Physical impacts of recreational terminal fishing rigs in NSW, Australia

Shane McGrath

March 2012

This thesis has been submitted for the degree of Doctor of Philosophy with the School of Environmental Science and Management,

Southern Cross University, Lismore, NSW,

Australia
Declaration of Originality

I, Shane Patrick McGrath, consent to this report being made available for photocopying and loan, provided always that my work is fully acknowledged, and further that the granting of such a license in no way inhibits me from exercising any of my exclusive rights under the Copyright Act 1968. I have read the Abstract and I am satisfied that it is an accurate description of my thesis. I understand this license is granted in the interests of education and research, and that no royalties are payable.

Shane Patrick McGrath
Acknowledgements

This study would not have been possible without the financial support of the Fisheries Conservation Technology Unit (FCTU) of the NSW Department of Primary Industries (DPI) and the Recreational Fishing Trusts.

I would like to thank my two NSW DPI supervisors associated with my thesis, Paul Butcher and Matt Broadhurst for allowing me to undertake my studies as part of their research program. I would also like to thank the rest of the incredible FCTU which is composed of Alex Hulme, Chris Dowling, Lachlan Roberts, Craig Brand, Michael Wooden and Sebastian Sven Uhlmann. Without the help, guidance, support and encouragement from everyone in and associated with the FCTU, I really don’t think I would have completed my thesis.

First of all, I must thank Stuart Cairns for all his statistical support for the analysis of my data, without your help I would have had just a lot of numbers that I was unsure what they meant. Further, must also thank Stuart for forcing me to use R-stats. I must also thank my university supervisor, Amanda Reichelt-Brushett for deciding to be my supervisor at such a late stage in the development of my thesis. I am so grateful for your reviews, comments and support that you provided during the completion of my last research chapter. Thank you also to Steve Smith for the supervision and support that was provided during the completion of my thesis, it was quiet the rollercoaster. I also must thank Les Christidis from his support and encouragement during overcoming the final hurdles during my candidature.

Thank you also to Brendan Smith, Gavin Spencer, Adam Sheridan, Nigel Ferguson, Trent Rogash, Shane Taylor, Jason Sleeman, Jay Guthrie, Kristian Williams, Andy Carroll, Matt Harrison, Ben Black, Steve Dalton, Anna Scott, Daniel Harvey and Damien Harvey for their scientific input, support and encouragement. I am sure that without their contributions I would not have finished. Although, without some of their social support, I may have finished much earlier.
I would also like to thank my father (Ken McGrath), step mother (Dianne Hill) and brother (Rhys McGrath) for their constant support and help during completing my studies at university. I definitely would not have made it to university or ended up making completing my thesis without your support and encouragement. I would also like to thank my mother (Mary McGrath) who may be gone from this world but will surely never be forgotten. I must also thank my beautiful partner Tara Clark for her continued support, comments and encouragement during the completion of my thesis.

Thank you to everyone and to anyone that I have forgotten, you all made this project possible.
Abstract

Globally, recreational fishing is one of the most popular leisure activities with participation rates estimated at > 40% in some developed countries. Such high participation put more strain on an already depleted fisheries resource; however, while fishing pressure has increased so has the number of released individuals. This is due to anglers releasing at least part of their catch, either voluntarily or in response to regulations that include legal sizes and personal quotas. Australia is no different with a combined release rates of > 30% for all species. More specifically, three of the most important coastal species targeted by recreational anglers are mulloway (*Argyrosomus japonicas*), yellowfin bream (*Acanthopagrus australis*) and snapper (*Pagrus auratus*), with > 11 million individual fish released annually. However, there are many concerns regarding their post release fate and the consequences of catch-and-release angling. Given this, two field and five aquaria experiments were completed to quantify impacts associated with current catch-and-release methods.

The two field experiments were completed to identify and assess the occurrence of factors associated with the post-release mortality of angled *P. auratus*. The results from these experiments support the general consensus from previous work that hook ingestion is the primary factor influencing mortality, although the proportion of fish affected can be minimised by simply cutting the line and releasing the fish. However, the utility of this approach is based on the premise that hook-ingested individuals eventually eject their hooks, often after sufficient decay. The third experiment (initial aquaria based experiment) aimed to identify the technical parameters that contribute to increased hook decay by exposing different hook designs to seawater to investigate temporal and structural degradation. Degradation was mainly influenced by the hook material and wire diameter. The fourth experiment involved selecting three hook types (from the previous experiment) with similar absolute sizes made from different materials (stainless steel and nickel-plated and red-lacquer carbon steel) and then either modified (using small notches) or left unmodified. Individuals of the three species of fish listed above were then allowed to ingest one of the selected hook types (during angling), to determine the rate of hook ejection and their long-term fate. Hook ejection was the primary factor that influenced mortality in all three species, with only one death among those fish that ejected their hooks (133 fish in total).
compared to almost half of those that retained their hooks. This research supports the use of hooks that oxidise faster as they subsequently become weaker and are ejected more readily. However, ingested nickel-plated hooks could be detrimental to fish health as they resulted in significantly more deaths among *A. australis* and *A. japonicus* when compared to the other two hook types. This was investigated further in the final experiment which quantified the absorption of metals by *A. japonicus* that ingested nickel-plated hooks. Nickel-plated hooks resulted in elevated concentrations of nickel in the liver and blood, which were significantly higher than wild and control individuals.

This thesis demonstrates that hook ingestion results in increased mortality, and that cutting the line on angled fish increases their chance of survival as they will eventually eject their hooks. Hook ejection also augments survival; however, this is strongly influenced by hook decay. This may prove problematic, as the increased metal concentrations may negatively impact fish health. Therefore, future research should aim to investigate and develop hook designs that degrade quickly once immersed in seawater, which would inevitably increase the rate of hook ejection and reduce fish mortalities. Future research would also benefit from more ethically acceptable practices of determining the effects of ingested hook, which could included using haematological analysis methods rather than more destructive sampling methods (i.e. liver, flesh and gill samples).
# Table of Contents

List of Figures ...................................................................................................................... ix
List of Tables ........................................................................................................................ x

Chapter 1: General Introduction ..................................................................................... 12
1.1 Commercial fishing .................................................................................................. 13
1.2 Recreational fishing ................................................................................................. 14
   1.2.1 Management ................................................................................................. 15
   1.2.2 Effects of recreational fishing ...................................................................... 16
   1.2.3 Factors affecting post-release mortality ...................................................... 17
   1.2.4 Reducing post-release mortality ................................................................. 19
   1.2.5 Indirect and physiological responses to catch-and-release ......................... 23
1.3 Distribution and life history ..................................................................................... 25
   1.3.1 Mulloway (Argyrosomus japonicus) ............................................................ 25
   1.3.2 Yellowfin bream (Acanthopagrus australis) ................................................ 27
   1.3.3 Snapper (Pagrus auratus) ............................................................................ 27
1.4 Research aims and thesis outline .............................................................................. 28

Chapter 2: Immediate and post-release survival of angled snapper (*Pagrus auratus*) during two catch-and-release fishing events in NSW .................................................... 31
Abstract ............................................................................................................................. 32
2.1 Introduction .............................................................................................................. 33
2.2 Materials and methods ............................................................................................. 35
   2.2.1 Experiment 1- Botany Bay, Sydney, January 2008 ...................................... 35
   2.2.2 Experiment 2- Coffs Harbour, June 2009 and 2010 .................................... 37
   2.2.3 Data collection and statistical analyses ...................................................... 37
2.3 Results ...................................................................................................................... 38
   2.3.1 Experiment 1 .............................................................................................. 38
   2.3.2 Experiment 2 .............................................................................................. 43
2.4 Discussion ................................................................................................................ 46

Chapter 3: Reviewing hook degradation to promote ejection after ingestion by marine fish ......................................................................................................................... 51
Abstract ............................................................................................................................. 52
3.1 Introduction .............................................................................................................. 53
3.2 Materials and methods ............................................................................................. 56
   3.2.1 Hook types .................................................................................................. 56
   3.2.2 Hook assessment ....................................................................................... 58
   3.2.3 Statistical analyses .................................................................................... 60
3.3 Results ...................................................................................................................... 61
   3.3.1 Percentage of hook weight remaining ......................................................... 61
   3.3.2 Percentage of hook point remaining ............................................................. 63
   3.3.3 Tensile strength .......................................................................................... 66
   3.3.4 Compression strength ................................................................................ 67
3.4 Discussion ................................................................................................................ 69
3.4.1 Suggested minimum hook strength required ................................................ 70
3.4.2 Maximising the decay to reduce hook strength ............................................ 70
3.4.3 Conclusion ................................................................................................... 72

Chapter 4: Fate of three Australian teleosts after ingesting conventional and modified stainless- and carbon-steel hooks ............................................................................................ 74
Abstract ............................................................................................................................. 75
4.1 Introduction .............................................................................................................. 76
4.2 Materials and methods .......................................................................................... 78
  4.2.1 Collection of fish ........................................................................................ 79
  4.2.2 Hooks used ................................................................................................... 79
  4.2.3 Experimental procedure ............................................................................... 81
  4.2.4 Data collected and statistical analyses ........................................................ 82
4.3 Results ...................................................................................................................... 83
  4.3.1 Fate of mulloway .......................................................................................... 85
  4.3.2 Fate of yellowfin bream ............................................................................... 91
  4.3.3 Fate of snapper ............................................................................................ 92
4.4 Discussion ................................................................................................................ 93

Chapter 5: Absorption of metals in mulloway (Argyrosomus japonicus) after ingesting nickel-plated carbon-steel hooks ............................................................................................... 99
Abstract ........................................................................................................................... 100
5.1 Introduction ............................................................................................................ 101
5.2 Materials and methods ........................................................................................ 104
  5.2.1 Collection of fish ........................................................................................ 104
  5.2.2 Experimental procedure ............................................................................... 104
  5.2.3 Metal analyses ........................................................................................... 105
  5.2.4 Data collected and statistical analyses ........................................................ 106
5.3 Results .................................................................................................................... 108
  5.3.1 Metal analyses ........................................................................................... 109
5.4 Discussion .............................................................................................................. 117

Chapter 6: Conclusions and future directions ........................................................ 125
6.1 Conclusions ............................................................................................................ 125
6.2 Future directions and management implications .................................................... 128

References ......................................................................................................................... 131

Appendices ........................................................................................................................ 153
Appendix 1: Southern Cross University, animal research authority ......................... 153
Appendix 2: NSW Department of Primary Industries, animal research authority ......... 155
Appendix 3: University of New England, animal research authority ......................... 157
Appendix 4: University of New England, animal research authority ......................... 159
List of Figures

Figure 1: The size-frequency (TL mm) distribution of snapper, Pagrus auratus caught during the field study in Botany Bay (Experiment 1). Dark bars = treatment fish, Light bars = control fish. ..................39

Figure 2: The mean ± s.e. differences in the plasma glucose (mmol L⁻¹) between treatment, control and wild snapper, Pagrus auratus at the conclusion of experiment 1. ..................................................41

Figure 3: The size-frequency (TL - mm) distribution of snapper, Pagrus auratus caught during the two years (dark bars = 2009, light bars = 2010) for experiment 2 in Coffs Harbour. .........................43

Figure 4: The depth-frequency (m) distribution for snapper, Pagrus auratus caught during the two years (dark bars = 2009, light bars = 2010) for experiment 2 in Coffs Harbour. .........................44

Figure 5: The measurements recorded from all (a) J- and (b) circle hooks, and the location of the three small notches (1–3) cut into the shaft, bend and point of the modified J-hooks. .......................58

Figure 6: The specialised fittings used on the (a) Chatillon digital force gauge to measure the (b) compression and (c) tensile strengths (N) of hooks before immersion in seawater and then after eight and 28 days. ..............................................................................................................................59

Figure 7: (a) the differences in mean (+ s.e.) percentage of weight remaining for hooks sampled at $T_{8}$ and $T_{28}$ and the relationships between the percentage of weight remaining and hook (b) front (c) bend and (d) gape lengths. .................................................................................................................62

Figure 8: Scatter plots of the relationships between the percentage of hook weight remaining and wire diameter for hooks that were (a) stainless steel, (b) carbon steel, (c) modified, (d) unmodified, (e) circle designs and (f) J-designs, and the relationship between the percentage of hook weight remaining and shaft length for hooks (g) with and (h) without bait-holder barbs. ..........64

Figure 9: The differences in mean (+ s.e.) percentage of the point remaining for hooks sampled at (a) $T_{1}$ and $T_{28}$ and those made from (b) stainless and carbon steel. .............................................................................................................65

Figure 10: Scatter plots of the relationships between the percentage of the point remaining and wire diameter for hooks that were (a) modified, (b) unmodified, (c) circle designs and (d) J-designs. ....65

Figure 11: The differences in mean (+ s.e.) tensile strength between (a) $T_{0}$ and $T_{8}$, and (b) stainless and carbon steel. ................................................................................................................................ 66

Figure 12: Scatter plots of the relationships between the tensile strength and wire diameter for hooks sampled at (a) $T_{0}$ and (b) $T_{28}$, and those that were (c) modified, (d) unmodified, and (e) with and (f) without bait holders ......................................................................................................................66

Figure 13: (a) the differences in the mean (+ s.e.) compression strength of hooks sampled at $T_{0}$ and $T_{28}$, and (b) the relationship between front length and compression strength. ..................................................67

Figure 14: Scatter plots of the relationships between the compression strength and wire diameter for hooks that were (a) circle, (b) J-designs and (c) with and (d) without bait-holder barbs, and scatter plots of the relationship between the compression strength and shaft length for hooks that were (e) modified and (f) unmodified. .............................................................................................................69
Figure 15: The cumulative percentage of mortality of (a, b & c) mulloway *Argyrosomus japonicus*, (d, e & f) yellowfin bream *Acanthopagrus australis*, and (g, h & i) snapper *Pagrus auratus* after ingesting the six treatment hooks during the experiment. .................................................................86

Figure 16: The cumulative percentage ejection of the ingested treatment hooks by (a, b & c) mulloway *Argyrosomus japonicus*, (d, e & f) yellowfin bream *Acanthopagrus australis*, and (g, h & i) snapper *Pagrus auratus*. ..........................................................................................................87

Figure 17: The predicted proportion of weight loss per day of the ejected and dissected, and the six hook treatment groups (a & b) mulloway, *Argyrosomus japonicus* (c & d) yellowfin bream, *Acanthopagrus australis* and (e & f) snapper *Pagrus auratus*. .........................................................90

Figure 18: The logistic regression and predicted line value for the effect of total length (mm) on hook ejection by yellowfin bream, *Acanthopagrus australis*. .................................................................................................................91

Figure 19: Infection caused by the irritation of a stainless-steel hook that could not be passed by a snapper. ..............................................................................................................................................97

Figure 20: Scatter plot of the temporal differences in the percentage of weight loss (a) and the mean predicted proportion of weight loss per day (b) of the ejected and dissected hooks from mulloway, *Argyrosomus japonicus* ........................................................................................................110

Figure 21: The differences in mean (+ s.d.) metal concentrations in treatment, control and wild mulloway *Argyrosomus japonicus* samples for: (a) liver nickel, (b) blood nickel, (c) liver iron, (d) blood chromium, (e) liver chromium, (f) muscle chromium and (g) blood copper samples. *p < 0.05; **p < 0.01; ***p < 0.001..................................................................................................................113

Figure 22: Regression analyses of the relationship between TL (mm) and metal concentration in mulloway, *Argyrosomus japonicas* for (a) nickel muscle, (b) cobalt muscle, (c) manganese blood samples, and (d) stomach pH and manganese liver concentration. ..............................................................................................................116

Figure 23: Schematic representation of metal concentrations (µg g⁻¹) in (a) blood samples and (b) edible muscle samples of the treatment (■), control (●) and wild (▲) mulloway. The unbroken black lines (—) show the range of samples and the placement of the shape depict the mean, the broken grey lines (…) acceptable limits for human consumption (i.e. 10.0 and 1.0 µg g⁻¹ wet weight for copper and nickel, respectively) set by the Australian National Health and Medical Research Council. ..............................................................................................................121

List of Tables

Table 1: The pooled categorical parameters for angled-and-released snapper, *Pagrus auratus*, at the end of experiment 1 and the 2009 and 2010 tournaments (Experiment 2). ........................................41

Table 2: Mean (= s.e.) continuous parameters from experiment one and two (2009 and 2010), for angled-and-released *Pagrus auratus*. ..............................................................................................................45

Table 3: The pooled venting and release weight data from experiment 2 (2009 and 2010). ..........45
Table 4: Specifications and initial mean (± s.e.) continuous technical parameters for the 23 hooks examined in the study. Lengths, weight, absolute size ($n = 36$) and force ($n = 12$) are in mm, mg, mm² and N, respectively.

