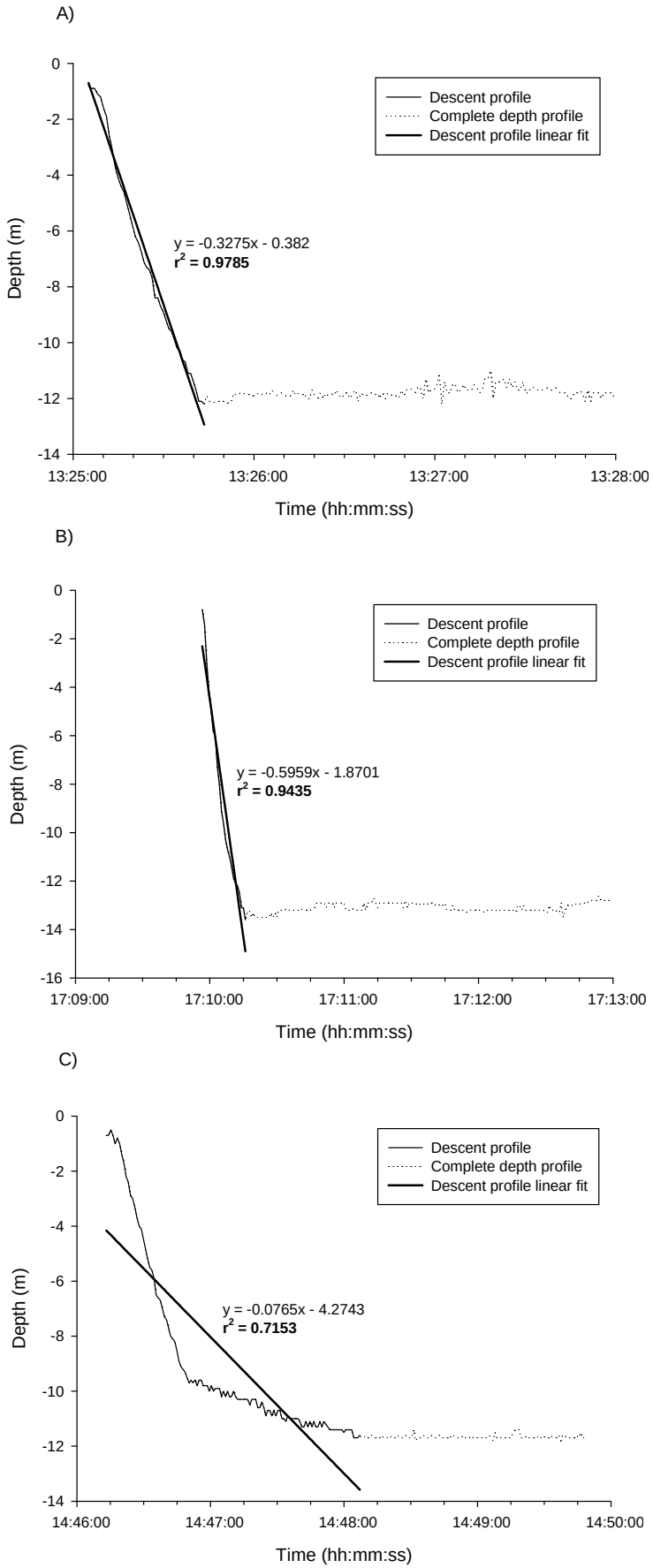
5.3.2.4. Depth profiles

Example depth profiles for mulloway are presented in Figure 5.20. The profiles for the majority of fish (3/5) showed little variation between trials in terms of descent rates or variability in descent rates (Fig 5.20A). However, the remaining two fish showed very different depth profiles following release. One fish swum very quickly to the bottom (Fig. 5.20B), whilst the other showed an initial rapid descent from the surface down to ~10 m depth followed by a dramatic reduction in descent rate through the final 2 m of water with descent through the first 10 m of the water column taking almost as long as the final 2 m (Fig. 5.20C). Following arriving at the bottom, all fish remained at that depth for the remainder of the trial.

5.3.2.5. Descent rates

Mean descent rate for mulloway was 15.1 ± 4.1 m/min (range 6.6 – 28.7 m/min). Descent rate was highly variable for one fish ($r^2 = 0.72$, Fig. 5.20C), but much less variable in all four other trials (0.94 to 0.99) with an overall mean r^2 of 0.92 ± 0.05.

Figure 5.20. Depth profiles of three mulloway following untreated release.



5.4. Discussion

5.4.1. Snapper

5.4.1.1. Symptoms

As has been previously demonstrated for snapper (Chapter 4, Stewart, 2008; Lenanton et al., 2009; Butcher et al., 2012), this species does suffer barotrauma when caught from deep water (~>10 m) with the prevalence of externally observed barotrauma-related symptoms increasing with depth. Similar results have been found by other authors for snapper with symptoms like abdominal distension, anal prolapse, stomach eversion and excessive buoyancy occurring more frequently with increasing depth (Stewart, 2008; Lenanton et al., 2009; Butcher et al., 2012). Many of the observations made from hyperbaric chamber experiments on snapper (Chapter 4) were confirmed by observations recorded in this study to also occur in the field-caught fish. For example, between 50 and 100% of fish decompressed from 30 m in the second chamber experiment in Chapter 4 were observed to have a distended abdomen with a similar proportion (79-95%) occurring in angled fish caught from 10-40 m deep water. Butcher et al. (2012) also recorded 100% of snapper to have a bloated abdomen when caught from >15 m deep water.

Results presented in Chapter 2 indicate that all snapper caught from >14 m depth suffer swimbladder rupture. Rupture of the swimbladder and resultant sudden release of gas into the abdominal cavity may induce eversion of the stomach and the likelihood of this occurring increase with the amount of gas which is contained within the swimbladder. This is determined by the depth the fish is acclimated to, with fish acclimated to greater depths releasing larger amounts of gas when they are brought to the surface and this is in turn reflected by the increasing prevalence of stomach eversion (4, 38 & 58%) and excessive buoyancy (4, 48 & 92%) that occurred with increasing depth (10-20, 20-40 & >40 m, respectively). In-chamber observations of the escape of gas bubbles from the area around the cloaca when the body tissues could no longer constrain the increasing volume of gas resulted in 34% of fish being negatively buoyant when depressurized from 30 m in Chapter 4 and was also observed in 28% of fish on approach to the surface when caught from >20 m depth in the field.

5.4.1.2. Post-release behaviour

Perhaps unsurprisingly, the behaviour exhibited by almost all (98%) snapper upon release, regardless of capture depth, surface interval or whether released vented or untreated, was to immediately swim towards the bottom. The single untreated fish which did not exhibit this behaviour was so buoyant that it was unable to submerge. Similarly, in 56 out of the 57 trials, regardless of release treatment or depth of capture, snapper swum all the way to the seafloor and remained in close proximity to the seafloor for the remainder of the trial. Importantly, no fish floated back to the surface after being returned to depth. This demonstrates conclusively that unless the fish is unable to submerge, a released

barotrauma-affected snapper wants to return through the entire depth of the water column to the bottom. The use of release weights or 'shot lines' to return fish all the way to the bottom is therefore also confirmed by these results to be an appropriate release technique as it mimics the natural post-release behaviour preference of the fish to return all the way to the bottom, even if they are unable to submerge on their own.

The potentially detrimental effects of barotrauma-related injuries (e.g. organ displacement, stomach eversion, exophthalmia, embolisms) on the welfare of affected fish require rapid mitigation if the fish is to be given the best chance of survival when released (Butcher et al, 2012). Results of hyperbaric chamber experiments presented in Chapter 4 showed mortality to be 0% when snapper were recompressed following simulated capture compared with 16% mortality when fish were not recompressed. Repressurizing the fish to capture depth has also been shown to relieve or mitigate the effects of many barotrauma-related injuries and decrease post-release mortality in many other species (e.g. St. John & Syers 2005; Parker et al. 2006; Hannah & Matteson 2007; Jarvis & Lowe 2008; Pribyl et al., 2012). It is therefore clear that all three release methods presented here eventually allow for fish to return to depth, albeit at different rates. Releasing fish untreated resulted in the slowest (although not statistically) return to depth, whereas venting snapper allowed for gas to be released from the body cavity rapidly and alleviated excessive buoyancy, thus allowing them to be able to swim away slightly faster than untreated fish. Butcher et al. (2012) came to similar conclusions. Due to the process involved in releasing fish using the release weight treatment, whereby the fish are attached to a heavy lead weight and physically towed rapidly all the way to the bottom, average descent rate was significantly higher than for either vented or untreated fish. Maximising the speed at which fish are able to return to depth may have important benefits for released barotrauma-affected fish (Butcher et al., 2012). These include: a lower probability of pelagic predation (Keniry et al., 1996; Overton et al., 2008); increased dissolved oxygen and cooler temperatures at depth (Marty et al., 1995; Shasteen & Sheehan, 1997); reduced energy expenditure (Strand et al., 2005) if the fish does not have to descend using its own propulsion (release weight) or is less buoyant (vented); and reduced exposure to the sun, predation by birds or being struck by boats if excessively buoyant and unable to submerge (Keniry et al., 1996; Gravel & Cooke, 2008).

Despite high variability in the descent rates seen from the depth profiles for each different release treatment, the patterns which occurred consistently between treatments are likely reflective of the variation in handling and manipulation of fish prior to, and during, release. The depth profiles for untreated fish showed the descent of fish to occur at a relatively constant rate from release until the fish reached the bottom compared with vented fish which descended slightly faster overall than untreated fish, but their descent was far more variable often with multiple increases and decreases in descent speed. We propose that this is in part a result of the stress associated with the increased handling required to successfully vent a fish (i.e. removal from livewell, physical restraint, identification of venting location and scale removal), as well as causing a localised puncture wound and physical trauma associated with the actual penetration of the body cavity by the venting device (Butcher et al., 2012). Incorrect venting technique may also cause mortality if inadvertent puncture of internals organs or the spinal cord occurs (Rummer & Bennett 2005; Jarvis & Lowe, 2008). The implications of the temporal variation among release methods do provide some basis for prioritization of their use by fishers as suggested by Butcher et al. (2012), however, there are some other important factors to consider when making a release method choice for barotrauma-affected snapper. The increased speed of

descent found for vented fish over untreated fish in the present study and that of Butcher et al. (2012) may support venting over untreated release, however as described above the potential for increased handling stress and inadvertent detrimental injury via incorrect venting technique outweighs the minor benefit of getting the fish to the bottom slightly quicker than if released untreated. Similarly, the stress associated with handling a fish in order to insert a release weight successfully through the skin of the upper or lower jaw, followed by that caused by rapidly plummeting through the water column to the seafloor, may be considerable. It has also previously been suggested that a struggling fish attached to a release weight may also attract predators on descent as well as when disoriented on the bottom after release (Danylchuk et al., 2007; Butcher et al., 2012). Hannah et al. (2008) similarly showed that handling-related injuries played a large role in the failure of barotrauma-affected rockfish *Sebastes* spp. to submerge when released.

Even though we did not find a significant effect of the amount of time a fish spends at surface pressure after capture (surface interval) on snapper survival in this study, it has been shown to significantly affect survival in many other fish species (Feathers & Knable, 1983; Burns et al., 2002; Parker et al., 2006; Hannah et al., 2008; Jarvis & Lowe, 2008). Jarvis & Lowe (2008) found that fish held at the surface for 10 min or less had a 78% probability of survival following recompression, and this probability of survival increased to 83% if fish were released within 2 min of landing. Similarly, even when held in water at the surface, Burns et al. (2002) found that mortality of reef fishes captured from 40 m ranged from 20% when held at the surface for 3 min to 100% when held at the surface for 18 min. Fish held at the surface for long periods of time may experience thermal stress which may have a detrimental synergistic effect when combined with reduced blood flow as a result of intravascular bubble formation following decompression, especially in warmer surface waters where oxygen demand is higher and oxygen concentration is lower (Feathers & Knable, 1983).

5.4.2. Mulloway

5.4.2.1. Symptoms

Many of the observations of barotrauma symptoms made from hyperbaric chamber experiments on mulloway (Chapter 3) were confirmed by observations recorded in this study to also occur in the field, despite the small number of trials undertaken (n=5). A bloodshot cloaca, slight anal prolapse, abdominal distension and excessive buoyancy were all recorded in mulloway caught from 8-12 m deep water. These symptoms were similarly prevalent to those seen in fish used in hyperbaric chamber experiments where C&R was simulated from 10 m (Chapter 3). Results presented in Chapter 2 show that the swimbladder of mulloway does not rupture unless caught from depth exceeding 17.5 m indicating that all of the five mulloway caught in this study would have possessed intact swimbladders. Because the expanded gas was not able to escape from the swimbladder, the barotrauma symptoms and injuries observed were therefore not as severe as those seen in fish used in hyperbaric chamber experiments where C&R was simulated from 30 or 50 m (Chapter 3). The only symptom of barotrauma recorded in the closely-related black jewfish *Protonibea diacanthus* when caught from similar water depths to those found in this study (<15 m) was abdominal distension (Phelan, 2008). Injuries such as exophthalmia, stomach eversion and escape of gas from the body cavity resulting from swimbladder perforation

and sudden release of gas into the abdominal cavity were therefore avoided in these trials. A similar suite of injuries considered to be detrimental or critical such as corneal haemorrhaging, exophthalmia and stomach eversion also increased in frequency when black jewfish were caught from >15 m deep water (Phelan, 2008).

5.4.2.2. Post-release behaviour

Because of the small sample size and shallow capture depths, all five mulloway were released untreated. Nonetheless, all fish five fish immediately began swimming towards the bottom upon release. All five fish successfully returned to capture depth, albeit at varying rates, and remained in close proximity to the bottom for the remainder of each trial. Mean descent rate for mulloway (15.1 ± 4.1 m/min) following release was similar to that of untreated snapper (14.3 ± 1.8 m/min) (see 5.3.1.5 above). As for snapper, these results demonstrate a released barotrauma-affected mulloway wants to return through the entire depth of the water column to the bottom. The use of release weights or 'shot lines' to return fish all the way to the bottom is therefore also confirmed by these results to be an appropriate release technique as it mimics the natural post-release behaviour preference of the fish to return all the way to the bottom.