Table 5: Summary of parameters included in parsimonious multiple regression models in relation to percentage total hook weight and point remaining and tensile and compression strengths (N – newtons of force) following immersion in seawater for eight and then 28 days.

Table 6: Initial technical specifications (mean ± s.e.) of the six treatment hooks investigated for their rates of ejection and decay and influences on the mortality of mulloway, yellowfin bream and snapper after being ingested. Lengths ($n = 12$), weight ($n = 54–56$) and absolute size are in mm, mg and mm², respectively.

Table 7: Elemental composition of the three hook types (% composition), $n = 2$.

Table 8: Summary of fixed variables tested in parsimonious generalized linear mixed models for their independence on the mortality and hook ejection of hook-ingested mulloway, yellowfin bream and snapper over 61, 35 and 41 days, respectively.

Table 9: Significant categorical (counts) and continuous (mean ± s.d.) fixed effects ($p < 0.05$) identified in generalized linear mixed models affecting the mortality and hook ejection of mulloway, yellowfin bream and snapper over 61, 35 and 41 days, respectively.

Table 10: The percentage recovery for Cobalt, Chromium, Copper, Iron, Manganese and Nickel concentrations (mg kg⁻¹) in Lobster Hepatopancreas (TORT 2), (National Research Council, Canada), for the present work and the certified values.

Table 11: Ranges and means (± s.d.) for total length (TL - mm), stomach (S) and intestinal (I) pH ranges of the treatment, control and wild fish used in the experiment.

Table 12: The percentage metal composition (mean ± s.d.) of the size 2 nickel-plated hooks ($n = 3$) used.

Table 13: Metal concentrations (± s.d.) in the aquaculture food ($n = 4$), pond water and sediment ($n = 3$), and seawater at the NMSC ($n = 3$).

Table 14: Summary of fixed variables tested in parsimonious generalized linear mixed models for their independence on the concentrations of metals in the blood (B), liver (L) and muscle (M) of treatment, control and wild mulloway over 42 days.

Table 15: Summary of fixed variables tested in parsimonious generalized linear mixed models for their independence on the concentrations of metals in the blood (B) and liver (L) of treatment mulloway.

Table 16: The significant interaction ($p < 0.05$) between pierced stomach wall and organ in relation to metal concentrations (mean ± s.d. in mg kg⁻¹) in the liver and blood of treatment mulloway.

Table 17: The significant interaction ($p < 0.05$) between the presence of food in the stomach and metal concentrations (mean ± s.d. in mg kg⁻¹) in the liver and muscle of mulloway.
Chapter 1: General Introduction

Globally, recreational fishing pressures is high and it has previously been estimated that more than 40% of the population participate in recreational fishing in developed countries, and due to population growth, this is likely to increase (Cowx 2002; Cooke and Cowx 2004). Similar trends are evident in recreational fisheries in Australia (Henry and Lyle 2003). In 2000/01, a national recreational and indigenous fishing survey on fishing activity estimated that > 19% of Australian residents participated in some form of recreational fishing (mostly using hook-and-line) and contributed > $1.8 billion to the Australian economy (Henry and Lyle 2003). During the survey period it was estimated that fishers caught > 260 species of elasmobranchs, teleosts, crustaceans and cephalopods, with a total harvest of 135 million individuals. However, due to bag limits; minimum-legal lengths and the growing awareness of the need to conserve fish stocks, more than 40% of these individuals were released (Henry and Lyle 2003).

Of the > 260 species caught; mulloway (*Argyrosomus japonicus*), yellowfin bream (*Acanthopagrus australis*) and snapper (*Pagrus auratus*) are three coastal fish species released in large numbers (>11 million) by recreational fishers in Australia (Henry and Lyle 2003). Such large release numbers have raised concerns about unaccounted fishing mortality and led to several relevant studies on post-release survival (Broadhurst *et al.* 1999; Broadhurst and Barker 2000; Broadhurst *et al.* 2005; Broadhurst *et al.* 2007; Butcher *et al.* 2007; Grixti *et al.* 2010). These studies showed that the re-occurring factor primarily influencing mortality is anatomical hook location, with hook ingestion resulting in increased mortalities compared to mouth hooking.

For conservation and economic purposes, it is critical that the survival of released fish is optimised. Several methods have been developed to reduce fish from ingesting hooks during angling and thus improve survival. These generally include modifications to hook designs such as the attachment of metal appendages, lead and use of circle hooks (i.e. Willis and Millar 2001; Cooke and Suski 2004; Beckwith and Rand 2005; Butcher *et al.* 2008b). While these changes to terminal tackle
have proven to reduce mortalities, they also reduce hooking efficiency and overall catch, which is not popular with recreational anglers. In addition, hook design modifications are rarely fully effective at alleviating hook ingestion and post-release impacts.

Survival of hook-ingested fish has previously been shown to be increased through faster ejection rates (Broadhurst et al 2007). For example, Broadhurst et al (2007) observed that 76% of yellowfin bream ejected ingested nickel-plated hooks after an average of 20 days. The high rate of ejection was primarily attributed to hook decay. Most hooks oxidised to about 95% of their original weight, and had decayed sufficiently to break into smaller pieces.

Oxidising ingested hooks could have further sub-lethal effects on fish. As the hook decays, there is an increase in the concentration of metal ions readily available. While some metals are essential for metabolic processes, they can cause severe damage if they are at levels exceeding what is required (Berkowitz et al. 2008). With saltwater increasing the rate of the electro-chemical reaction, it is imperative that fishing hooks incorporate an anti-corrosive layer to limit corrosion. However, these coatings often include metals (i.e. nickel, tin, zinc and cadmium) that can be toxic (Noga 2010). Therefore, it is essential to establish whether or not there are any associated effects of ingested decaying hooks prior to recommending specific hook types. The primary objectives of this thesis were to provide valuable information on the post-release survival of hook-ingested mulloway, yellowfin bream and snapper; and the potential for increased hook ejection rates to mitigate mortality.

1.1 Commercial fishing
Mulloway, snapper and yellowfin bream are important to NSW commercial fisheries, where they are targeted and caught incidentally by several gear types, including gillnets, hook and line, seines, trawls and traps (Stewart and Ferrell 2002; Uhlmann and Broadhurst 2007; Scandol et al. 2008). In 2006/07, the combined annual commercial catch for these three species on the east coast of Australia (Queensland, NSW and Victoria), was in excess of 1052 mt and was worth more than $8.4 million.
AUD (Scandol et al. 2008; Department of Primary Industries 2009; Department of Primary Industries and Fisheries 2012). Due to the importance of these three species to the commercial fisheries, there have been several studies investigating unaccounted fishing mortality (Stewart and Ferrell 2002; Sumpton and Jackson 2005; Uhlmann and Broadhurst 2007; Broadhurst et al. 2008a; Broadhurst et al. 2008b). These studies determined that there is a clear relationship between the amount of damage caused to fish and air exposure during their discard from commercial fishing gear, and the probability of mortality. Specifically, Sumpton and Jackson (2005) observed high mortality rates (over 85%) in trawl captured juvenile snapper exposed to air for more than 15 minutes. Similarly, Uhlmann and Broadhurst (2007) observed that a combination of air exposure and damage increased the probability of discard mortality in yellowfin bream. However, Broadhurst et al (2008b) found that mortality in discarded yellowfin bream could be effectively reduced by limiting the duration of air exposure and damage through improved handling procedures. Similar differences in mortality following escape or release from various commercial fishing gears has been observed for other species (Chopin and Arimoto 1995; Chopin et al. 1996; Kennelly and Gray 2000; Broadhurst et al. 2006b).

1.2 Recreational fishing

On a global scale, recreational fishing is a multimillion dollar industry providing employment in many countries (Shrestha et al. 2002; Henry and Lyle 2003; Steinback et al. 2004). For example, in the USA, it was estimated that recreational saltwater fishing alone generated over US$30.5 billion in 2001, of which approximately $12 billion was income that created $≈ 350 000 jobs (Steinback et al. 2004). In Australia similar economic trends have also been recorded; however, expenditure was estimated at only AU$1.8 billion (Henry and Lyle 2003).

Globally, the pressures of recreational fishing are high. It is estimated that in several developed countries in Europe, Asia and America > 40% of the population participate in recreational fishing, and due to population growth, this is likely to increase (Cowx 2002; Cooke and Cowx 2004). This increase in recreational fishing pressure will put more strain on an already depleted resource.
However, one positive outcome is that while angling effort has increased, the number of released individuals has also increased as the majority of anglers release at least part of their catch (typically between 30 and 76%; Burke et al. 1994; Cooke and Cowx 2004), either voluntarily or in response to regulations that include legal sizes and personal quotas (Arlinghaus et al. 2007a). Such so-called ‘catch-and-release’ fishing is widely promoted as being socially and environmentally responsible (Arlinghaus et al. 2007a), although, to ultimately satisfy this definition, it needs to result in few associated mortalities or negative welfare effects for survivors (Muoneke and Childress 1994; Arlinghaus et al. 2007b).

In Australia, similar participation and release rates have also been observed. In particular, yellowfin bream, snapper and mulloway are three of the more popular coastal species and are particularly important due to their economic value. More specifically, these species are targeted by recreational anglers and have a combined total recreational catch of > 17 million individuals per annum, and are released at rates exceeding 62, 66 and 46%, respectively. This equates to a combined total of more than 11 million individuals being released per annum (Henry and Lyle 2003). As for the commercial sector, there have been concerns over the potential for at least some mortalities and/or sub-lethal impacts to these angled-and-released individuals and this has led to several quantitative studies (Broadhurst et al. 1999; Broadhurst and Barker 2000; Broadhurst et al. 2005; Broadhurst et al. 2007; Butcher et al. 2007; Grixti et al. 2010). However, there is a need to obtain more information on the potential factors that are associated with mortality of fish released by recreational anglers, so that mortalities can be mitigated.

1.2.1 Management

The current NSW recreational fishing regulations for mulloway, snapper and yellowfin bream are: a legal minimum length (LML) of 450, 300 and 250 mm TL, respectively; and a personal quota (bag limit) of 5 (only 2 fish longer than 70 cm), 10 and 20 day\(^1\), respectively. A recreational fishing license is also required unless the angler is: under the age of 18 years or assisting a person under 18;
Aboriginal or Torres Strait Islander; or a pensioner. While these regulations are imperative for controlling harvest rates, they are limited as they are unable to restrict biases in the sizes of fish that are targeted. In contrast, commercial fisheries are heavily regulated and include closures to fishing areas which reduce the likely event of undersized bycatch and subsequent discards (Ley et al. 2002). For example, recreational anglers exert fishing effort either knowingly or unknowingly in areas where there are high abundances of juvenile or undersize fish below the LML (Pollock and Williams 1983; Kumar et al. 1995). Furthermore, the majority (< 87%) of angling effort in Australia is concentrated in coastal areas, which is primarily where juveniles of the three species examined in this study spend their lives (Kailola et al. 1993; Griffiths and Heemstra 1995; Griffiths 1996; Henry and Lyle 2003).

Recreational fishing has increased both globally and within Australia (Henry and Lyle 2003; Bartholomew and Bohnsack 2005). It could therefore be hypothesised that there has been a consistent increase in fishing effort. Coincident with this increased effort is the heightened chance of potential disregard for current regulations and laws associated with recreational angling. For example, West and Gordon (1994) reported that more than 50% of mulloway (A. japonicus), captured and retained by anglers were under the LML. West and Gordon (1994) also indicated that the high retention observed was due to both a lack of angler knowledge and blatant disregard for current fishing regulations. While it may be relatively easy to implement controls within the commercial fishing sector, through licensing conditions, peer pressure and rigorous penalties, it is more difficult to improve compliance within the recreational fishery as enforcement is primarily limited by the number of fisheries officers.

1.2.2 Effects of recreational fishing

Both the direct and indirect effects of recreational line fishing have been investigated on a national (e.g. Broadhurst et al. 2005; Butcher et al. 2006; Grixti et al. 2010) and international scale (e.g. Arlinghaus et al. 2007a). The most obvious effects of recreational fishing include: a direct loss of resource (through harvesting); impacts on released fish (e.g. unaccounted mortality and sub-lethal effects); and impacts on the environment (e.g. discarded fishing gear, physical damage to habitat;
It is difficult to obtain a definitive figure on the indirect effects of recreational fishing on fish stocks as limited information is available on the survival of fish once they are released. By contrast, the direct loss can be measured more easily through surveys or catch records. There are a number factors associated with mortality that have been identified by quantifying the short-term fate of fish and the key factors influencing mortalities of released fish (for reviews see Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Lewin et al. 2006; Arlinghaus et al. 2007a).

With large numbers of fish released by recreational anglers in Australia on an annual basis, combined with the fate of released individuals being relatively unknown, it is imperative that the factors associated with recreational angling mortalities are identified and mitigated (Muoneke and Childress 1994; McLeay et al. 2002; Henry and Lyle 2003). Once these factors are identified it will allow us to better understand the processes causing mortalities and this, in turn, will help to develop better strategies that will aid in conserving fish stocks. These improved conservation strategies can then be implemented to reduce the mortalities of released or discarded individuals.

1.2.3 Factors affecting post-release mortality

While there is limited information available on the fate of most fish after being released from capture by hook and line, studies to date suggest there is large variability in mortality between species (Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007a). This variability has been partly attributed to several abiotic and biotic factors that fish are exposed to during the angling-and-release procedure (Broadhurst et al. 2005; Meka and McCormick 2005; Butcher et al. 2006; Hall et al. 2009).

Factors associated with catch-and-release that have been identified as predictors of mortality range from angling time to the size and type of bait used (Meka 2004; Butcher et al. 2006). The most common factors influencing mortality are: hypoxia; depressurisation; water temperature; and anatomical hook location (Storck and Newman 1992; Furimsky et al. 2003; Millard et al. 2003;
This latter factor is particularly important, with several studies demonstrating a clear correlation between mortality and the depth of hooking for many species (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005).