Results of hyperbaric chamber experiments presented in Chapter 3 showed mortality to be 38% when mulloway were recompressed to 30 m depth following simulated capture, compared with 100% mortality when fish were not recompressed clearly demonstrating the usefulness of recompression in order to mitigate the effects of many barotrauma-related injuries and decrease post-release mortality. Even though we were not able to examine the post-release behaviour and depth profiles of mulloway released after being vented or released using a release weight, these release methods were not required for mulloway caught from the relatively shallow depths presented here. We were also unable to perform any trials on large mulloway or mulloway caught from deeper water. Mulloway grow to extremely large sizes (>75 kg & >180 cm: Silberschneider et al., 2009) and the effects of barotrauma in several species have been shown to be affected by fish size. Hannah et al. (2008) found that larger body size negatively influenced submergence success in blue rockfish *Sebastes mystinus*, thus reducing the probability of survival. Similarly, Rudershausen et al. (2007) found length had a significant positive relationship with stomach eversion in vermilion snapper *Rhomboplites aurorubens* and red grouper *Epinephelus morio* which may cause delayed mortality (Schirripa et al. 1999; Burns & Restrepo 2002; Burns et al., 2002). Mulloway are also caught from water depths of up to 150 m in southern Australia (Kuiter, 1993) and capture depth has been shown to be the critical factor affecting mortality and the development of detrimental decompression injuries in almost every study of the effects of barotrauma in many fish species (e.g. Collins et al. 1999, St John & Syers 2005), including mulloway (Chapter 3). Any future investigation into the effect of differing release methods on post-release behaviour in mulloway should attempt to examine larger fish and fish caught from deeper water than those in this study.

Nonetheless, results presented for snapper (see 5.4.1.2 above) and other species (e.g. Brown et al., 2008) make it clear that all three release methods could theoretically allow mulloway to return to depth, with venting and the use of release weights likely to be successful even if the fish is unable to submerge by itself. However, it has been reported for a similar species, the black jewfish, that venting is an unsuitable method for releasing

larger fish due to the large volume of gas contained in the swimbladder (Phelan, 2008). These authors suggested that release weights were more suitable for such large animals as they still allowed prompt return to depth. Once again however, the stress associated with the increased handling required to successfully vent a fish or release it using a release weight (i.e. removal from livewell and physical restraint) together with physical trauma associated with the penetration of the body cavity by the venting device or mouth/lips by the hook on the release weight, suggest that untreated release is likely to be the preferred release method for barotrauma-affected mulloway unless the fish is unable to submerge by itself.

The small number of field-based mulloway release trials presented here did not appear to show an effect of surface interval on release behaviour even though fish were kept at the surface for between 5 and 35 min. However, results of the hyperbaric chamber experiments presented in Chapter 3 showed clearly that surface duration is an important determinant of post-release mortality in mulloway. Mortality of fish acclimated to 30 m and repressurized after a surface interval of 2 min was 46%, but almost double (88%) when acclimated to the same depth but repressurized after a longer surface interval (10 min). Similar conclusions regarding the importance of minimising surface interval in order to maximise post-release survival has been reached by many other researchers examining determinants of post-release mortality in various barotrauma-affected fish species (e.g. Koenig, 2001; Burns et al., 2002; Jarvis & Lowe, 2008).

6. GENERAL DISCUSSION

6.1. Species-specific differences

The difference between snapper and mulloway in terms of each species' susceptibility to barotrauma was stark. The external symptoms, injuries sustained, mortality rates and recovery capacity of the two species differed substantially.

6.1.1. Symptoms

Mulloway exhibited many of the >70 physical signs of barotrauma reported in the literature (Rummer & Bennett, 2005). Exophthalmia, corneal emphysema, gill haemorrhage, bloodshot cloaca, abdominal distension, stomach eversion and rupture of the body wall all occurred in at least one mulloway after simulated capture from pressure equivalent to >30 m water depth (Chapter 3). In addition, several symptoms occurred in very high proportions of fish subject to certain experimental conditions. For example, for mulloway decompressed from 30 m and not repressurized, abdominal distension and stomach eversion occurred in all individuals, a bloodshot cloaca was apparent in 88% of fish, 63% exhibited exophthalmia and 50% had suffered body wall perforation. In comparison, abdominal distension was the only symptom that occurred in a significant proportion of individual snapper when decompressed from 30 m; 100% when repressurized and 47% when not repressurized. Other symptoms were recorded in only a few individual snapper; 5 (out of 64) possessed a bloodshot cloaca, 2 suffered haemorrhaging from the gills and only 1 had an everted stomach. Body wall perforation occurred in a similar proportion of snapper (34%) as for mulloway (50%) when subject to the same experimental treatment (i.e. not repressurized from 30 m). For rockfish *Sebastes* spp., Jarvis & Lowe (2008) have shown that species that show a high degree of barotrauma, like mulloway in the present study, are expected to have low survival following recompression relative to those species showing fewer external signs of barotrauma.

6.1.2. Injuries

Injuries due to barotrauma were similarly far higher for mulloway than for snapper. The number of injuries (20) caused by rapid decompression recorded from post-mortems of mulloway mortalities were also considerably higher than those recorded from snapper (13), indicating the greater susceptibility of mulloway to barotrauma when compared with snapper. Furthermore, the injuries which were most prevalent in mulloway (exophthalmia, haemorrhaging from the gills, viscera displacement, stomach eversion, liver trauma, hepatic vein damage and splenomegaly) were all considered by Phelan, (2008) to be detrimental, critical or fatal injuries in the closely-related black jewfish *Protonibea diacanthus*. Even though post-mortems performed on the small number of snapper mortalities confirm that many snapper would have almost certainly suffered the same types of injuries, their effects were not significant enough to kill the fish. The prevalence of most barotrauma injuries among snapper were relatively low, but consistent with those

previously recorded in snapper (Butcher et al., 2012) and many other species (Rummer & Bennett, 2005; St. John & Syers, 2005), some of which were likely reversible (Burns & Restrepo, 2002; Nichol & Chilton, 2006; Parker et al., 2006; Butcher et al., 2012; Chapter 4).

6.1.3. Mortality

Mulloway mortality was shown to be related to acclimation depth with mortality from 30 or 50 m equivalent depth (46 & 50%, respectively) far higher than mortality from 10 m (13%). In contrast, despite being subjected to simulated C&R from the greatest pressures the chambers were capable of operating at (8.08 bar ≈ 70 m water depth), there were no snapper mortalities in pilot experiments. It was not until snapper were decompressed from pressure equivalent to 30 m water depth and left at surface pressure that some mortality was evident, albeit at a relatively low level (16%). In contrast, mulloway subjected to an identical experimental treatment suffered 100% mortality, and even when repressurized to depth after 10 min, mortality was still very high (88%). Even when the surface interval before recompression was much smaller (2 min), mulloway still suffered ~50% mortality when subjected to simulated C&R from pressure equivalent to 30 or 50 m depth. In contrast to previous research on snapper (Stewart, 2008; Lenanton et al., 2009; Butcher et al., 2012), this research showed mortality in snapper due to barotrauma to be affected only by surface interval (Chapter 4, experiment 2). Mulloway mortality due to barotrauma, on the other hand, was shown to be positively related to both capture depth (Chapter 3, experiment 1) and surface interval (Chapter 3, experiment 2).