Mortality of released mouth-hooked individuals has been recorded to be as low as 0% for some species (Aalbers et al. 2004; Lyle et al. 2007). Similarly, mortality rates for the three species investigated in this research has been previously observed to be as low as 0 to 4.3% for mouth hooked fish (Broadhurst et al. 1999; Broadhurst and Barker 2000; Broadhurst et al. 2005; Broadhurst et al. 2007; Butcher et al. 2007; Grixti et al. 2010). These studies also estimated varying short-term (< 10 days) mortality rates of 3–38% for yellowfin bream (Broadhurst et al. 2007; Butcher et al. 2007), 3–51% for snapper (Broadhurst et al. 2007; Grixti et al. 2010), and up to 23% for mulloway (Butcher et al. 2007). These mortality rates are similar to those observed for other Australian species (Kumar et al. 1995; Butcher et al. 2006), and are considerably lower than the upper mortality limits of 87.5% recorded for hook-ingested yellowfin bream (Butcher et al. 2007). This variability has been observed in a number of other marine species worldwide and has been partially attributed to catch-and-release handling procedures (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007a).

The large variation in mortality rates observed between studies is most probably due to the limited availability of information on the effects of various fishing stressors and severity of physical damage that fish are exposed to during catch-and-release (Nuhfer and Alexander 1992; Chopin and Arimoto 1995). For example, the severity of damage to critical areas (i.e. gills and oesophagus), and consequent bleeding was directly correlated with frequency of mortality (Nuhfer and Alexander 1992; Broadhurst et al. 2007; Grixti et al. 2010). Several studies have also supported these results and concluded that injury to vital organs and/or excessive bleeding is strongly related to mortality (Muoneke and Childress 1994; Broadhurst et al. 2005; Grixti et al. 2007; Grixti et al. 2010). Further
investigation demonstrated that hook ingestion can result in mortality rates > 16 times higher than those recorded for mouth-hooked individuals (Butcher et al. 2007; Grixti et al. 2010).

With respect to mulloway, snapper and yellowfin bream it was concluded that the anatomical hook location, or more specifically hook ingestion, was a significant predictor of mortality (Butcher et al. 2007; Grixti et al. 2010). These studies observed high mortality rates for fish that ingested hooks and it was suggested that further investigation was needed to help reduce the occurrence of hook ingestion.

1.2.4 Reducing post-release mortality

The most obvious methods of reducing post release mortality is through minimising stress or limiting the extent of physical damage (Wertheimer et al. 1989; Meka 2004; Butcher et al. 2007). Mortality can be reduced through several improved procedures; better handling methods, changes to hook-removal methods, modifying hook designs, gear modifications and reducing air exposure (Ferguson and Tufts 1992; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007a). For example, a mortality rate of 72% was recorded for rainbow trout (Oncorhynchus mykiss), when they were exercised and exposed to air, whereas only 12% died when fish were exercised only (Ferguson and Tufts 1992). Previous studies have identified that novice anglers injured more fish and had longer handling times during hook removal than more experienced anglers (Cooke and Suski 2004; Meka 2004). Furthermore, Meka and McCormick (2005) indicated that minimizing the duration of angling will reduce sub-lethal physiological disturbances.

Passive actions, such as changes in terminal tackle selection and gear modifications can also be used to reduce mortalities (Wertheimer et al. 1989; Willis and Millar 2001). Circle hooks are an example of a commonly investigated change to terminal tackle to minimise hook ingestion, and ultimately mortalities (Falterman and Graves 2002; Cooke et al. 2003a; Meka 2004). The concept behind the success of circle hook designs that reduce fish mortalities is the orientation of the hook
point, which is curved back towards the shaft, thus allowing the hook to have a smaller gape. This reduces the chance that the hook will become deeply embedded within the digestive tract of the fish, and increases the rate of mouth-hooking when compared to conventional J-hooks (Falterman and Graves 2002; Cooke et al. 2003b). The greater rates of mouth-hooking, should lead to reductions in mortality (Falterman and Graves 2002; Millard et al. 2003).

While the benefits of circle hooks are quite positive, they by no means alleviate all the factors contributing to mortality. Cooke et al (2003a), found that when compared to three other conventional hook types, circle hooks had a higher probability of eye hooking and therefore permanently impairing the vision of released fish. They concluded that fish may have limited foraging ability and predator evasion due to sustained eye injuries. It is highly probable that such injuries may lead to increased long-term mortalities (Cooke et al. 2003b; Cooke et al. 2005). Circle hooks have also been reported to have half the hooking efficiency of other hook types (Cooke et al. 2003a; Cooke et al. 2005).

Another simple positive design change to commonly-used hooks is the removal of barbs from the point (Taylor and White 1992; Turek and Brett 1997; Schaeffer and Hoffman 2002; Meka 2004). Generally, this leads to shorter removal times and thus results in fewer handling injuries (Meka 2004). However, while barbless hooks have been shown to reduce damage and therefore mortalities, they are also associated with lower landing and hooking efficiencies than barbed J-hooks (Schill and Scarpella 1997; Schaeffer and Hoffman 2002; Meka 2004).

An alternative design modification that has been shown to reduce hook ingestion in snapper was developed by Willis and Millar (2001), and involves attaching a horizontal metal appendage (measuring either 20 or 40 mm) to the eye of the hook. This modification physically prevents the ingestion of the hook by effectively increasing its absolute size. The addition of the appendage was estimated to reduce the annual discard mortality by 78 and 96%, respectively, if it was assumed that all of the released hook-ingested fish died. Further, Butcher et al (2008b) also observed reduced hook
ingestion rates in yellowfin bream with a similar hook design modifications. Similarly, several other studies have recorded reductions in by-catch and hook ingestion in relation to the inclusion of metal appendages (Hall et al., 2006; Swimmer et al., 2011; Hataway and Stokes 2012; Serafy et al., 2012).

One problem with methods that limit the rate of hook ingestion is that they are also suggested to reduce catches through lower hooking efficiencies compared to conventional configurations (Willis and Millar 2001). Thus, anglers may not readily accept them because of their projected reduction in catches. Possible avenues that have been suggested to minimise hook ingestion whilst maintaining catches include active fishing methods (i.e. having the fishing line under tension during bait fishing) and using lures instead of bait when fishing (Diggles and Ernst 1997; Grixti et al. 2007).

Mortalities may also be reduced by limiting the extent of internal damage from hook ingestion (Butcher et al. 2007; Wilde and Sawynok 2009; Grixti et al. 2010). For example, Butcher et al (2007) reduced the likelihood of mortality for hook-ingested yellowfin bream from 87.5 to 7.7% when fish were released with their lines cut rather than forcefully removing the ingested hook. Due to the correlation between hook removal and mortality, and in accordance with overseas studies (e.g. Jordan and Woodward 1992; Aalbers et al. 2004), recommendations have been made to release hook-ingested fish with their lines cut, rather than using the harmful process of removing the hooks, which has been observed to cause severe internal damage (Broadhurst et al. 2007; Butcher et al. 2007; Grixti et al. 2010). The long-term utility of minimising negative impacts of releasing fish with hooks still ingested was recently investigated for yellowfin bream (Broadhurst et al. 2007; Butcher et al. 2010). However, there is an absence of similar longer-term data for mulloway and snapper (although see Grixti et al. 2010; which recorded an ejection rate of 13% among hook-ingested snapper).

While many marine fish have been observed to survive with ingested hooks and eventually ejecting their hooks, after up to 16 weeks (Hall et al. 2009; Stein et al. 2012), very little is known about the mechanisms that facilitate this process. It is most likely that the ability of some species to
cope with ingested hooks is due to their morphology and feeding traits (e.g. Aalbers et al. 2004; Broadhurst et al. 2007; McGrath et al. 2009). Yellowfin bream and snapper, for example, typically consume molluscs and crustaceans and their digestive tract is presumably accustomed to either ejecting or passing hard structures (Russell 1983; Kailola et al. 1993). Sparids also have pharyngeal teeth which help to digest hard and sharp materials by breaking the indigestible parts into smaller pieces (Alexander 1970). In addition to biological traits, both the mortality of fish and their hook ejection are strongly influenced by hook decay, mainly because this dictates how long the point and barb remain sharp (and the potential for associated internal damage) and the overall structural integrity of the hook (Aalbers et al. 2004; Broadhurst et al. 2007; Grixti et al. 2008).

Broadhurst et al (2007) suggested that hook ejection is possibly encouraged by corrosion and that small slices in the shaft of the hook (termed ‘bait holder barbs’) increase the chance of the hook breaking into smaller pieces and thus further increase the likelihood of the hook being ejected or passed by the fish. Therefore, an appropriate strategy to reduce mortalities from hook ingestion is through the promotion of hooks that are ejected more readily. This would include the use of hooks that have a high oxidation rate and incorporate areas that encourage corrosion. The concept behind this approach is supported by several studies which have demonstrated that, compared to forcefully removing ingested hooks, releasing fish with their lines cut resulted in lower mortality, with most individuals subsequently ejecting or passing their hooks (reviewed by Hall et al. 2009). For example, Aalbers et al (2004) and Butcher et al (2007) showed that this strategy reduced the mortalities of white seabass (Atractoscion nobilis), and yellowfin bream (Acanthopagrus australis) by 24 and 79%, respectively. Broadhurst et al (2007) also demonstrated that 76% of hook-ingested yellowfin bream were able to eject their hooks after an average of 20 days. Similarly, Lyle et al (2007) and Aalbers et al (2004) observed ejection rates of 30 and 39% for sand flathead (Platycephalus bassensis), and white seabass (Atractoscion nobilis), after 29 and 130 days, respectively.
While research shows that some fish are able to eject or pass ingested hooks, there is limited information available on the actual mechanisms that contribute to the shedding of the hook and the possibility that ejection can be promoted by particular designs and material composition of the hooks. The general consensus from the few available studies is that hook ejection is influenced by hook decay, especially at the point and barb (Aalbers et al. 2004; Broadhurst et al. 2007; Grixti et al. 2008). An example of this was observed in yellowfin bream, where hook-ingested fish were observed for up to 105 days after ingesting nickel-plated carbon steel hooks. Ejected hooks had oxidised, on average, 4% more than those that were not ejected and many of the ejected hooks had decayed sufficiently to break apart, which was often at or near bait-holder barbs. It was consequently presumed that these may have facilitated hook ejection (Broadhurst et al. 2007).

1.2.5 Indirect and physiological responses to catch-and-release

During angling, fish can be affected by sub-lethal factors that can contribute to mortalities (Meka 2004). These factors may have a cumulative effect on physiological stress and cause physiological disruptions (Barton et al. 1986). To assess the effects of cumulative or interactive factors, adequate information describing the catch-and-release history of individual fish must be recorded and the individual tracked for the occurrence of mortality. A simple way of achieving this is by limiting the number of individuals in an experimental tank or cage (Broadhurst and Barker 2000; Broadhurst et al. 2007). The extent of stress can be obtained via blood sampling and measuring cortisol and glucose levels (Kumar et al. 1995; Broadhurst and Barker 2000; McLeay et al. 2002; Broadhurst et al. 2007). Concentrations of cortisol are an indication of the initial stress response via stimulation of the nervous and endocrine systems and the release of catecholamines and corticosteroids (i.e. adrenaline and cortisol; Pankhurst and Sharples 1992; Barton 1997). If fish are exposed to stress for extended periods of time, secondary responses can occur affecting blood cell homeostasis and concentrations of metabolites such as glucose (Mazeaud et al. 1977; Wedemeyer and McLeay 1981; Carragher and Rees 1994). If the changes in blood plasma osmolality are great enough, or are prolonged, changes in
growth and morphological condition can occur, and can increase susceptibility to infections (Pankhurst and Sharples 1992; Barton 1997).

Previously accumulated physiological stress has been determined to have negative effects on behaviour, growth, predator evasion and disease resistance (Carragher and Pankhurst 1991; Chopin and Arimoto 1995; Gregory and Wood 1999). For example, increased mortalities were observed in brown trout (Salmo trutta L.) due to bacterial and fungal infections after exposed to chronic levels of stress (Pickering and Pottinger 1989). The majority of investigations into catch-and-release fishing have focused on short-term mortalities and have not included longer-term factors such as the ability of fish to avoid predation and recover from injuries or stress induced by recreational fishing (Chopin and Arimoto 1995; Broadhurst et al. 2006b; Butcher et al. 2010).

The majority of longer-term studies have focused on obvious and specific factors that are likely to reduce mortality rather than investigating the potential sub-lethal effects associated with angling and releasing fish (Aalbers et al. 2004; Broadhurst et al. 2007; McGrath et al. 2009; Grixti et al. 2010). For example, Broadhurst et al. (2007) observed hook-ingested bream for up to 105 days and only recorded mortalities, hook ejections and stress levels (cortisol and glucose) at the conclusion of the experiment. Similarly, McGrath et al. (2009) and Aalbers et al. (2004) monitored hook-ingested fish for up to 21 and 90 days, respectively, and recorded the same response variables. Some of these studies recorded oxidation in the ingested hooks; however, it was only suggested that increased oxidation was beneficial with respects to hook ejection, without determining if the oxidising ingested hook had any sub-lethal effects (Broadhurst et al. 2007; McGrath et al. 2009; Grixti et al. 2010). Further, the majority of the hooks used in the above studies contain nickel which could potentially have severe sub-lethal effects on fish health as it is classified as a heavy metal (Berkowitz et al. 2008).

While there has been limited research on the effects of hook ingestion, even less work has been conducted on the sub-lethal effects of an oxidising ingested hook and the associated negative
health implications. However, several studies have investigated the use of liver lesions, or increases in melenomacrophage centres (MMCs) within the liver as biomarkers for environmental degradation and pollution (Hartley et al. 1996; Manera et al. 2000; Stentiford et al. 2003). In particular, Schmalz et al (2002) observed that the presence of necrotic areas in the liver of fish that were exposed to mercury and other heavy metals was related to the increases in the formation of MMCs. Similarly, it was suggested that an increase in melanin or changes in pigmentation of the liver may protect against further cellular damage by absorbing free metal ions (Hartley et al. 1996). Hartley et al (1996) also indicated that increased areas of MMCs may be directly related to increased exposure to multiple chemicals and that excessive intake of iron could cause the destruction of red blood cells. While nickel is essential in trace levels to all organisms, at levels exceeding these requirements, it can cause severe damage which can result in deactivation of essential enzyme reactions, damage cellular structure and cause DNA modification (Berkowitz et al. 2008). It is also theorised that metal absorption will increase whilst in the digestive system, due to the lower acidity of digestive fluids increasing the availability of free ions (Whitehead et al. 1996). With iron and nickel being the main (96.0 and 2.5%, respectively) elemental components of nickel-plated hooks (along with Cr, C, Mn, CU and Co in trace amount), there is a need to establish if there are any sub-lethal effects associated with nickel-plated hook types prior to recommending their continued use.

1.3 Distribution and life history

1.3.1 Mulloway (Argyrosomus japonicus)

Mulloway (Argyrosomus japonicus) are members of the Sciaenidae family which consists of more than 270 species worldwide; 20 of which have been recorded in Australian waters (Paxton and Eschmeyer 1994; Kuiter 2006; Froese and Pauly 2009). Mulloway are a coastal species inhabiting estuaries and reefs up to 150 m deep. Their distribution ranges around the southern coast of Australia from Exmouth Gulf, Western Australia to the Burnett River, Queensland (Kailola et al. 1993; Edgar 1997). Additionally, they can also be found in waters surrounding Africa, China, India, Japan, Korea and Pakistan (Kailola et al. 1993). Adult fish (>100 cm total length- TL) generally inhabit nearshore
reef environments but can occasionally be found inshore around the mouths of estuaries and rocky foreshores. While sub-adults (< 100 cm $TL$) primarily inhabit inshore habitats (estuaries and surf zones), juveniles (<15 cm $TL$) are typically found in estuaries (Griffiths and Heemstra 1995; Griffiths 1996).

Adult mulloway are opportunistic predators but are primarily considered as benthic carnivores, feeding on small fish and a variety of soft-bodied marine invertebrates like squid and octopus (Marais 1984; Starling 1992; Kailola et al. 1993; Griffiths 1997). However, their dietary composition changes with different life stages. The diet for juvenile mulloway has been recorded to consist of alpheid, mysid and penaeid shrimps, and small fish (Marais 1984; Hall 1986; Fielder et al. 1999).

Limited information has been collected on the age and growth of mulloway in Australia. Evidence from South Africa reports that mulloway live for up to 42 years and reach a maximum $TL$ of 181 cm and weigh up to 75 kg (Griffiths and Hecht 1995; Griffiths and Heemstra 1995). Griffiths (1996) reported that growth rate and sexual maturity vary between males and females, with 50% reaching sexual maturity at 107 and 92 cm $TL$, respectively. Similarly, observations in Australia indicate that 50% of females and males reach sexual maturity at 68 and 51 cm $TL$, respectively (Silberschneider and Gray 2008).

In Australia, spawning generally occurs in nearshore coastal habitats from December to February; however, this varies geographically and is believed to be linked to oceanography and water temperature (Hall 1986; Griffiths 1996; Silberschneider and Gray 2008). The eggs are pelagic and hatch $\approx$ 1.5 days after spawning. After hatching, larval development occurs at sea and the larvae recruit into estuaries when they reach 2 cm $TL$ (Beckley 1990; Griffiths 1996; Silberschneider and Gray 2008).
1.3.2 **Yellowfin bream (*Acanthopagrus australis*)**

Yellowfin bream (*Acanthopagrus australis*) are members of the Sparidae family which consists of 110 species, 11 of which are found in Australian waters (Kailola *et al.* 1993; Carpenter and Johnson 2002; Orrell and Carpenter 2004; Kuiter 2006). They are endemic to the east coast of Australia and their distribution ranges from Townville, Queensland to Lakes Entrance, Victoria (Edgar 1997). Yellowfin bream generally inhabit estuaries, beaches and nearshore reefs; and can also be found in the lower freshwater reaches of rivers (Kailola *et al.* 1993). Their diet primarily consists of molluscs, crustaceans, worms and small fish (Kailola *et al.* 1993; Carpenter and Niem 2001).

Yellowfin bream can reach a maximum TL of approximately 75 cm, weigh up to 4.5 kg and have been reported to live for 14 years (Kailola *et al.* 1993; Carpenter and Niem 2001; Ochwada *et al.* 2008). They are a slow-growing fish and generally take about 3 years to reach sexual maturity (23 cm TL; Pollock 1982a). Yellowfin bream generally spawn in winter around the entrances to rivers (Pollock 1982b). After spawning, the majority of eggs drift out to sea and they recruit into estuaries when they reach about 13 mm (Pollock 1984).

1.3.3 **Snapper (*Pagrus auratus*)**

Snapper (*Pagrus auratus*) also belong to the family Sparidae and primarily inhabit sub-tropical and temperate waters of the Indo-Pacific including the Atlantic, Indian and Pacific oceans (Kailola *et al.* 1993; Carpenter and Johnson 2002; Orrell and Carpenter 2004). In Australia, adult snapper mostly inhabit nearshore reefs and can be found on offshore reefs in depths up to 200 m around the southern coastline from Hinchinbrook Island, Queensland to Barrow Island, Western Australia (Kailola *et al.* 1993). Juvenile snapper inhabit estuaries, bays and other shallow sheltered coastal environments (Kailola *et al.* 1993). Adult snapper primarily feed on crustaceans, molluscs and small fish, while the juvenile diet mostly comprises prawns and squid (Starling 1992; Kailola *et al.* 1993).
Snapper are a slow-growing species and, have been recorded to 130 cm TL, >16 kg and up to 35 years old (Francis et al. 1992; McGlennon et al. 2000). However, their growth rate varies within their distribution range and this is related to their habitat type. For example, in New South Wales (NSW), snapper attain sexual maturity at 3 years of age and 30 cm TL, whereas in Victoria they mature at 4 years and 27 cm TL (Kailola et al. 1993; Grant 2002). Once mature, spawning is triggered when the water temperature reaches 18 °C and, therefore, their spawning period also varies across their distribution range. For example, in Victoria, they spawn in summer (December-February) and in Queensland they spawn in the winter months between June-August (Kailola et al. 1993; Kuiter 2006). Snapper may also repeatedly spawn within a breeding season (Kailola et al. 1993).

Despite limited information on the early life history of snapper in Australian waters, it is assumed that fertilised eggs float around with the ocean currents until they hatch (Kailola et al. 1993). When they reach about 12 mm TL, they begin to recruit into estuaries, bays and other shallow sheltered coastal environments (Kingsford and Atkinson 1994; Trnski 2002; Fowler and Jennings 2003).

1.4 Research aims and thesis outline
Mulloway, snapper and yellowfin bream are targeted and caught by commercial and recreational anglers throughout Australia and large numbers of these species are subsequently released. While most of the harmful processes associated with catch-and-release fishing have been recorded for many other fish species (for reviews see Muoneke and Childress 1994; Cooke and Suski 2004; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007a). There is limited information on the post-release fate of these Australian species and the procedures that can be implemented to minimise their mortality; this is an essential step in ensuring the sustainability of the fisheries. It is therefore important to determine the primary factors that cause mortality and secondary long-term sub-lethal impacts which may influence survival. Such knowledge will lead to improved catch-and-release practices which can be implemented through education of the recreational fishing community.
The specific aims and objectives of the research covered in this thesis were specific to each chapter:

- **Chapter 2,** “Immediate and post-release survival of line-caught snapper (*Pagrus auratus*) during two catch-and-release fishing events in NSW”, aimed to quantify mortality (both immediate and 5 days after capture) and the prevalence of known mortality-causing factors for snapper, and suggest simple methods that might help mitigate these problems.

- **Chapter 3,** “Reviewing hook degradation to promote ejection after ingestion by marine fish”, used results from Chapter 2 on hook ingestion, and previous observations by Broadhurst *et al* (2007), that ejection of ingested hooks is influenced by corrosion. This chapter aimed to determine the technical characteristics affecting the temporal decay and structural integrity of a range of hooks commonly used to target small (< 40 cm TL) coastal species. These indices of decay can then serve as proxies, prior to the completing smaller-scale trials with fish to test and confirm hypothesised explanatory variables.

- **Chapter 4,** “Fate of three Australian teleosts after ingesting conventional and modified stainless- and carbon-steel hooks” investigated the effects of six different hook types studied in Chapter 3 on mulloway, yellowfin bream and snapper once they were ingested. The objective were to determine if wire material (stainless-steel and carbon steel) and/or modifications (comprising notches along the shaft, bend and under the point of the hook) affected the rates of mortality, hook breakage and ejection. The results were used to validate the findings from Chapter 3.

- **Chapter 5,** “Absorption of metals in mulloway (*Argyrosomus japonicus*) after ingesting nickel-plated carbon-steel hooks” further investigated the effects of ingesting nickel-plated hooks on fish, as Chapter 4 suggested that metals from ingested hooks could influence mortalities. This chapter sought to quantify the metal accumulation within the tissues and blood of mulloway that ingested nickel-plated fishing hooks.
- The concluding chapter discusses and synthesises the outcomes of the entire thesis and suggests possible mitigation strategies and further research that could contribute to fisheries sustainability.

- As this thesis is written as a series of papers, there is some inevitable repetition in the introductory sections of each chapter.
Chapter 2: Immediate and post-release survival of angled snapper (*Pagrus auratus*) during two catch-and-release fishing events in NSW

Publication

The data collected from Botany Bay in this chapter was incorporated in the following publication;

*Journal of Fish Biology* (Published)


This paper was a collaborative work by S. P. McGrath, P. A. Butcher, M. K. Broadhurst and S. C. Cairns. Shane McGrath contributed 20% of the research design, 10% of the data analysis and 90% of the interpretation of the data.
Abstract

Two experiments were completed with snapper (*Pagrus auratus*) to quantify their short-term mortality after being exposed to catch-and-release angling. In experiment 1, 157 snapper were angled and released from a coastal bay into holding cages and 48 control fish were similarly confined and left for up to four days. Mortality rates in the treatment group were 7.6% (12 fish) compared to no deaths in the control group. The categorical variables ‘hook location’ and ‘hook removal’ significantly influenced mortality ($p < 0.001$), with 66.7 and 50.0% of fish that died ingesting hooks or had their hook removed, respectively. However, only 8.3% (13 fish) ingested their hooks and apart from one body hooked fish all were hooked in the mouth. Experiment 2 was completed as part of an established catch-and-release tournament and involved 415 anglers. A total of 636 snapper were angled and released in nearshore waters over two years (2009 and 2010). The anatomical hook location was mainly limited to the mouth (96.5 and 97.5 %), and only a few fish ingested their hooks (0.3 and 0.9%) in 2009 and 2010, respectively. The hook location, combined with the majority of fish being caught in shallow offshore water may account for the majority of fish swimming away quickly after release. However, four fish failed to swim away after the catch-and-release process and this was primarily attributed to hook ingestion and/or incorrect venting methods. The results from these experiments were positive for improving post-release survival, and are consistent with previous studies on snapper and other species that hook ingestion increases mortalities. In addition, the results from experiment 2 supported lure fishing as a possible avenue for reducing the rate of hook ingestion.
2.1 Introduction

The family Sparidae comprises more than 110 species, which primarily inhabit sub-tropical and temperate waters of the Indo-Pacific and the Atlantic (Kailola et al. 1993; Carpenter and Johnson 2002; Orrell and Carpenter 2004). In Australia, snapper (*Pagrus auratus*) are one of the more popular recreationally targeted species, and are commonly angled at various sizes (between 10 and 100 cm total length – *TL*). They generally inhabit near and offshore waters around the south from Hinchinbrook Island in Queensland to Barrow Island in Western Australia (Starling 1992; Kailola et al. 1993). Their popularity among anglers was evident from a 12-month survey of recreational angling in 2000 and 2001, which estimated that of >18 million fish (belonging to the Sparidae family) captured, more than 3.8 million were snapper (Henry and Lyle 2003).

At least 66% of snapper caught were released due to bag limits, legal minimum lengths (LML) and the growing awareness of the need to conserve fish stocks (Henry and Lyle 2003). This high release rate could primarily be attributed to juvenile snapper (below the LML) spending their lives in estuaries and enclosed waters (Kailola et al. 1993), which is where more than 87% of angling effort is concentrated (Henry and Lyle 2003). The need to validate the assumption that the majority of released snapper survive led to three studies investigating the key contributing factors of mortality (Broadhurst et al. 2005; Lenanton et al. 2009; Grixti et al. 2010). Similar research has also been undertaken on the fate of many other released species (Broadhurst et al. 2005; Butcher et al. 2008a; Grixti et al. 2008) which have identified that there are a number of factors influencing mortality. These factors include but are not limited to; angling time, size and type of bait used (Meka 2004; Butcher et al. 2006), handling methods (Neal and Lopez-Clayton 2001; Hall et al. 2012), pressurisation (Morrissey et al. 2005), hypoxia (Furimsky et al. 2003) and water temperature (Storck and Newman 1992). However, the most common factor influencing mortalities is the anatomical hook location (Millard et al. 2003; Butcher et al. 2006; Butcher et al. 2007; Grixti et al. 2008; McGrath et al. 2009). More fatalities occur for fish that ingested their hooks than those hooked in the mouth (Butcher et al. 2008b; Grixti et al. 2008).
Several relevant reviews have indicated a strong relationship between mortality and hook ingestion for many species (for reviews Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007a). While a number of studies have identified methods of limiting hook ingestion, such as metal appendages (Willis and Millar 2001), circle hooks (Cooke and Suski 2004), active fishing methods (Grixti et al. 2007) and lure fishing (Diggles and Ernst 1997), hook ingestion will still occur. Further, Butcher et al. (2007) observed that mortalities of hook-ingested fish increase if the hook is removed rather than simply cutting the line. While the protracted benefits of cutting the line and reducing hook ingestion have been demonstrated for several species (e.g. Broadhurst et al. 2007; Butcher et al. 2007; McGrath et al. 2009), there is limited information on the mortalities of larger released individuals. However, it is important to conserve mature individuals because they have higher fecundity and the larvae have substantially better survival potential (Pankhurst and Conroy 1987; Birkeland and Dayton 2005; Willis et al. 2003). With anglers shifting away from dead weigh-in to catch-and-release tournaments, it is imperative that further investigations into the prevalence of factors that are known to affect survival of these larger individuals are done. The direct benefits of catch-and-release compared to dead weigh-in tournaments are immediately evident because they promote the release of fish in the best possible condition (Hall et al. 2009). However, the ethical debate surrounding catch-and-release tournaments is widely argued as they can increase stress and have sub-lethal effects because fish may be kept for extended periods of time in live wells with poor water quality (Arlinghaus et al. 2007a; Hall et al. 2009). Conversely, some forms of immediate release tournaments may not be as detrimental to fish health because captured fish are released quickly after being measured or weighed. These tournaments may still have some negative impacts because some fish are angled from deep water, and this can cause barotrauma (Gravel and Cooke 2008; Hall et al. 2009; Nguyen et al. 2009).

The potential for the above factors to affect the survival and other sub-lethal effects on released fish warrants further investigation. The aims of this study were to quantify mortality and the
prevalence of known mortality-causing factors for snapper (immediate and short term up to 4 days), and suggest simple methods that might be implemented to help mitigate these problems.

2.2 Materials and methods

2.2.1 Experiment 1- Botany Bay, Sydney, January 2008

Three months before experiment 1, approximately 150 juvenile wild snapper were caught from coastal waters around Coffs Harbour (30° 18' S, 153° 08' E), Australia, by commercial fishers using traps (≈ 2 x 3 x 2 m, soaked for 24 h). All fish were held in a 200 L live-well for transport to shore where they were collected by researchers. Collected fish were then transported to the aquaria facilities at the National Marine Science Centre (NMSC), Coffs Harbour, according to the methods described by Butcher et al (2007). They were then distributed among five 3000 L holding tanks (≈ 30 individuals per tank) and allowed to acclimate for at least two weeks before being used in the experiment. The fish were fed commercially available 4 mm sinking fish pellets and Australian sardine (Sardinops neopilchardus) at a rate of approximately 1% biomass day⁻¹.

The experiment was undertaken in Botany Bay, Sydney (34° 00' S, 151° 12' E), over seven days in January 2008 and involved 24 boat-based anglers (distributed among 15 boats) and four researchers on two boats. Three days prior to starting the experiment, 48 control fish were removed from tanks at the NMSC and transported to Botany Bay in two 380 L tanks supplied with oxygen. Upon arrival, fish were anaesthetized with benzocaine (ethyl-p-aminobenzoate, 25 mg L⁻¹; Barker et al. 2002) and transferred by boat in 70 L black polyethylene (PE) bins and placed in either of two anchored large circular cages made from 16-mm knotless polyamide netting and measuring 2.3 m ø x 2.5 m.