The overall low levels of mortality and lack of any delayed mortality found for snapper in this study suggest that in the absence of other factors which could affect survival other than barotrauma, the majority of snapper should make a full recovery after deep water C&R. Other factors which do affect mortality in snapper include hooking location (Broadhurst et al., 2005; Grixti et al., 2010; McGrath et al., 2011; Broadhurst et al., 2012), attempted deep hook removal (Grixti et al., 2010; McGrath et al., 2011), handling (including tagging) (Broadhurst et al., 2005; 2012), time spent in onboard holding tanks (Broadhurst et al., 2005) and confining fish in cages after release (Broadhurst et al., 2005; Stewart, 2008; Lenanton et al., 2009; Butcher et al., 2012). Mulloway are similarly affected by such additional deleterious factors (Broadhurst & Barker, 2000; Butcher et al., 2007; McGrath et al., 2011) and the presence of these other causes of mortality of would likely serve to further increase the already high levels of mortality for mulloway caught from >10 m deep water.

6.1.4. Recovery capacity

A large number of injuries caused by rapid decompression were recorded from post-mortems of both mulloway and snapper, but mortalities were far higher in mulloway than in snapper (see 6.1.2 above). This indicates clearly that snapper have far greater capacity to recover from barotrauma than do mulloway. Mulloway also suffered from substantial delayed mortality with 46-50% of mulloway decompressed from 30 or 50 m dying an average of 64 d after simulated C&R. In contrast, there was no delayed mortality for snapper at all. Calculations of free vertical range (FVR) made in Chapter 2 and post-mortem examinations revealed that all snapper and mulloway decompressed from 14 or

17.5 m, respectively, would have suffered swimbladder perforation as a result of their simulated capture. Some species have the capacity to repair damaged swimbladders in <4 d (Burns & Restrepo; 2002; Nichol & Chilton, 2006). Staged post-mortems done on several snapper which were unintentionally decompressed from pressure equivalent to 30 m water depth due to a power failure in the work presented in Chapter 4 showed that swimbladder healing likely occurs at a similar rate (Fig. 4.24). In contrast, post-mortems of mulloway which died from 5 to 219 d after simulated C&R from 30 and 50 m depth revealed that a large proportion (~70%) had empty swimbladders. This suggests that the ability of mulloway to heal perforations and reinflate the swimbladder is far less than for snapper. Much of the delayed mortality in mulloway was likely due to the constant swimming behaviour exhibited by mulloway in order to generate hydrodynamic lift and so maintain position in the water column to compensate for their lack of buoyancy usually provided by the inflated swimbladder. This relentless expenditure of energy eventually resulted in loss of condition resulting in increased susceptibility to infections and parasites. This mechanism has been previously suggested to also lead to delayed mortality in various reef fish species via progressive loss of condition as a result of an inability to meet the energetic demands required to regulate their position in the water column by actively swimming if they have damaged swimbladders (Burns et al., 2002). In the wild, many such fish would swim away apparently healthy only to succumb to disease, predation or starvation potentially long after release.

6.2. Ecological & physiological determinants

The species-specific differences in susceptibility to barotrauma reported in this study appear to be related to differences in swimbladder morphology, physiology and also to the degree of vertical movement within the water column each species naturally exhibits (e.g. Lea et al., 1999; Parker et al., 2006; Hannah & Matteson, 2007). For example, snapper, which showed a high degree of resilience to barotrauma, have a small swimbladder which is completely enclosed by muscle tissue except where it is exposed to the visceral cavity. This small area of swimbladder membrane is thin and relatively inflexible so that it ruptures quickly and easily when hyperinflated through a relatively small perforation. In contrast, mulloway, which showed a high degree of susceptibility to barotrauma, have a thick, flexible swimbladder which takes up a large volume within the visceral cavity. This allows for much greater hyperinflation before catastrophic rupture occurs via large perforations. Thus, the extent of organ damage may differ among species with different body shapes, swimbladder morphologies, or both, even from similar capture depths, with the large degree of swimbladder hyperinflation evident for mulloway likely to result in greater damage to organs via displacement, crushing or torsion. The more spectacular swimbladder perforations in mulloway are also likely responsible for the increased prevalence of stomach eversion, liver damage and hepatic vein trauma, and thus mortality, compared with snapper. Other authors have also suggested swimbladder morphology to be an important determinant of susceptibility to barotrauma in various other species (e.g. Feathers & Knable, 1983; Jarvis & Lowe, 2008). Jarvis & Lowe (2008) found that rockfish species with thicker swimbladder morphology had lower survival than species with thinner swimbladders. Species with thick swimbladders were suggested to suffer greater prevalence of stomach eversion due to gas escapement into the body cavity at high pressure, and thus susceptibility to barotrauma. Delayed mortality in other species with stomach eversion may be a result of internal organ torsion associated with the occurrence of stomach eversion, internal organ damage, or both, resulting from the overinflated

swimbladder crushing organs (Keniry et al. 1996; Rummer and Bennett 2005; Jarvis & Lowe, 2008). The low susceptibility of snapper to barotrauma may also be due to the possession of multiple haemoglobin isomorphs (Stephens et al., 2002) which potentially permits them to be resilient to hypoxia caused by overinflation of the swimbladder markedly reducing cardiac function by impeding venous blood return together with the physical obstruction of water flow over the gills caused by an everted stomach filling the buccal cavity (Rummer & Bennett, 2005; Jarvis & Lowe, 2008; Phelan, 2008; Butcher et al., 2012).

The ecology and behaviour of the two species may also help to explain their differential susceptibility to barotrauma. Snapper most often occur in schools up off the seafloor with schooling occasionally occurring at the surface (Kailola et al., 1993). In addition, anecdotal reports from fishers indicate that, at times, snapper may be attracted from depth almost all the way to the surface using berley. Mulloway on the other hand, are a far more demersal species, most often found in close proximity to the bottom (Kailola et al., 1993). Results presented in Chapter 2 show that density at equilibrium and rates of gas secretion and absorption into and out of the swimbladder are also much faster for snapper than for mulloway (~4 & 7 times, respectively), indicating that snapper are able to more quickly change their position in the water column whilst maintaining near-neutral buoyancy. Mulloway, on the other hand, are restricted to much slower changes in depth and generally remain much closer to the bottom. The >10-fold difference in the rate of gas absorption than secretion (Chapter 2) means that vertically mobile species such as snapper, with autonomous physiology always driving them toward neutral buoyancy, would be neutrally buoyant at a depth much shallower than their mean depth. As shown in Chapter 2, the upper extent of the vertical range is a function of the neutral buoyancy depth and the physical limits of positive buoyancy. Because capture depth will probably be closer to neutral buoyancy depth for demersal species like mulloway, than for semi-pelagic species like snapper, semi-pelagic fish captured at similar depths should show less barotrauma.

6.3. Guidelines for releasing barotrauma-affected snapper and mulloway to maximise post-release survival

The following procedure for releasing barotrauma-affected snapper and mulloway in order to maximise post-release survival is therefore recommend. An initial attempt should be made to release the fish untreated as quickly as possible. This avoids the potential for handling-related stress associated with venting or release weight attachment and minimises surface interval. If the fish is unable to submerge by itself due to excessive buoyancy, exhaustion, or both, the fish should be returned to depth using a release weight or similar device. This avoids the potential for inadvertent puncture of internal organs or the spinal cord if vented incorrectly. The implementation of the least invasive assisted release methods (i.e. release weights or shot lines) have also been recommended in order to achieve recompression in several other barotrauma-affected species when unable to submerge independently (e.g. Jarvis & Lowe, 2008; Sumpton et al., 2010; Pribyl et al., 2012).