Anglers were asked to target all sizes of wild snapper using conventional gear and tackle over two days. Anglers were provided with an angler kit and a 110 L PE holding cage (56 cm ø x 70 cm in depth). The angler kits contained waterproof data sheets and a small flag. Once an angler caught a
fish, they immediately measured $TL$, released it into the holding cage (which was suspended in the water beside their boat), recorded the relevant data and raised their flag to inform researchers that the cage was ready for collection. Due to limited numbers of 110 L cages, some treatment and control fish were placed in groups of up to four, and a further 20 treatment fish were also divided evenly between two large treatment cages (see above). All snapper in multi-stocked cages were identified via $TL$. At the conclusion of the study the absolute hook size was also determined for the hooks used by anglers following the methods described by Ralston (1982).

For each fish, researchers confirmed the angler’s data and assessed the condition and recorded any damage. Cages were then transported to one of two 240 m lines that were anchored to the sea floor within 500 m of the fishing area. The cages were then placed 3 m apart and lowered to the bottom in approximately 3 m of water. In addition, control fish were transferred without exposure to air from the stock sea cages and similarly confined and attached to the line. The water temperature ($^\circ$C) and dissolved oxygen concentration (mg L$^{-1}$) were collected daily from the monitoring site using a Horiba U10 meter during the experiment.

During the experiment, no fish were fed and cages were checked for mortalities after four days. Also, prior to commencing the experiment, 10 snapper were angled and sampled for blood using the procedures described by Broadhurst et al (2005). These samples were used to determine the baseline glucose levels. All control and treatment fish were sampled for blood (Broadhurst et al. 2005), measured ($TL$) and had their fin damage and scale loss assessed. The blood samples were used to ascertain whether there were any differences in glucose concentrations within and between treatment and control fish after the monitoring period, or any differences between these groups and wild fish at the end of the experiment.
2.2.2  Experiment 2 - Coffs Harbour, June 2009 and 2010

The second experiment was completed offshore from Coffs Harbour (30° 18' S, 153° 08' E), over two days in June 2009 and 2010. The events involved 196 and 219 anglers competing in an established catch-and-release tournament. The rules from the tournament stipulated that all anglers were to use lures only and treble hooks were to be barbless. All fish were to be handled with care and lifted from the water using knotless landing-nets.

During the tournament, anglers were instructed to measure TL (nearest mm), then take a photograph of the fish whilst being measured and finally complete a data sheet describing the capture and release processes. The data sheets also included a classification for ‘condition’ on release (Table 1), which included either no negative impacts from angling (C1 - fish swam away after <10 s; and, C2 - fish swam away after 10 - 40 s) to severe negative impacts (C3 - fish struggled to swim away after > 40 s; and, C4 - the fish didn’t swim away).

2.2.3  Data collection and statistical analyses

During experiments 1 and 2, anglers recorded data on the time of capture, hook type and size, water depth, anatomical hook location separated as mouth, body or hook-ingested, hooking damage and removal, blood presence and TL. Anglers also recorded the time of capture and release into the cage, bait type, line strength, rig type and trace length, fishing method either anchored or drifting; time fish was played for and air exposure once landed; and landing method (type of net used) for experiment 1. Additional information in experiment 2 on the presence/absence of an inflated swim bladder, if the fish was vented and/or if a release weight was used, were also recorded.

The collected blood samples from experiment 1 were analysed for concentrations of glucose (mmol L\(^{-1}\)) derived using colorimetric clinical kits (Roche Diagnostics, USA) using an enzymatic spectrophotometric assay, which was done according to the manufacturer’s instructions. A one-factor
ANOVA was used to examine differences in the mean concentrations of glucose (mmol L\(^{-1}\)) in the blood plasma among wild (base line), treatment and control fish at the end of experiments.

Two-sample Kolmogorov-Smirnov tests were used to compare the size-frequency distributions of angled and control fish in experiment 1; and fish captured between 2009 and 2010 in experiment 2. A two-tailed Fisher’s exact test was used to determine the independence of the treatment of fish on mortality at the end of experiment 1. All variables describing the capture and release of snapper in experiment 1 were collated as either categorical (Table 1) or continuous variables (Table 2). The categorical variables were hook (J or circle; Table 1) and rig type separated into: (i) long trace (> 50 cm with a running sinker and swivel); (ii) short trace (< 50 cm with a running sinker and swivel); (iii) paternoster (sinker attached below hooks); (iv) running sinker on the hook; and (v) hook only. Bait type was also separated into; (i) lure; (ii) mackerel (*Euthynnus affinis*); (iii) mullet (*Mugil cephalus*); (iv) Australian sardine (*S. neopilchardus*); (v) prawn (*Metapenaeus macleayi*); and (vi) squid (*Natodarus gouldi*).

Logistic regression models incorporating these explanatory variables were fitted separately to the survival data for experiment 1, in an attempt to explain any mortality (Agresti 1996). In addition, the regressions were also fitted to assess the effect of the different types of fishing methods in relation to hook location (shallow versus hook ingestion) in experiment 1. Due to the low numbers of mortalities and fish that ingested hooks (Table 1), the logistic regressions were fitted using the method of conditional maximum likelihood (Agresti 1996) with the LogXact 8 software package (Cytel Software 2007).

2.3 Results

2.3.1 Experiment 1

A total of 157 snapper between 87 and 288 mm *TL* (mean ± s.e. of 184.30 ± 3.48 mm *TL*) were angled and released into the cages. In addition, a further 48 control fish between 219 and 325 mm *TL* (mean
were placed in cages. Two-sample Kolmogorov-Smirnov tests detected significant differences between the size-frequency of angled and control fish ($p < 0.01$, with the latter individuals being longer; see Figure 1). The ambient water temperature and dissolved oxygen during the experiment ranged from 22.1 to 23.4°C (mean ± s.e. of 22.5 ± 0.1 ºC) and 8.11 to 9.18 mg L$^{-1}$ (mean ± s.e. of 8.55 ± 0.10 mg L$^{-1}$), respectively.

**Figure 1:** The size-frequency (TL mm) distribution of snapper, *Pagrus auratus* caught during the field study in Botany Bay (Experiment 1). Dark bars = treatment fish, Light bars = control fish.

The majority of treatment fish were caught using paternoster (35.0%) and hook only rigs (26.1%), baited with prawn (40.8%) and squid (41.4%) while fishing in shallow water (mean ± s.e. of 7.4 ± 0.2 m) using light line (2.8 ± 0.1 kg), long traces (78.3 ± 3.2 cm), and relatively small absolute hook sizes (317.3 ± 9.4 mm$^2$). The majority (98.1 and 95.5 %) of the fish were played and exposed to air for less than 30 s, respectively. Most fish were also lifted from the water with little or no support (96.2%) before being restrained using either dry (17.2%) or wet (81.5%) bare hands. The majority of the fish were caught using J-hooks (67.5%) and the hook location was mainly limited to the mouth.
(91.1%), with 8.3% of fish ingesting their hooks. The majority of fish had their hooks removed (93.0%) and very few fish showed any signs of hook damage (6.4%), or blood loss (7.6 %) and no fish had any scale loss or fin damage.

After four days, mortality rates in the treatment group were 7.6% (12 fish) compared to no deaths in the control group. (Fisher’s exact test, \( p > 0.05 \)). Of the 12 fish that died, 66.7% had ingested their hooks and the majority of deaths occurred immediately after release. Logistic regression analysis detected several significant \( (p < 0.05) \) main effects of the various categorical and continuous factors on mortality (Tables 1 and 2). The categorical factors of hook location and removal were highly significant \( (p < 0.001) \), with hook ingestion varying from 3.4 to 66.7% and hook removal from 96.6 to 50.0%, respectively for live and dead fish. Blood loss also significantly affected mortality \( (p < 0.01) \) with the presence of blood varying from 5.5 to 33.3% for live and dead fish, respectively. The only significant \( (p < 0.05) \) continuous factor was absolute hook size (mean ± s.e.) which varied from 310.8 ± 9.4 to 391.4 ± 44.1 mm² for live and dead fish, respectively. In addition, logistic regression analysis identified significant main effects of one categorical (rig type) and one continuous (absolute hook size) factor on hook location \( (p < 0.05) \). More specifically, paternoster and hook only rigs accounted for all hook-ingested fish, and the absolute hook size (mean ± s.e.) varied from 311.2 ± 39.3 to 381.2 ± 9.5 mm² for shallow and hook-ingested fish, respectively.

A one-factor ANOVA detected no significant differences in the concentrations of plasma glucose that were sampled from the wild (mean ± s.e. of 1.2 ± 0.1 mmol L⁻¹), control (1.6 ± 0.1 mmol L⁻¹) and treatment (1.7 ± 0.2 mmol L⁻¹) fish at the end of the experiment (ANOVA \( F_{2,29} = 2.49, p > 0.05 \); Figure 2).
Figure 2: The mean + s.e. differences in the plasma glucose (mmol L\(^{-1}\)) between treatment, control and wild snapper, *Pagrus auratus* at the conclusion of experiment 1.

Table 1: The pooled categorical parameters for angled-and-released snapper, *Pagrus auratus*, at the end of experiment 1 and the 2009 and 2010 tournaments (Experiment 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alive</td>
<td>Dead</td>
<td>2009</td>
<td>2010</td>
</tr>
<tr>
<td>Hook location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Gills</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Ingested</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mouth</td>
<td>140</td>
<td>3</td>
<td>305</td>
<td>312</td>
</tr>
<tr>
<td>Hook type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circle</td>
<td>46</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J- hook</td>
<td>99</td>
<td>7</td>
<td>316</td>
<td>320</td>
</tr>
<tr>
<td>Hook removed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>140</td>
<td>6</td>
<td>316</td>
<td>316</td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Hooking damage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>137</td>
<td>10</td>
<td>289</td>
<td>283</td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>2</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td>Play time (sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>63</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10-30</td>
<td>79</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>31-60</td>
<td>2</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>1</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Air exposure (sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>42</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>15-30</td>
<td>98</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>31-60</td>
<td>5</td>
<td>2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Scale loss</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------</td>
<td>---------------------</td>
<td>----</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fin damage</td>
<td>Yes</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>145</td>
<td>12</td>
<td>NA</td>
</tr>
<tr>
<td>Scale loss</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Blood present</td>
<td>No</td>
<td>137</td>
<td>8</td>
<td>253</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
<td>63</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>145</td>
<td>12</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
<td>63</td>
<td>104</td>
</tr>
<tr>
<td>Fish landing method</td>
<td>Knotless</td>
<td>2</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Knotted</td>
<td>4</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>No net</td>
<td>139</td>
<td>12</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fishing method</td>
<td>Anchored</td>
<td>86</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Drifting</td>
<td>59</td>
<td>7</td>
<td>NA</td>
</tr>
<tr>
<td>Fish restraining method</td>
<td>Dry bare hands</td>
<td>26</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Not restrained</td>
<td>1</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Towel</td>
<td>1</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Wet bare hands</td>
<td>117</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>Rig Type</td>
<td>Long trace (&gt; 50 cm)</td>
<td>33</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Short trace (&lt; 50 cm)</td>
<td>14</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Paternoster</td>
<td>50</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Free sinker only on hook</td>
<td>12</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Hook only</td>
<td>36</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>Bait</td>
<td>Lure</td>
<td>0</td>
<td>0</td>
<td>325</td>
</tr>
<tr>
<td></td>
<td>Mackerel</td>
<td>10</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Mullet</td>
<td>7</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Australian sardine</td>
<td>11</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Prawn</td>
<td>58</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Squid</td>
<td>59</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>Inflated bladder</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>285</td>
<td>Yes</td>
<td>NA</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>35</td>
</tr>
<tr>
<td>Vented</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>283</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>Yes</td>
<td>NA</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Release weight used</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>285</td>
<td>Yes</td>
<td>NA</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>282</td>
<td>38</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
2.3.2  

Experiment 2

A total of 316 (ranging between 265 to 955mm; mean ± s.e. of 511.65 ± 7.95 mm $TL$) and 320 (ranging between 300 to 920 mm; mean ± s.e. of 512.22 ± 8.13 mm $TL$) snapper were caught and released during the 2009 and 2010 tournaments, respectively. A Kolmogorov-Smirnov test detected no significant differences between the size-frequency of the fish captured between 2009 and 2010 ($p > 0.05$; Figure 3).

![Figure 3](image)

**Figure 3:** The size-frequency ($TL$ - mm) distribution of snapper, *Pagrus auratus* caught during the two years (dark bars = 2009, light bars = 2010) for experiment 2 in Coffs Harbour.

The anatomical hook location was mainly limited to the mouth (96.5 and 97.5%), and only a few fish ingested the hook (0.3 and 0.9%) in 2009 and 2010, respectively. All fish captured in 2009...
and 98.7% in 2010 had their hook removed. The damage caused from hooking was relatively low (8.5 and 11.6%), but the presence of blood was nearly double (19.9 and 32.5%) in 2010. The majority of fish were captured in waters < 30m (79.4 and 98.4%) and the mean (± s.e.) depth of capture between 2009 and 2010 was 19.7 ± 0.4 to 18.7 ± 0.3 m, respectively. A one-factor ANOVA detected no significant differences in the depth of capture between 2009 and 2010 (ANOVA F1, 634 = 3.69, p > 0.05; Figure 4). Anglers also observed more fish with inflated swim bladders in 2009 (18.4%) than 2010 (10.9%), respectively. While the fish that were vented (10.4 and 12.5%; Table 3) or released using weights (9.8 and 11.9%) were relatively similar in 2009 and 2010, respectively. The majority of fish released in 2009 and 2010 swam away quickly (i.e. C1) after release (89.1 and 89.7%), respectively. However, four fish released in the 2010 tournament failed to swim away (C4) after release. One of these fish ingested the hook and the other three were vented. It was also observed that anglers vented fish (0.9 and 6.3%) that had no signs of inflated swim bladders during each year, respectively.

Figure 4: The depth-frequency (m) distribution for snapper, *Pagrus auratus* caught during the two years (dark bars = 2009, light bars = 2010) for experiment 2 in Coffs Harbour.
Table 2: Mean (± s.e.) continuous parameters from experiment one and two (2009 and 2010), for angled-and-released *Pagrus auratus*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ (± s.e.)</td>
<td>Alive</td>
<td>Dead</td>
<td>2009 (± s.e.)</td>
<td>512.6</td>
<td>$511.7 \pm 8.0$</td>
</tr>
<tr>
<td>$TL \text{ (mm)}$</td>
<td>Angled</td>
<td>184.3 ± 3.4</td>
<td>184.5 ± 3.5</td>
<td>182.3 ± 13.1</td>
<td>512.6 ± 5.7</td>
<td>511.7 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>258.1 ± 3.4</td>
<td>258.1 ± 3.4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Line strength (kg)</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Trace length (cm)</td>
<td>78.3 ± 3.2</td>
<td>79.5 ± 3.4</td>
<td>63.6 ± 7.4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Water depth (m)</td>
<td>7.4 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>7.3 ± 0.6</td>
<td>19.2 ± 0.3</td>
<td>19.7 ± 0.4</td>
<td>18.7 ± 0.3</td>
</tr>
<tr>
<td>Absolute hook size (mm$^2$)</td>
<td>317.3 ± 9.4</td>
<td>310.8 ± 9.4</td>
<td>391.4 ± 44.1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>NA, not applicable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The pooled venting and release weight data from experiment 2 (2009 and 2010).