7. CONCLUSIONS

7.1. Swimbladder gas exchange rates & buoyancy control

The volume of the mulloway swimbladder was found to be ~4.9% of total fish volume, which was larger than that for snapper (~4.2%). Despite this, overall snapper density was not significantly different to that of seawater, indicating that snapper at equilibrium are neutrally buoyant, whereas mulloway were denser than seawater and therefore negatively buoyant. This difference in buoyancy is almost certainly related to their differing utilization of the water column. The energetic costs of maintaining position in the water column is reduced by the neutral buoyancy of the semi-pelagic snapper. In contrast, the negatively buoyant mulloway are a far more demersal species. The rates of swimbladder gas secretion and resorption for snapper were also ~4 and 7 times faster than that for mulloway, again highlighting the difference in ecology and behaviour of the two species with the semi-pelagic snapper able to more quickly change position in the water column whilst maintaining near-neutral buoyancy than mulloway. Neither secretion nor resorption rates were affected by water pressure, water temperature or time at pressure for either species suggesting that gas is secreted at a constant rate whenever a certain level of pressure differential exists between the gas gland and the swimbladder. The ability to estimate acclimation rates of fish to different water depths is vital for any experiments that aim to investigate changes to fish physiology with depth. Our results confirm that rates of swimbladder gas exchange to be highly variable between species with inference of gas exchange rates from even closely-related or congeneric species likely to produce unreliable results.

7.2. Mulloway

Hyperbaric chamber experiments simulating the pressure changes experienced by mulloway during C&R have shown capture depth to be the most significant factor in determining whether a fish survives following release. Mortality increased dramatically when C&R was simulated from pressures greater than those found at 10 m water depth (i.e. 30 & 50 m). Chamber experiments were also able to show that swimbladder rupture occurs in mulloway if decompressed from pressure equivalent to water depths >17.5 m. It is unlikely that the occurrence of increased mortality and swimbladder rupture are purely coincidental. We therefore consider swimbladder rupture to likely be a key predictor of mortality in mulloway. The effects of swimbladder rupture and resultant release of gas directly into the body cavity caused abdominal distension, stomach eversion, exophthalmia, viscera haemorrhage, torn mesentery, liver trauma, hepatic vein damage, splenomegaly and in some cases, body wall perforation. With the exception of abdominal distension, these injuries did not occur in fish subjected to simulated C&R from pressure equivalent to 10 m depth and associated mortality was low. The majority of mulloway landed from water <10 m deep therefore, should survive if handled and released carefully. For depths >10 m, these experiments have also shown surface interval to be an important determinant of post-release mortality in mulloway. An increase in surface interval from 2 to 10 min before repressurization to 30 m depth effectively doubled mortality from ~50% to almost 90%. Surface interval also influenced how quickly mortality occurred; a 2 min

surface interval resulted in exclusively delayed (up to 219 d after simulated C&R) mortality, whereas a 10 min surface interval resulted primarily in immediate death. Immediate mortality was mitigated slightly (12%) by repressurization (returning the fish to capture depth). Depth profile trials indicate that the use of release weights or similar devices to return fish to the bottom is an appropriate release technique for mulloway unable to submerge as it mimics the natural post-release behaviour preference of the fish to return all the way to the bottom. However, the stress associated with the increased handling required to successfully vent a fish or release it using a release weight, together with physical trauma associated with the penetration of the body cavity by the venting device or mouth/lips by the hook on the release weight, suggest that untreated release is likely to be the preferred release method for barotrauma-affected mulloway unless the fish is unable to submerge by itself.

In order to maximise the post-release survival of mulloway, the following is therefore recommended:

- Avoid C&R fishing in water deeper than that which causes swimbladder rupture (i.e. ~17.5 m).
- If mulloway caught from water deeper than this must be released, the fish should be released untreated with the minimum surface interval possible.
- If the fish is unable to submerge by itself, the fish should be returned to depth using a release weight or similar device.

7.3. Snapper

The hyperbaric chamber experiments simulating the pressure changes experienced by snapper during capture in this study have shown it to be a relatively robust species to the effects of barotrauma. Despite using the greatest pressures the chambers were capable of simulating (8.08 bar ≈ 70 m water depth), there were no mortalities recorded. Capture depth is therefore not a significant determinant of mortality in snapper due to barotrauma, even though experimental results showed that swimbladder rupture in snapper occurs if decompressed from pressure equivalent to water depths of >14 m. The only experimental C&R simulation which resulted in any snapper mortality, albeit at low levels (16%), occurred when fish were left at atmospheric pressure after simulated capture from depth. Mortality as a result of this treatment was immediate (<15 min after simulated capture) and likely caused by the formation of emboli in the blood vessels of vital organs. No mortalities were recorded when identically-treated snapper were repressurized after a 10 min surface interval, suggesting that even this source of mortality is mitigated by repressurization (returning the fish to capture depth). These data show that in the absence of other factors which could affect survival other than barotrauma (e.g. deep hooking, handling, air exposure), that all snapper should make a full recovery after deep water C&R if quickly returned to depth. Depth profile data makes it clear that all three release methods examined (untreated, venting and release weight) facilitate return to depth, however the stress associated with the increased handling required to successfully vent a fish or release it using a release weight, together with physical trauma associated with the penetration of the body cavity by the venting device or mouth/lips by the hook on the release weight, indicate that untreated release is the preferred release method for barotrauma-affected snapper. Depth profile trials also indicate that the use of release weights or similar devices to return fish to the bottom is an appropriate release technique for snapper unable to

submerge as it mimics the natural post-release behaviour preference of the fish to return all the way to the bottom.

In order to maximise the post-release survival of snapper, the following is therefore recommended:

- Release the fish untreated as quickly as possible in order to minimise handling-related stress and surface interval.
- If the fish is unable to submerge by itself, the fish should be returned to depth using a release weight or similar device.

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9. APPENDICES

9.1. Estimation of yellowfin bream (*Acanthopagrus australis*) swimbladder gas secretion rate

On 23 May and 25 July 2012, a 3rd year student from the University of Western Sydney (UWS), Andrew Jacovides, used the custom-built hyperbaric chamber setup at the Cronulla Fisheries Research Centre of Excellence (CFRC) to estimate swimbladder gas secretion rate for yellowfin bream *Acanthopagrus australis* as part of his 3rd year Marine Biology major project. The yellowfin bream were collected from Port Hacking by rod and line from ~4 m water depth so that the potential for barotrauma when captured were minimised, and were housed at the CFRC Aquarium Facility in either circular fibreglass 5,000 L flow-through aquaria or in a 1 million L flow-through seawater pond.

9.1.1. Estimation of swimbladder volume

The relationship between fish length (fork length – FL), weight (g), volume (ml) and swimbladder volume (ml) was first calculated using the methods outlined in Chapter 2 for snapper and mulloway.

The fish ranged in size from 12.4 to 32.7 cm FL (mean ± SE: 21.6 ± 2.5 cm FL) and 43 to 903 g in weight (mean ± SE: 364 ± 106 g) (Table 9.1). The relationship between fish volume and body weight for yellowfin bream was linear:

Fish volume = 0.99 × body weight + 1.50, $r^2 = 0.9997$.

The linear relationship between swimbladder gas volume and fish volume (Gas volume = 0.04 × fish volume + 2.27, $r^2 = 0.9854$) was a better fit than the linear relationship with fish weight (Gas volume = 0.04 × body weight + 2.28, $r^2 = 0.9850$).

The mean (± SE) percent of fish volume that was swimbladder gas was 6.03 ± 0.79%.