<table>
<thead>
<tr>
<th>Inflated swim bladder</th>
<th>Vented</th>
<th>Release weight used</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
<td>238</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>17</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>21</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>23</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
2.4 Discussion

The rates of hook ingestion and immediate mortality were greater for snapper caught using baits than lures. More specifically, during experiment 1, hook ingestion, removal and the presence of blood were identified as the primary predictors of mortality. The total mortality for snapper was 7.6% in experiment 1, which was within the ranges (less than 12%) previously observed in other studies for snapper (Lenanton et al. 2009; Grixti et al. 2010). In addition, the similarity in the levels blood plasma glucose between treatments and controls at the conclusion of the experiment, and those sampled from the wild, suggest that the impacts and stresses associated with capture and release were relatively low. Further, the control, treatment and wild glucose parameters were within previously unstressed limits of *P. auratus* (Cleary et al. 2000).

Similar to previous observations for snapper by Broadhurst *et al* (2005) and Grixti *et al* (2010), the majority of mortalities in experiment 1 occurred shortly after capture, with anatomical hook location accounting for the majority of the deaths in the latter study. More specifically, like with many other species, hook ingestion resulted in substantially more mortalities in snapper when compared to mouth hooking (Broadhurst *et al*. 2005; Lenanton *et al*. 2009; Grixti *et al*. 2010). For example, the mortality was reduced from 61.5 to 2.1% when hooking was limited to the mouth in experiment 1. Further, 8.3% of snapper in experiment 1 ingested their hooks, whereas hook ingestion in experiment 2 was relatively low (< 1%). The higher ingestion rate in experiment 1 could be attributed, but not limited to, the size of hooks used and fishing method. Due to previous studies on another sparid, black bream (*Acanthopagrus butcheri*) observing interactions between absolute hook size/fishing method and anatomical hook location (Grixti *et al*. 2007). Lure fishing can also be categorised as an active angling method which has previously been shown to reduce hook ingestion in other species (Diggles and Ernst 1997; Grixti *et al*. 2007). Similarly, the type of rig used also contributed to the anatomical hook location, with more than 46.2% of the hook-ingested snapper being caught using a design that was essentially a hook attached to the fishing line with no weight. These rig designs function similar to the slack line fishing method (there is no tension on the line) that was
investigated by Grixti et al (2007), which resulted in black bream (A. butcheri) being twice as likely to ingest the bait than if the line was kept under tension. Observations by Beckwith and Rand (2005) also support line tension as a factor influencing hook ingestion, with longer leaders and unfixed lead weights resulting in higher proportions of hook ingested red drum (Sciaenops ocellatus). While lure fishing with single hooks in general has been shown to result in less hook ingestion and is potentially quiet positive, there was still some damage and up to 32.5% of captured individuals in experiment 2 had signs of blood loss, the bleeding would generally be assume to be exterior bleeding caused by damage to the upper and lower mandible due to the high portion of mouth hooking. This high portion of bleeding individual could result in increase predation, due to the likeliness of the blood attracting other predators.

Previous research on another Sparidae, yellowfin bream (Acanthopagrus australis) showed that mortalities from hook ingestion were exacerbated if the hook was forcefully removed rather than just cutting the line (Butcher et al. 2007). The results from experiment 1 supported this relationship, with 100% mortality occurring for three fish that had their ingested hooks removed. Conversely, only 54.5% of snapper died when they were released with the line cut. Grixti et al (2010) also recorded similar increased mortalities when hooks were removed from hook-ingested fish rather than just cutting the line, and reported that the removal of hooks resulted in severe internal damage/bleeding and subsequently led to mortalities. Experiment 1 supports Grixti et al (2010) observations on bleeding, with the presence of blood being six times more likely to result in death. Similar trends associated with blood loss have been observed in other studies with the same species (Broadhurst et al. 2005; Lenanton et al. 2009). Conversely, in experiment 2 nearly a third of the captured fish were observed to have blood loss. However, fewer deaths were observed, and this was attributed to the hooking location where the bleeding occurred.

There are many methods that can be used to mitigate hook ingestion. One of the more common approaches is the use of circle hooks because they have been shown to limit hook ingestion
when compared with conventional J-hooks (Cooke and Suski 2004). However, the effectiveness of circle hooks to reduce mortality is species specific because it depends on hook size, fishing style, foraging behaviour and mouth morphology (Cooke and Suski 2004). There was some evidence of the usefulness of circle hooks to minimise hook ingestion in experiment 1, with circle hooks being ingested at nearly double the rate of J-hooks (11.8 vs. 6.6%, respectively). Although it was non-significant, this suggests that more factors are associated with hook ingestion than hook design and type. However, the absolute hook size was identified as a factor that significantly contributed to mortality in experiment 1, with larger hooks resulting in more deaths than smaller hooks and this could be due to larger hooks causing more damage and being ingested.

While experiment 1 investigated most factors associated with mortality, it failed to encompass the potentially deleterious process of angling fish from depth and the prevalence of barotraumas, experiment 2 addressed this deficit. In experiment 2, anglers recorded that nearly double (18.4 versus 10.9%) the amount of fish had inflated swim bladders in 2009 compared to 2010. The impact of barotrauma can be softened by venting and/or the use of release weights during the release procedure (Butcher et al. 2012). However, venting could potentially be more harmful if done incorrectly (Wilde 2009; Butcher et al. 2012). For example, in 2010, > 6.3% of fish were recorded as being vented even though there were no signs of swim bladder inflation. This highlights the fact the angler knowledge is limited and further education may be required before these practices can be fully supported.

As snapper were not held for a monitoring period after being caught in experiment 2, it is difficult to estimate short-term mortality after release. However, previous studies with snapper and other sparids have observed that the majority of mortalities generally occur immediately after release (Broadhurst et al. 2005; Butcher et al. 2007; Grixti et al. 2008). The classification system used in experiment 2 to assess the condition of snapper immediately after release may help to predict long-term mortality rates. In experiment 2, four individuals failed to swim away after release and these fish either ingested their hook or were vented by anglers. An explanation for the latter factor causing
mortalities could be incorrect venting methods used by the anglers which subsequently led to internal damage from piercing vital organs with the needle (Kerr 2001; Wilde 2009). This emphasises the fact that further education is needed if potentially deleterious release methods are incorporated in conservation strategies. However, when venting is completed correctly there are no effects on survival in water < 20 m (Butcher et al. 2012). In addition, the usefulness of venting on the survival of fish is dependent on the methods used (Childress 1989; Shasteen and Sheehan 1997) and survival may vary among species (McLeay et al. 2002; Wilde 2009). Despite the above, the overall predicted survival from experiment 2 was assumed to be relatively high with 85.1% of released fish swimming away in less than 10 s. The high predicted survival rate could be attributed to most fish being caught from shallower waters (on average < 20 m) and the low hook ingestion rate (Grixti et al. 2010; Butcher et al. 2012).

While the results from experiment 2 are limited in terms of being able to estimate definitive mortality rates, the method of collecting these data from catch-and-release events is relatively simple compared to the value of the information that is acquired. Further research should incorporate air exposure, scale loss and fin damage assessment. This data may help provide further supportive evidence that longer handling times result in an increase in physiological damage which has previously been correlated fish size (Meka 2004). The incorporation of a short (1 h) observation time may also aid in the prediction of mortality, as this study and other research has observed most mortalities to occur initially after release (Broadhurst et al. 2005; Grixti et al. 2010). In addition, future research would benefit from the inclusion of a reflex impairment index as previous research suggests it is a proxy for mortality (Davis 2010).

The above results clearly suggest that lure fishing is another possible avenue to reduce the occurrence of hook ingestion because relatively few fish ingested their lures. More specifically, bait fishing resulted in a hook ingestion rate that was more than eight times higher when compare with the rate of hook ingestion for lure caught snapper. Nevertheless, despite all the evidence to support lures
and different hook designs as methods of reducing hook ingestion, fish will still inevitably ingest hooks. Therefore it would be beneficial to investigate the effectiveness of different hook designs and their breakage and ejection after ingestion. Further investigation may also be needed to determine the effectiveness of different hook designs (i.e. circle hooks) and sizes to limit hook ingestion, due to feeding mechanism varying between species. In the meantime, it would seem appropriate to minimise hook ingestion and use lures for catch-and-release fishing practices rather than bait.
Chapter 3: Reviewing hook degradation to promote ejection after ingestion by marine fish

Publication

*Marine and Freshwater Research* (published)


This paper was a collaborative work by S. P. McGrath, P. A. Butcher, M. K. Broadhurst and S. C. Cairns. Shane McGrath contributed 70% of the research design, 40% of the data analysis and 30% of the interpretation of the data.
Abstract

A widely recommend strategy for releasing fish that have ingested hooks is to simply cut the line. The utility of this approach is based on the premise that the individual will eventually eject the hook, often after sufficient oxidation and decay. While this outcome has been demonstrated for several species, few quantitative data are available on the factors influencing hook degradation. This was addressed this issue by quantifying the technical parameters affecting the percentage weight and point remaining and differences in tensile and compression strengths for 828 hooks comprising 23 designs (absolute sizes between 227 – 611 mm² and used to target fish < 40 cm total length). After immersion in seawater for 8 and 28 days, 12 replicate from each hook design were removed, re-photographed, re-weighed and destructively tested for compression and tensile strengths (using a force gauge) to provide indices of decay. Degradation was mainly affected by the wire material and diameter and could be significantly promoted by choosing carbon-steel designs, either with a wire diameter of < 0.9 mm for the examined sizes of hooks or, alternatively, bait-holder barbs (or similar modifications) along the shaft. By rapidly oxidising and weakening after ingestion, such designs ultimately could help to reduce negative impacts to released fish.
3.1 Introduction

Globally, recreational fishing (and especially angling) is a popular pastime, with participation rates estimated at >40% in some developed countries (Cowx, 2002; Cooke and Cowx, 2004). Most anglers release at least part of their catch (typically between 30 and 76% – Burke et al. 1994; Cooke and Cowx, 2004); either voluntarily or in response to regulations that include legal sizes and personal quotas (Arlinghaus et al. 2007a). Such so-called ‘catch-and-release’ fishing is widely promoted as being socially and environmentally responsible (Arlinghaus et al. 2007a), although to ultimately satisfy this definition, there needs to be few associated mortalities and negative welfare effects among survivors (Muoneke and Childress 1994; Arlinghaus et al. 2007b).

Recognition of the need to validate catch-and-release angling as a tool to manage recreational fisheries has resulted in several relevant studies; most of which have sought to quantify mortalities and identify the key determinate factors (for reviews see Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007a). Although there are considerable species-specific differences, in most cases the numbers of fatalities are usually much lower than the numbers of survivors (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005). Further, mortality can often be attributed to consistent, re-occurring factors, among which anatomical hook location is the most important (Bartholomew and Bohnsack 2005). More specifically, hook ingestion (throat, oesophagus or stomach) often has a strong main effect or interaction with other parameters (e.g. fish size, bait type, fishing method and rig configuration) on mortality (Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007a).

Previous studies have demonstrated that the impacts of hook ingestion can be minimised by adopting three general approaches, including: (i) spatial and/or temporal closures to fishing (Bohnsack et al. 2004); or maintaining fishing and promoting either (ii) mouth hooking (Cooke and Suski 2004), or (iii) hook ejection after ingestion (Aalbers et al. 2004; Broadhurst et al. 2007). Of these strategies, closures to fishing are the most extreme, but may be warranted where there is considerable risk to a
particular species (Powles et al. 2000). The second and third approaches are less controversial and, as such, have received the most attention (Willis and Millar 2001; Broadhurst et al. 2007).

In particular, considerable work has been done to investigate the ways in which hook ingestion can be minimised (i.e. ii above). Recognised strategies range from simple changes to the type and/or size of bait (Pauley and Thomas 1993) and the method of fishing (Grixti et al. 2007), to more complex terminal rig modifications, including modified J-hooks (Willis and Millar 2001; Butcher et al. 2008b) or specific designs such as circle (Cooke and Suski 2004) or Shelton hooks (Jenkins 2003). Such hooks can effectively reduce ingestion, although many often have lower hooking efficiencies than conventional configurations, which can restrict their acceptance by anglers (Butcher et al. 2008b). Further, even if recommended designs are adopted, they are rarely effective at eliminating hook ingestion. For example, in a review of 71 papers comparing the ingestion rates of J- and circle hooks, Cooke and Suski (2004) concluded that while the latter designs promoted mouth hooking among some fish (by up to 96%), their effectiveness was species-specific. In some cases, circle hooks actually increased ocular tissue damage.

A more appropriate strategy to mitigate the mortality associated with anatomical hook location in some fish might be to promote the use of hooks that are rapidly ejected (i.e. iii above). The concept for such an approach is supported by several studies which have demonstrated that, compared to removing ingested hooks, releasing fish with their lines cut resulted in less mortality, with most individuals subsequently ejecting their hooks (reviewed by Hall et al. 2009). For example, Aalbers et al (2004) and Butcher et al (2007) showed that this strategy reduced the mortalities of white seabass (Atractoscion nobilis), and yellowfin bream (Acanthopagrus australis) by 24 and 79%, respectively. Broadhurst et al (2007) also demonstrated that 76% of hook-ingested yellowfin bream were able to eject their hooks after an average of 20 days. Similarly, Lyle et al (2007) and Aalbers et al (2004) observed ejection rates of 30 and 39% for sand flathead, (Platycephalus bassensis), and white seabass, after 29 and 130 days, respectively.
While it is clear that some fish can eject ingested hooks, less information is available on the mechanisms by which this occurs and whether ejection can be promoted through the use of particular designs and materials. The consensus from the few available studies is that hook ejection is strongly influenced by hook decay; especially at the point and barb, both of which can cause extensive internal injuries to fish (Aalbers et al. 2004; Broadhurst et al. 2007; Grixti et al. 2008). For example, Broadhurst et al (2007) observed that over 105 days, nickel-plated carbon steel hooks that were ejected by yellowfin bream had on average oxidised 4% more than those that were not ejected. Further, many of the ejected hooks had decayed sufficiently to break apart, often at or near bait-holder barbs (located on the shaft), which may have facilitated their ejection, and most had blunt points.

Intuitively, the rate of hook decay is likely to be affected by a number of technical factors, including the type, diameter and length of wire used in their fabrication. In any study that seeks to assess the full range of such potential explanatory variables, it is important that there is sufficient replication. One problem, however, is that there are severe logistical and ethical issues associated with forcefully causing the required number of fish to ingest hooks. Ideally, appropriate indices of decay might serve as proxies, prior to the conduct of smaller-scale trials with fish to test and confirm hypothesised explanatory variables.

Since Broadhurst et al (2007) demonstrated no significant differences in the rate of oxidation (measured as weight loss and breakage) between hooks that were ingested by yellowfin bream and those placed in plastic containers and immersed in seawater, the latter could be used to represent an appropriate method of acquiring relevant data on hook decay. Such an approach does not replicate the forces on an ingested hook, but also measuring tensile and compression strength would at least facilitate a comparison among different designs (e.g. Edappazham et al. 2008), and provide direction for more refined studies with fish.
Given the above, the main aim of this study was to determine the technical characteristics affecting the temporal oxidation and integrity of a range of hooks (absolute sizes – see Ralston 1982-between 227 and 611 mm²) commonly used to target small (<40 cm TL) coastal species. A secondary aim was to use this information to provide directions for the refinement and design of hooks that might more readily decay and be ejected by fish.

3.2 Materials and methods

3.2.1 Hook types

Thirty-six replicates of 20 different hooks commonly sold by Australian tackle stores (and available worldwide) were purchased for the experiment (Table 4). All hooks were measured for their wire diameter (ø) and shaft, front, gape and bend lengths to the nearest 0.01 mm, weighed (to the nearest 0.0001g) and had their steel type, coating, presence or absence of bait-holder barbs (see Broadhurst et al., 2007 for details) and design (i.e. J or circle) recorded (Figure 5; Table 4). Absolute hook size (mm²) was calculated as the product of the shaft and bend lengths (Ralston 1982). The hooks were classified as either J- or circle designs, based on whether the point was approximately perpendicular or parallel to the shaft (Figure 5a and b; Cooke and Suski 2004).

Three of the J-hooks had virtually identical sizes and shapes (Table 4; hooks 18, 19 and 20), but were constructed from different materials: stainless steel, and red-lacquer and nickel-plated carbon steel. Based on their design uniformity, these three hooks were chosen for a more detailed assessment of the utility of modifications to increase degradation among different materials. Using a rotary tool (24-mm disc), 36 of the hooks (termed ‘modified’ – hooks 18a, 19a and 20a; Table 4) made from each of the three materials had three small notches cut into the shaft, bend and point (to approx 80% of the wire diameter - Figure 5a). The notches were designed to increase the surface area and subsequent oxidation of the hooks and, therefore reduce their strength (Broadhurst et al. 2007). The remaining 36 hooks made from each material were left unmodified (Table 4). The modified hooks were also weighed after being altered, and were only used if they comprised >98% of their original mass.
Table 4: Specifications and initial mean (± s.e.) continuous technical parameters for the 23 hooks examined in the study. Lengths, weight, absolute size (n = 36) and force (n = 12) are in mm, mg, mm² and N, respectively.

<table>
<thead>
<tr>
<th>Hook No.</th>
<th>Steel type</th>
<th>Manufacturer’s coating</th>
<th>Wire diameter</th>
<th>Bait-holder barbs</th>
<th>Shaft Length</th>
<th>Front Bend</th>
<th>Gape</th>
<th>Absolute size</th>
<th>Weight</th>
<th>Tensile force</th>
<th>Compression force</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>C Black matt</td>
<td>1.04</td>
<td>N</td>
<td>22.64 ± 0.05</td>
<td>11.30 ± 0.01</td>
<td>13.24 ± 0.02</td>
<td>11.09 ± 0.01</td>
<td>299.71 ± 0.75</td>
<td>282.45 ± 0.39</td>
<td>139.25 ± 10.70</td>
<td>149.42 ± 6.98</td>
</tr>
<tr>
<td>2</td>
<td>C Black nickel</td>
<td>1.02</td>
<td>N</td>
<td>26.05 ± 0.02</td>
<td>11.33 ± 0.04</td>
<td>23.45 ± 0.03</td>
<td>17.91 ± 0.06</td>
<td>610.85 ± 0.75</td>
<td>336.57 ± 0.31</td>
<td>144.67 ± 6.70</td>
<td>145.17 ± 5.68</td>
</tr>
<tr>
<td>3</td>
<td>C Red lacquer</td>
<td>0.98</td>
<td>Y</td>
<td>27.28 ± 0.01</td>
<td>10.03 ± 0.00</td>
<td>10.97 ± 0.00</td>
<td>9.34 ± 0.00</td>
<td>299.37 ± 0.18</td>
<td>265.86 ± 0.50</td>
<td>112.25 ± 2.13</td>
<td>45.83 ± 2.13</td>
</tr>
<tr>
<td>4</td>
<td>C Black nickel</td>
<td>1.33</td>
<td>N</td>
<td>28.39 ± 0.02</td>
<td>12.30 ± 0.02</td>
<td>12.64 ± 0.01</td>
<td>8.87 ± 0.01</td>
<td>358.88 ± 0.31</td>
<td>517.86 ± 1.94</td>
<td>201.75 ± 6.70</td>
<td>66.92 ± 4.71</td>
</tr>
<tr>
<td>5</td>
<td>C Black nickel</td>
<td>0.96</td>
<td>Y</td>
<td>26.85 ± 0.01</td>
<td>10.30 ± 0.01</td>
<td>10.76 ± 0.07</td>
<td>10.89 ± 0.01</td>
<td>288.79 ± 1.87</td>
<td>263.60 ± 0.38</td>
<td>112.25 ± 2.76</td>
<td>104.00 ± 11.30</td>
</tr>
<tr>
<td>6</td>
<td>C Black nickel</td>
<td>1.14</td>
<td>Y</td>
<td>28.29 ± 0.02</td>
<td>10.97 ± 0.07</td>
<td>11.54 ± 0.02</td>
<td>10.36 ± 0.01</td>
<td>326.57 ± 0.68</td>
<td>384.69 ± 0.48</td>
<td>201.75 ± 6.70</td>
<td>66.92 ± 4.71</td>
</tr>
<tr>
<td>7</td>
<td>C Black nickel</td>
<td>1.00</td>
<td>N</td>
<td>26.34 ± 0.17</td>
<td>11.63 ± 0.00</td>
<td>11.59 ± 0.01</td>
<td>9.61 ± 0.00</td>
<td>305.43 ± 2.01</td>
<td>336.42 ± 13.30</td>
<td>225.08 ± 36.36</td>
<td></td>
</tr>
<tr>
<td>8*</td>
<td>C Black matt</td>
<td>1.24</td>
<td>N</td>
<td>18.13 ± 0.00</td>
<td>12.12 ± 0.00</td>
<td>12.52 ± 0.01</td>
<td>9.83 ± 0.01</td>
<td>226.93 ± 0.18</td>
<td>389.50 ± 0.26</td>
<td>237.75 ± 25.09</td>
<td>305.42 ± 23.30</td>
</tr>
<tr>
<td>9</td>
<td>C Red lacquer</td>
<td>1.08</td>
<td>Y</td>
<td>27.50 ± 0.01</td>
<td>11.35 ± 0.01</td>
<td>11.98 ± 0.01</td>
<td>9.90 ± 0.01</td>
<td>329.54 ± 0.41</td>
<td>330.93 ± 0.72</td>
<td>119.33 ± 0.69</td>
<td>65.42 ± 1.43</td>
</tr>
<tr>
<td>10</td>
<td>C Nickel</td>
<td>1.06</td>
<td>Y</td>
<td>24.99 ± 0.01</td>
<td>10.55 ± 0.01</td>
<td>11.96 ± 0.01</td>
<td>10.81 ± 0.01</td>
<td>298.88 ± 0.38</td>
<td>263.70 ± 0.66</td>
<td>175.25 ± 11.52</td>
<td>60.00 ± 1.66</td>
</tr>
<tr>
<td>11</td>
<td>C Bronze</td>
<td>1.06</td>
<td>Y</td>
<td>27.54 ± 0.07</td>
<td>10.47 ± 0.00</td>
<td>11.14 ± 0.03</td>
<td>8.90 ± 0.03</td>
<td>299.26 ± 0.19</td>
<td>317.26 ± 0.46</td>
<td>120.08 ± 15.37</td>
<td>43.33 ± 1.89</td>
</tr>
<tr>
<td>12</td>
<td>C Nickel</td>
<td>1.08</td>
<td>N</td>
<td>24.20 ± 0.07</td>
<td>10.10 ± 0.01</td>
<td>11.48 ± 0.01</td>
<td>9.90 ± 0.01</td>
<td>329.54 ± 0.41</td>
<td>451.01 ± 1.03</td>
<td>297.51 ± 0.90</td>
<td>156.92 ± 3.85</td>
</tr>
<tr>
<td>13</td>
<td>C Nickel</td>
<td>1.05</td>
<td>Y</td>
<td>27.78 ± 0.01</td>
<td>9.92 ± 0.06</td>
<td>10.92 ± 0.02</td>
<td>9.38 ± 0.01</td>
<td>277.91 ± 0.56</td>
<td>293.83 ± 0.49</td>
<td>156.92 ± 3.85</td>
<td>162.17 ± 2.30</td>
</tr>
<tr>
<td>14</td>
<td>C Bronze</td>
<td>1.04</td>
<td>Y</td>
<td>25.45 ± 0.01</td>
<td>10.13 ± 0.00</td>
<td>10.92 ± 0.02</td>
<td>9.38 ± 0.01</td>
<td>277.91 ± 0.56</td>
<td>293.83 ± 0.49</td>
<td>156.92 ± 3.85</td>
<td>162.17 ± 2.30</td>
</tr>
<tr>
<td>15</td>
<td>C Red lacquer</td>
<td>0.94</td>
<td>Y</td>
<td>36.32 ± 0.08</td>
<td>11.34 ± 0.00</td>
<td>12.42 ± 0.00</td>
<td>10.36 ± 0.00</td>
<td>451.01 ± 1.03</td>
<td>297.51 ± 0.90</td>
<td>156.92 ± 3.85</td>
<td>162.17 ± 2.30</td>
</tr>
<tr>
<td>16</td>
<td>C Red lacquer</td>
<td>1.16</td>
<td>N</td>
<td>29.81 ± 0.02</td>
<td>11.16 ± 0.00</td>
<td>12.33 ± 0.00</td>
<td>9.94 ± 0.00</td>
<td>367.48 ± 0.20</td>
<td>406.04 ± 0.54</td>
<td>253.33 ± 12.50</td>
<td>287.08 ± 7.70</td>
</tr>
<tr>
<td>17</td>
<td>S Na</td>
<td>1.06</td>
<td>N</td>
<td>28.50 ± 0.01</td>
<td>10.45 ± 0.00</td>
<td>11.76 ± 0.00</td>
<td>9.53 ± 0.01</td>
<td>335.02 ± 0.13</td>
<td>320.94 ± 0.31</td>
<td>196.83 ± 7.04</td>
<td>177.92 ± 12.36</td>
</tr>
<tr>
<td>18</td>
<td>C Nickel</td>
<td>1.06</td>
<td>N</td>
<td>21.33 ± 0.01</td>
<td>11.21 ± 0.01</td>
<td>11.41 ± 0.01</td>
<td>9.96 ± 0.01</td>
<td>243.44 ± 0.16</td>
<td>279.58 ± 0.31</td>
<td>137.17 ± 5.14</td>
<td>131.33 ± 4.97</td>
</tr>
<tr>
<td>19</td>
<td>C Nickel</td>
<td>1.06</td>
<td>N</td>
<td>21.34 ± 0.01</td>
<td>11.21 ± 0.01</td>
<td>11.42 ± 0.01</td>
<td>9.96 ± 0.01</td>
<td>243.44 ± 0.16</td>
<td>279.58 ± 0.31</td>
<td>137.17 ± 5.14</td>
<td>131.33 ± 4.97</td>
</tr>
<tr>
<td>20</td>
<td>S Na</td>
<td>0.98</td>
<td>N</td>
<td>21.43 ± 0.02</td>
<td>11.47 ± 0.01</td>
<td>11.91 ± 0.01</td>
<td>10.44 ± 0.01</td>
<td>255.16 ± 0.24</td>
<td>228.50 ± 0.48</td>
<td>137.25 ± 3.05</td>
<td>126.67 ± 5.60</td>
</tr>
<tr>
<td>20a</td>
<td># S Na</td>
<td>0.98</td>
<td>N</td>
<td>21.42 ± 0.02</td>
<td>11.45 ± 0.01</td>
<td>11.90 ± 0.01</td>
<td>10.30 ± 0.04</td>
<td>254.92 ± 0.28</td>
<td>226.10 ± 0.39</td>
<td>137.08 ± 4.48</td>
<td>75.33 ± 7.33</td>
</tr>
</tbody>
</table>

*, circle hooks; #, modified with three notches on the shaft, bend and front (Figure 6a); C, carbon steel; S, stainless steel; Na, not applicable; Y, yes; N, no.
Figure 5: The measurements recorded from all (a) J- and (b) circle hooks, and the location of the three small notches (1–3) cut into the shaft, bend and point of the modified J-hooks.

3.2.2 Hook assessment

On the first day (termed $T_0$) of the experiment, 12 replicates of all 23 hook types (including the three modified designs) were tested separately for their compression ($n = 6$) and tensile ($n = 6$) strengths (N) using a Chatillon DFX-100 digital force gauge was attached to an adjustable Chatillon LTCM-100-EU motorised tester (Brooklyn, NY, USA; Figure 6a). The force gauge was rated to 500 N (accurate to ± 2.5 N), and recorded the strength at the point of maximum hook elasticity or separation within a specified sensitivity of ± 0.1 N. The speed of the tester was set at 100 mm min$^{-1}$ (with a speed accuracy of ± 15 mm min$^{-1}$). Compression was determined by positioning each hook between two purpose-built, stainless-steel cylinders (40-mm ø × 50-mm length) that were fitted with grooves in one of their ends to secure the bend and eye, while the other ends were screwed (5-mm thread) to the base plate and force gauge, respectively (Figure 6b). The tensile strength was tested by attaching the eye of the hook to a clip (491-N strength rated) secured in a small vice screwed to the base plate, and the bend of the hook to a stainless-steel (5-mm ø) hook screwed into the force gauge (Figure 6c).
Figure 6: The specialised fittings used on the (a) Chatillon digital force gauge to measure the (b) compression and (c) tensile strengths (N) of hooks before immersion in seawater and then after eight and 28 days.

The remaining 24 replicates of each hook design were photographed using a stereoscopic microscope (Leica, S6D, Heerbrugg, Switzerland) fitted with a micron scale before being individually placed into a 70 mL perforated cylindrical plastic container following the methodology of Broadhurst et al (2007). The images were then used to measure the length of each hook point to the nearest 0.01 mm. The contained hooks were evenly distributed between three 3000 L tanks (i.e. eight replicates of each hook type tank⁻¹). Each tank was supplied with seawater (~18°C) at a rate of 30 l min⁻¹. After eight days (termed T₈), half of the hooks were
removed from the tanks, cleaned of any oxidised metal (using a paper towel) and re-weighed to determine their proportional oxidation (expressed as the percentage of weight remaining). Photographs were taken to determine the point length remaining after oxidation for each hook. The difference between the oxidised and original point length was then converted to the percentage remaining. Fifty percent of the hooks were tested for their maximum tensile strength, while the remaining replicates were tested for maximum compression strength (as above). At the end of four weeks (termed $T_{28}$), the remaining hooks in the tanks were sampled as above.

3.2.3 Statistical analyses

The contributions of the various collected technical parameters towards explaining variability among the four response variables (the percentage weight and point remaining and tensile and compression strengths) were assessed using multiple linear regression analysis. Categorical parameters (including sample time, steel type and the presence or absence of modifications and bait-holder barbs) were incorporated into the models as ‘dummy’ variables. Of the dummy variables, only sample time had more than two levels (i.e. $T_0$, $T_8$ and $T_{28}$) when used to assess compression and tensile strengths. For these analyses, sample time was applied by referencing the $T_8$ and $T_{28}$ sampling periods against $T_0$. For the analyses of percentage point and weight remaining, the $T_{28}$ sampling period was referenced against $T_8$. All percentage data were $\sin^{-1}(\sqrt{x})$ transformed.