Table 9.1. Data collected on fish length (mm FL), body weight (g), fish volume (ml) and swimbladder gas volume (ml & % fish volume) for yellowfin bream.

Fork length (mm)	Body weight (g)	Fish volume (ml)	Swimbladder gas (ml)	Swimbladder gas (% of fish volume)
249	400.0	402.1	16.5	4.10
215	204.4	211.4	-	-
137	55.7	51	4.2	8.24
143	63.8	63.6	4.5	7.08
131	52.8	52.7	3.1	5.88
124	42.6	43	4.7	10.93
137	56.0	55.9	4.6	8.23
294	658.0	645.2	24	3.72
327	902.8	889.4	32	3.60
315	840.2	838.5	35	4.17
304	728	708.5	30.5	4.30

9.1.2. Estimation of swimbladder gas secretion rate

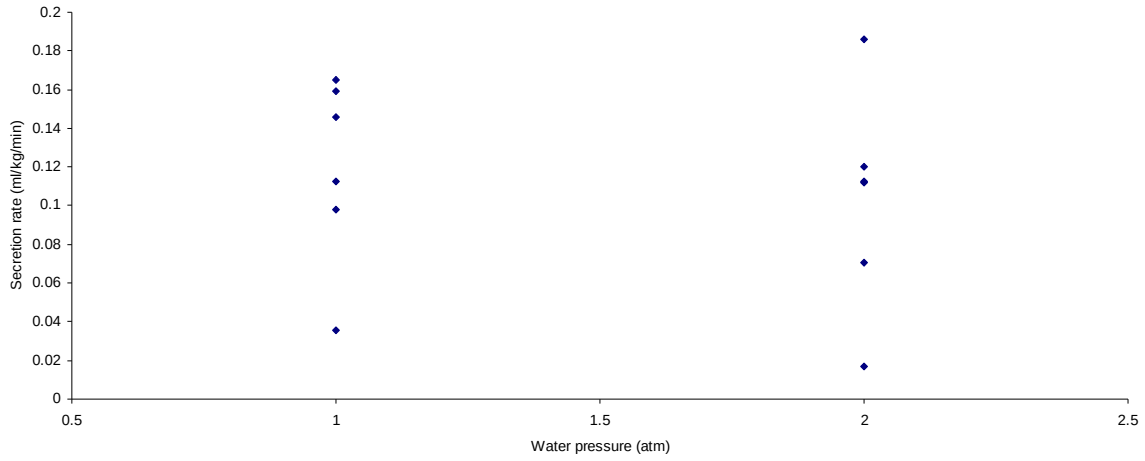
Swimbladder gas secretion rate was estimated using the methods outlined in Chapter 2 for snapper and mulloway, whereby yellowfin bream were exposed to 2 atm (equivalent to the pressure at 10 m water depth) and duration at pressure varied between one and 2 h.

The fish ranged in size from 13.6 to 24.1 cm FL (mean ± SE: 19.6 ± 1.0 cm FL) and 56 to 300 g in weight (mean ± SE: 167 ± 22 g).

The rate of gas secretion into the swimbladders of yellowfin bream did not appear to be affected by water pressure (Fig. 9.1).

The mean (± SE) rate of yellowfin bream swimbladder gas secretion was therefore calculated to be 0.111 (± 0.015) ml/kg/min.

Figure 9.1. Relationship between yellowfin bream swimbladder gas secretion rate and water pressure.



9.2. Detection, morphology & experimental confirmation of the role of the silver trevally (*Pseudocaranx georgianus*) swimbladder vent

During previous research into demersal fish traps in NSW (Stewart & Ferrell, 2002; 2003), it was noticed that as a trap containing large numbers of silver trevally (*Pseudocaranx georgianus*: Carangidae) neared the surface on ascent, it was preceded by large quantities of bubbles, presumably released by the fish in the trap (J. Stewart, pers. obs.). It has previously been recorded for another member of the family Carangidae, the samsonfish *Seriola hippos*, that large quantities of gas bubbles are released from the opercula region when angled from 90-110 m deep water, particularly during the last 10 to 20 m before reaching the surface (Rowland, 2009). It was subsequently shown that this gas release occurred via a membranous opening in the posterior region of the roof of the dorsal chamber of the swimbladder which allows swimbladder gas when under pressure to escape into a membranous tube that runs forward between the dorsal surface of the swimbladder and the vertebral column. Towards the anterior of the swim bladder, this membranous tube was shown to split laterally around the vertebral column before exiting under each operculum (Rowland, 2009). Rowland also reported that free-swimming samsonfish which followed hooked fish to the surface were also observed to release gas during ascent and SCUBA divers and rock lobster fisherman revealed that they had also witnessed samsonfish releasing bubbles when undertaking rapid vertical movements, suggesting this mechanism to occur as a part of the species' normal behaviour. Dissection of the congeneric yellowtail kingfish *S. lalandi* and amberjack *S. dumerili* revealed a homologous structure in each of these species, but not in species from several other carangid genera (*Carangoides*, *Caranx*, *Trachurus*, *Seriolina* & *Scomberoides*: Rowland, 2009).

Several silver trevally were therefore collected from Port Hacking by rod and line from ~4 m water depth so that the potential for barotrauma when captured were minimised, and were housed at the CFRC Aquarium Facility in either circular fibreglass 5,000 L flow-through aquaria or in a 1 million L flow-through seawater pond.

9.2.1. Detection of silver trevally swimbladder vent

A number of fish were subsequently euthanased with an overdose of anaesthetic (Aqui-S, New Zealand) and using a needle and syringe, air was introduced through the body wall of a submerged fish into the swimbladder (Fig. 9.2). Gas was subsequently revealed to be escaping via a single small (4-6 mm diameter) oval-shaped hole in an area of soft tissue in the pharyngo-cleithral membrane underneath each operculum (Fig. 9.3).

Figure 9.2. **A)** Location of syringe placement in order to introduce gas into the swimbladder of silver trevally, and **B)** gas bubbles escaping from beneath the operculum (circled).

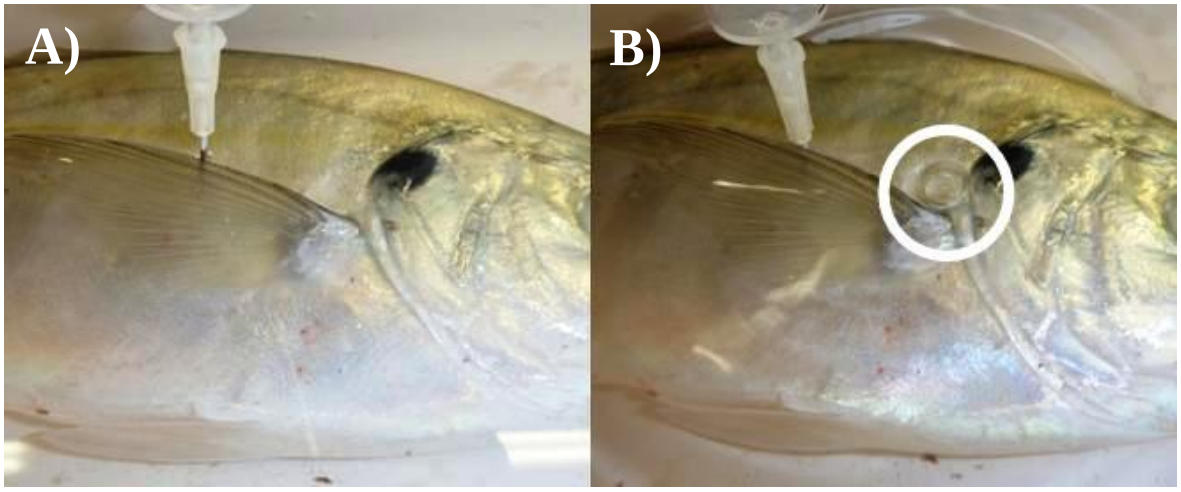
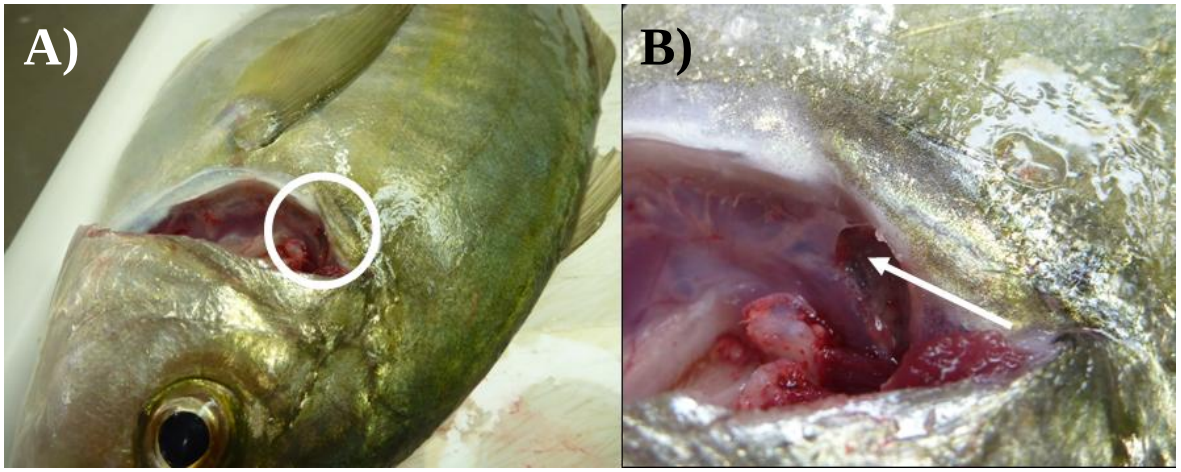


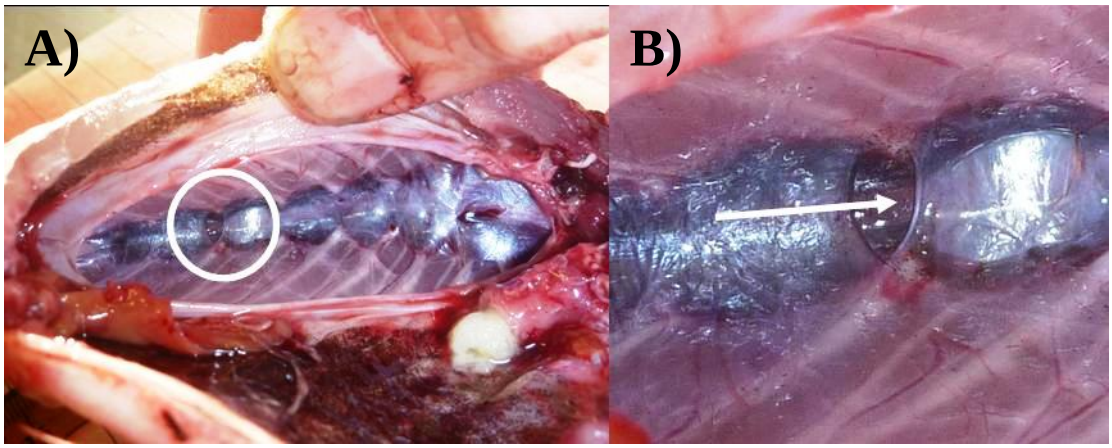
Figure 9.3. **A)** Location of external opening of silver trevally swimbladder vent (circled) in pharyngo-cleithral membrane tissue beneath operculum (gills and opercula removed), and **B)** close-up of opening (arrow).



9.2.2. Morphology of silver trevally swimbladder vent

Following the detection of the presence of this mechanism and its external opening, we attempted to determine the morphology of the structure allowing the escape of the gas by making a cast of it. This was achieved by introducing fast-drying cyanoacrylate glue (Loctite, Henkel Corp.) into the external opening of the swimbladder vent whilst simultaneously sucking gas from the swimbladder of a euthanased silver trevally using a needle and syringe through the body wall of the fish. This process drew the glue down the opening and into the swimbladder. The glue was then allowed to dry before the cast was dissected from the fish before being cleaned and photographed. The dissection of the dried glue cast revealed a membranous opening in the posterior region of the roof of dorsal chamber of the swimbladder (Fig. 9.4).

Figure 9.4. **A)** Location of internal membranous opening of silver trevally swimbladder vent in the posterior region of the roof of dorsal chamber of the swimbladder (circled), and **B)** close-up of opening showing direction of gas escape (arrow).

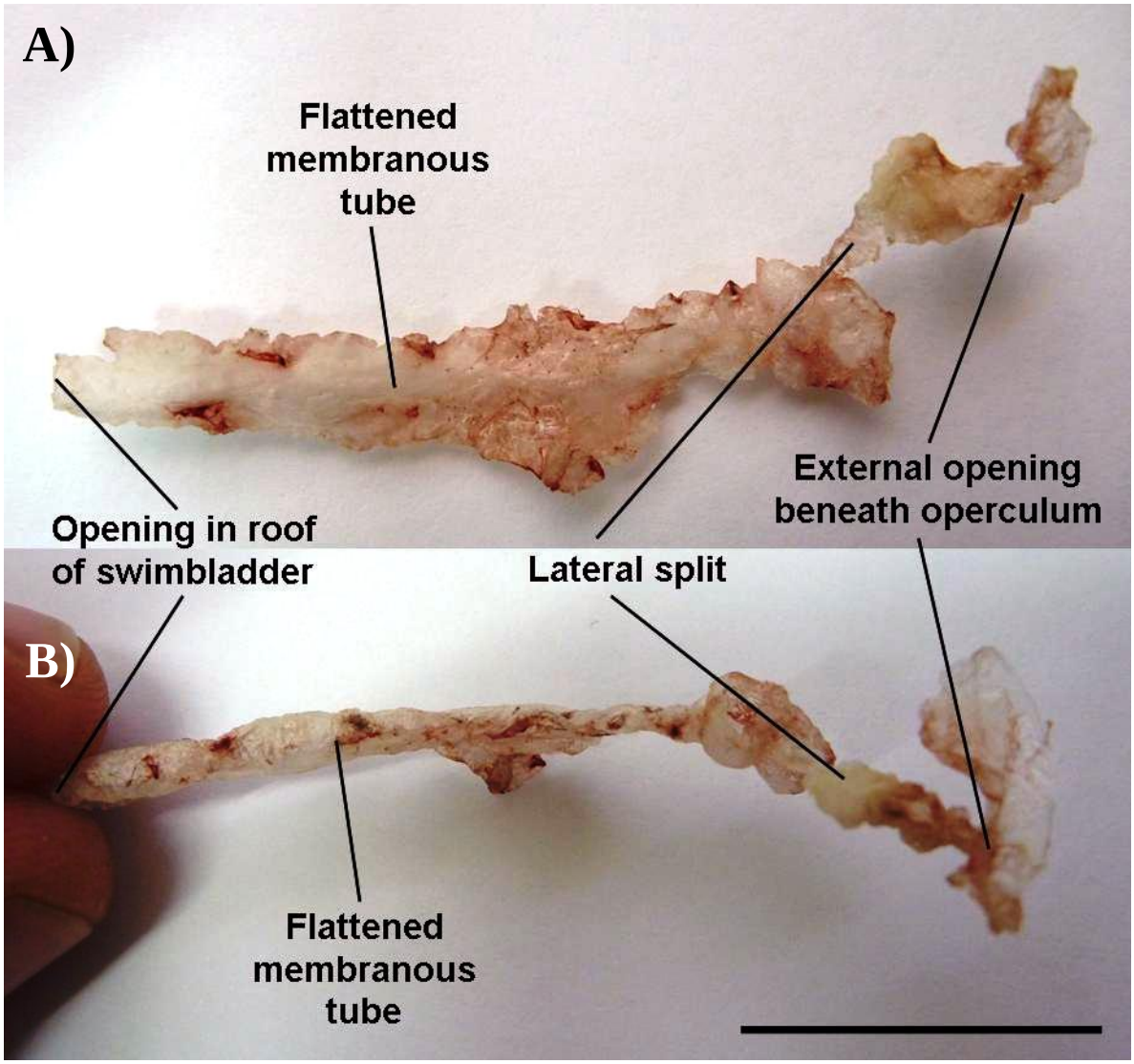


This opening led into a flattened membranous tube that ran anteriorly between the dorsal surface of the swim bladder and the vertebral column (Fig. 9.5). Toward the anterior of the swimbladder, the flattened membranous tube split laterally in two around either side of the vertebral column before exiting under each operculum (Fig. 9.5).

9.2.3. Experimental confirmation of the role of the silver trevally swimbladder vent

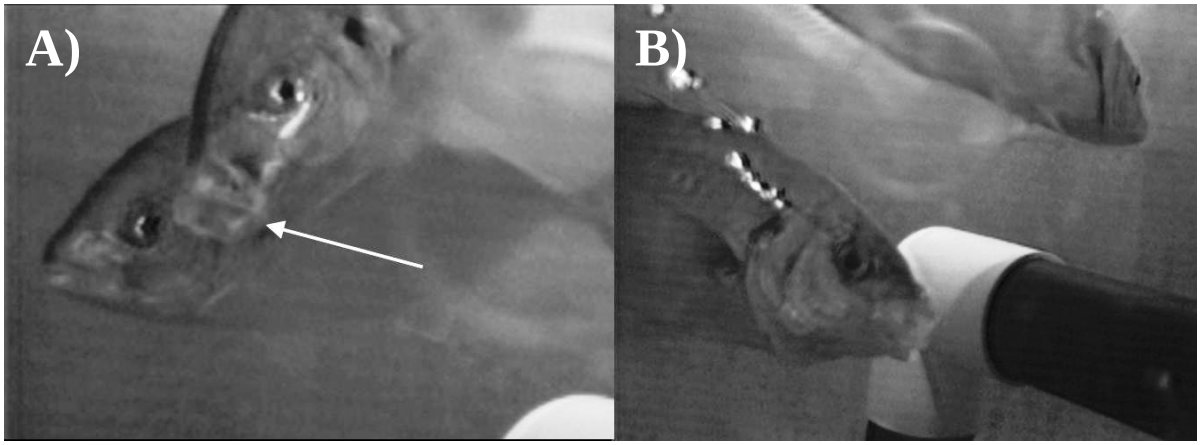
Following detection and anatomical characterisation of the morphology of the silver trevally swimbladder vent, we wanted to experimentally confirm the role played by this structure as a means by which the fish could vent excess swimbladder gas during ascent from depth. We therefore placed two silver trevally into one of the experimental hyperbaric chambers described in Chapter 2, pressurized the chamber to the equivalent of 30 m water depth and allowed the fish to acclimate over two days. The fish were then depressurized at the rate of ~1 m/s to sea level and their behaviour in the chamber was recorded by the camera setup described in Chapter 2. After ~20 s, at pressure equivalent to 10 m water depth, both fish flared their gills and everted their protrusible mouthparts, closely followed by the appearance of streams of bubbles originating from beneath both opercula on each fish (Fig. 9.6). In contrast to the behaviour of mulloway and snapper (Chapters 2 & 3, respectively) when depressurized, silver trevally showed no indications of stress whatsoever and continued to swim slowly around the chamber throughout. After depressurization, the fish were observed to be neutrally buoyant at surface pressure.

Figure 9.5. **A)** Ventral and **B)** lateral views of silver trevally swimbladder vent. Scale bar is 1 cm. Note that the glue was introduced into only one external opening so revealed the structure of only one of the two lateral splits.



The swimbladder vent therefore acts as a highly-effective "pressure release valve" (Rowland, 2009) by allowing expanding swimbladder gas to escape to the outside as the fish ascends through the water column. Control over the membranous opening of the swimbladder vent in the roof of the swimbladder may be an active or passive process, but the gill flaring and mouthpart eversion behaviour of fish recorded during depressurization in the hyperbaric chamber suggests the fish may have some control over whether or not to release the gas and the behaviours observed may facilitate this. Regardless, it is clear that when a critical pressure is reached inside the swimbladder during ascent, expanding gases exit via the flattened membranous tube and into the water column (Fig. 9.6). Observations of neutral buoyancy in fish depressurized from 30 m equivalent water depth which had vented gas (see above) suggests that this critical pressure is related to swimbladder volume for neutral buoyancy.

Figure 9.6. Silver trevally behaviour during depressurization taken from in-chamber video footage: **A)** gill flaring and eversion of the protrusible mouth, and **B)** appearance of streams of bubbles originating from beneath both opercula.



Venting of gas through the swimbladder vent continues whilst the internal pressure of the swimbladder exceeds the critical pressure during ascent through the water column. When the fish ceases ascending, internal swimbladder pressure drops below this critical limit and the venting of gas ceases, leaving the fish with an inflated fully-functioning swimbladder and neutral buoyancy. This highly-specialised structure thus enables silver trevally (and three members of the genus *Seriola*: Rowland, 2009) to undertake rapid vertical movements that might otherwise result in severe barotrauma injuries as a result of swimbladder hyperextension or rupture (Rummer & Bennett, 2005).

Rowland (2009) has suggested that the ability of a physoclist fish to vent excess swimbladder gases whilst retaining full swim bladder function would be advantageous in terms of: i) an increased ability to capture prey which are capable of rapid vertical movements (e.g. cephalopods which do not have a swimbladder or physostome fishes like clupeids); ii) avoiding predators that do not possess a swimbladder (e.g. sharks) in the pelagic environment; and iii) by permitting 'spawning-rushes' whereby gametes are released at the top of a high speed vertical ascent through the water column, a behaviour common in aggregating pelagic-spawning fishes.

9.3. Intellectual Property

No patentable inventions or processes were developed as part of this work. The work presented in this report remains the intellectual property of the authors, and they should be acknowledged when citing this work.

9.4. Staff

Staff who directly worked on this project:

Dr John Stewart – Senior Research Scientist
Dr Julian Hughes – Scientific Officer
Mr Cameron Doak – Fisheries Technician

Other titles in this series:

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