The most parsimonious models fitted to the results were derived by using a step-down procedure of model reduction, beginning a saturated model comparing all possible subsets (restricted to main effects and the first-order interactions between the categorical variables and wire diameter and the categorical variables and shaft length, plus the interaction between these two measured variables) using a penalised log-likelihood in the form of Akaike’s information criterion (AIC; Burnham and Anderson 2002). Final model (reduced) selection was made from among those models for which $\Delta$AIC < 2 in relation to the value of this statistic, determined for the most parsimonious model using an analysis of variance. This enabled further possible reductions in the number of variables in the final model. Terms were only considered significant at $p < 0.01$ (Table 5). All analyses were done using the R statistical package (R development Core Team 2009).
Table 5: Summary of parameters included in parsimonious multiple regression models in relation to percentage total hook weight and point remaining and tensile and compression strengths (N – newtons of force) following immersion in seawater for eight and then 28 days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% weight remaining</th>
<th>% point remaining</th>
<th>Tensile strength</th>
<th>Compression strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>Na</td>
</tr>
<tr>
<td>Sample time 1 ($T_8$ vs. $T_0$)</td>
<td>–</td>
<td>–</td>
<td>***</td>
<td>Na</td>
</tr>
<tr>
<td>Sample time 2 ($T_{28}$ vs. $T_0$)</td>
<td>–</td>
<td>–</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Sample time 3 ($T_8$ vs. $T_{28}$)</td>
<td>***</td>
<td>***</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Steel type (stainless vs. carbon)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>Na</td>
</tr>
<tr>
<td>Bait-holder barbs (presence vs. absence)</td>
<td>**</td>
<td>–</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>Modification (presence vs. absence)</td>
<td>**</td>
<td>***</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Hook design (circle vs. J)</td>
<td>***</td>
<td>***</td>
<td>Na</td>
<td>***</td>
</tr>
<tr>
<td>Wire ø (mm)</td>
<td>***</td>
<td>Na</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Shaft (mm)</td>
<td>***</td>
<td>–</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Front (mm)</td>
<td>***</td>
<td>–</td>
<td>Na</td>
<td>***</td>
</tr>
<tr>
<td>Bend (mm)</td>
<td>***</td>
<td>–</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>Gape (mm)</td>
<td>***</td>
<td>–</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Wire ø x sample time 1</td>
<td>–</td>
<td>–</td>
<td>Na</td>
<td>Na</td>
</tr>
<tr>
<td>Wire ø x sample time 2</td>
<td>–</td>
<td>–</td>
<td>***</td>
<td>Na</td>
</tr>
<tr>
<td>Wire ø x sample time 3</td>
<td>Na</td>
<td>Na</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Wire ø x steel type</td>
<td>***</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
</tr>
<tr>
<td>Wire ø x bait-holder barbs</td>
<td>Na</td>
<td>–</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>Wire ø x modification</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>Na</td>
</tr>
<tr>
<td>Wire ø x hook design</td>
<td>***</td>
<td>***</td>
<td>Na</td>
<td>***</td>
</tr>
<tr>
<td>Wire ø x shaft</td>
<td>***</td>
<td>–</td>
<td>Na</td>
<td>**</td>
</tr>
<tr>
<td>Shaft x sample time 1</td>
<td>–</td>
<td>–</td>
<td>Na</td>
<td>Na</td>
</tr>
<tr>
<td>Shaft x sample time 2</td>
<td>–</td>
<td>–</td>
<td>Na</td>
<td>Na</td>
</tr>
<tr>
<td>Shaft x sample time 3</td>
<td>Na</td>
<td>–</td>
<td>–</td>
<td>Na</td>
</tr>
<tr>
<td>Shaft x steel type</td>
<td>Na</td>
<td>–</td>
<td>Na</td>
<td>Na</td>
</tr>
<tr>
<td>Shaft x bait-holder barbs</td>
<td>**</td>
<td>–</td>
<td>Na</td>
<td>**</td>
</tr>
<tr>
<td>Shaft x modification</td>
<td>Na</td>
<td>–</td>
<td>Na</td>
<td>**</td>
</tr>
<tr>
<td>Shaft x hook design</td>
<td>Na</td>
<td>–</td>
<td>Na</td>
<td>Na</td>
</tr>
</tbody>
</table>

(* * * p < 0.001; ** p < 0.01; Ns, not significant at p > 0.01; Na, not included in model; – not considered in the model-fitting process; ø, diameter).

3.3 Results

3.3.1 Percentage of hook weight remaining

At the end of the experiment, none of stainless steel hooks appeared to be oxidized, while the carbon steel designs were reduced to as little as 95% of their original weights. The most parsimonious model accounted for 76% of the variation in the percentage of hook weight remaining and was reduced to ten significant main effects and five significant first-order interactions (with wire diameter and/or shaft length – Table 5). The four main effects that did not have a significant interaction with either wire diameter or shaft length were sample time 3 and front, bend and gape lengths (Table 5). These main effects presented as a significantly lower percentage of weight remaining for all hooks sampled at $T_{28}$ than at $T_8$ (mean reduced by 0.8%; Figure 7a) and
a lower percentage of hook weight remaining with increasing front lengths (up to 12 mm) and narrower bends and gapes \((p < 0.001; \text{Figure 7b–d})\).

**Figure 7:** (a) the differences in mean (+ s.e.) percentage of weight remaining for hooks sampled at \(T_8\) and \(T_{28}\) and the relationships between the percentage of weight remaining and hook (b) front (c) bend and (d) gape lengths.

Scatter plots of the significant interactions between wire diameter and the various categorical variables showed that, irrespective of wire diameter, the few stainless steel hooks retained their initial weights, while for the remaining carbon steel hooks, there was a positive relationship between wire diameter and the percentage of weight remaining (Figure 8a and b). The presence or absence of modifications also had significant effects on the percentage of weight remaining across different wire diameters; with unmodified hooks showing a similar positive relationship as above for carbon steel (Figure 8d). Although there were few data, this
relationship was not maintained for the modified hooks (Figure 8c). Both circle and J-hooks also presented
positive relationships between wire diameter and the percentage of hook weight remaining, although fewer data
meant that the relationship was weaker for circle hooks (Figure 8e and f). The scatter plots of the significant
interaction between shaft length and bait-holder barbs on the percentage of hook weight remaining showed that
there was a stronger positive relationship among hooks with bait-holder barbs than those without (Figure 8g
and h).

3.3.2 Percentage of hook point remaining

After 28 days of immersion in seawater, as little as 38% of the points remained on carbon steel hooks. The
model that best described the percentage of hook point remaining was reduced to four main effects and two
first-order interactions (both involving wire diameter) and accounted for 32% of the variation ($p < 0.001$; Table
5). The two main effects that did not have a significant interaction with wire diameter were sample time 3 and
steel type ($p > 0.01$; Table 5). Specifically, there was lower percentage of the hook point remaining at $T_{28}$ than
at $T_{8}$ (a mean reduction of 5.4%; Figure 9a) and while stainless steel hooks retained their initial point length,
carbon steel hooks had a mean reduction of 8.6% (Figure 9b).

A significant interaction was detected between the presence or absence of modifications and wire
diameter for the percentage of hook point remaining, with unmodified hooks showing a slightly positive
relationship; more so for wire diameters < 1.24 mm ($p < 0.001$; Figure 10a). There were few data for modified
hooks and so this limited the interpretation (Figure 10b). Hook design also significantly affected the
relationship between wire diameter and the percentage of hook point remaining, with J-hooks displaying a
similar trend as above for unmodified designs ($p < 0.001$; Figure 10d). Few data were available for circle
hooks (Figure 10c).
Figure 8: Scatter plots of the relationships between the percentage of hook weight remaining and wire diameter for hooks that were (a) stainless steel, (b) carbon steel, (c) modified, (d) unmodified, (e) circle designs and (f) J-designs, and the relationship between the percentage of hook weight remaining and shaft length for hooks (g) with and (h) without bait-holder barbs.
**Figure 9:** The differences in mean (+ s.e.) percentage of the point remaining for hooks sampled at (a) $T_8$ and $T_{28}$ and those made from (b) stainless and carbon steel.

**Figure 10:** Scatter plots of the relationships between the percentage of the point remaining and wire diameter for hooks that were (a) modified, (b) unmodified, (c) circle designs and (d) J-designs.
3.3.3 Tensile strength

The range of tensile strengths of hooks before and after immersion in seawater for 28 days was reduced from between 80 and 370 N to between 25 and 361 N, respectively. During testing, the majority of hooks straightened at maximum elasticity, with breakages limited to 16, 14 and 18% at $T_0$, $T_8$ and $T_{28}$, respectively.

The most parsimonious model accounted for 72% of the variation in tensile strength and comprised seven main effects and five first-order interactions (three and two involving wire diameter or shaft length, respectively; Table 5). Six of the seven main effects included in the model were significant at $p < 0.01$, as were the three significant first-order interactions with wire diameter. The two interactions with shaft length had $p > 0.01$ and are not discussed further.

The main effects of sample time 1 and steel type were not involved in significant interactions and manifested as overall reductions in tensile strength between $T_0$ and $T_8$ (a mean reduction of 10.6%) and between stainless and carbon steel (a mean reduction of 2.3%) (Figure 11a and b). Scatter plots of the wire-diameter interactions revealed that this variable had a stronger positive relationship with tensile strength at $T_0$ than at $T_{28}$ (Figure 12a and b), and among unmodified hooks or those without bait-holder barbs (compared to modified hooks and those with bait-holder barbs, respectively) (Figure 12c – f).

![Figure 11](image-url)

**Figure 11:** The differences in mean (+ s.e.) tensile strength between (a) $T_0$ and $T_8$, and (b) stainless and carbon steel.
3.3.4 Compression strength

The sampled hooks had compression strengths of between 29 and 405 N before the experiment and between 24 and 365 N after 28-days immersion in seawater. The percentages of hooks that broke during compression testing were 24, 27 and 30% for $T_0$, $T_8$ and $T_{28}$, respectively. The model that best explained the variation (71%) among compression strength comprised eight main effects and six interactions (all involving wire diameter and/or shaft length), of which six and three, respectively were significant at $p < 0.01$ (Table 5). The two main effects that did not have significant interactions with either wire diameter or shaft length were sample time 2 and front length. These effects presented as significantly greater reductions in compression strength at $T_{28}$ than at $T_0$ (a mean reduction of 21%), and with shorter front lengths ($p < 0.01$; Figure 13a and b).

![Figure 12: Scatter plots of the relationships between the tensile strength and wire diameter for hooks sampled at (a) $T_0$ and (b) $T_{28}$, and those that were (c) modified, (d) unmodified, and (e) with and (f) without bait holders](image-url)
Scatter plots of the significant categorical interactions showed that, although there were few data, there was a slightly stronger positive relationship between wire diameter and the compression strength of circle hooks than for J-hooks (Figure 14a and b). Conversely, the presence of bait-holder barbs negated any positive influence that wire diameter had on compression strength (Figure 14c and d). A significant interaction ($p < 0.01$) was found between shaft length and the presence or absence of modifications, with a clear negative relationship for unmodified hooks. The lack of a reasonable range of shaft lengths among the modified hooks precluded coherent interpretation of this result (Figure 14e and f). Although not plotted the interaction between wire diameter and shaft length shows that with increasing shaft length reduces the compression strength.

Figure 13: (a) the differences in the mean (+ s.e.) compression strength of hooks sampled at $T_0$ and $T_{28}$, and (b) the relationship between front length and compression strength.
Figure 14: Scatter plots of the relationships between the compression strength and wire diameter for hooks that were (a) circle, (b) J-designs and (c) with and (d) without bait-holder barbs, and scatter plots of the relationship between the compression strength and shaft length for hooks that were (e) modified and (f) unmodified.

3.4 Discussion

This study has isolated some of the key technical factors affecting the temporal oxidation and associated strength reduction in commonly used hooks after immersion, and in doing so, identified simple modifications by which decay can be promoted. Prior to a discussion of these results and their implications in terms of promoting hook ejection by fish, it is necessary to first consider the forces imposed on hooks during the catching process. This information can then be used to provide some indication of the required initial threshold for hook strength (and any associated modifications).
3.4.1 Suggested minimum hook strength required

There are few published studies quantifying the forces involved in hooking fish (Sakazume and Kanamori 1971; Mitsugi and Inoue 1985; Edappazham et al. 2008), although intuitively, once the barb penetrates the flesh or bone and the fish pulls away from the angler most of the load should be tensile strain. Fridman (1986) suggested that the approximate maximum tractive force (kg) of a fish could be estimated by dividing its weight in air (kg) by the cube root of its length (m). Applying this formula to the largest sizes of fish (i.e. \( \approx 40 \text{ cm TL} \)) targeted using the various hooks in this study, and assuming such fish typically weigh \(~1 \text{ kg}\) (e.g. Santos et al. 2002; Broadhurst et al. 2006a), their continuous tractive force would be some 13 N. Additional force from kinetic energy during the initial hooking might increase this estimate slightly (Fridman 1986), but it is still less than almost 85% of the minimum initial tensile strength detected here for the weakest hooks (i.e. those modified with notches – mean ± se of 92 ± 4 N), and 92% less than the average tensile strength (169 ± 5 N) of the remaining conventional designs (Table 4).

Based on the above estimate, all of the hooks tested in this study were considerably stronger than required, and therefore could be modified and/or redesigned to reduce their initial strength and increase their temporal decay during ingestion. It is also clear that the starting point for any such changes should involve those main factors identified to affect overall hook degradation, and in particular the wire material and diameter and, to a lesser extent, the bend, gape, front and shaft lengths.

3.4.2 Maximising the decay to reduce hook strength

Irrespective of all other parameters, the wire material had the strongest impact on oxidation and strength. Unlike all of the carbon-steel hooks, after 28-days immersion in seawater, the stainless-steel designs retained almost 100% of their initial strength, weight and point length; which means that the latter remained sharp. This result is important, since once ingested; stainless-steel hooks intuitively would have a much greater probability of penetrating soft tissue and vital organs during progression through the digestive tract (Broadhurst et al. 2007). Further, the maintenance of initial high tensile and compression strengths (between 127 ± 6 and 197 ± 7 N) among the unmodified stainless-steel hooks would greatly reduce their chances of breaking after ingestion. Both of these factors could translate to an increased mortality among fish that ingest hooks.
By comparison, after immersion for 28 days, the carbon-steel hooks were significantly oxidised; to the extent where some has less than 95 and 38% of their initial weights and hook points remaining, respectively. The amount of degradation and concomitant loss of strength among carbon-steel hooks varied among designs and was probably at least partly due to the alloy constitutes and the composition of the protective coating (Edappazham et al. 2008); both of which remain unknown. But it is also clear that a significant proportion of the degradation variability can be explained by the diameter of the wire.

In support of the few relevant previous studies (e.g. Edappazham et al. 2008), wire diameter had a consistent positive relationship with tensile and compression strengths and the percentages of weight and point remaining for most hooks, but especially conventional, carbon-steel J-designs. More specifically, owing to the linear increase in the surface area-to-volume ratio and reduction in mass, at narrower diameters the latter hooks were oxidized at a greater rate and were weaker. The importance of wire diameter was re-enforced by its consistent significant interactions with the presence or absence of modifications and bait-holder barbs. By reducing the diameter of the wire at multiple locations on the shaft, bend and/or front (by ~20%) and effectively providing a weak point, these changes largely negated any relationship between increasing wire diameter and strength.

Bait-holder barbs and similar modifications to wire diameter could be an appropriate strategy for increasing the probability of both stainless- and carbon-steel hooks breaking, and the latter oxidising, after ingestion. In support of such effects, Broadhurst et al (2007) attributed the bait-holder barbs on nickel-plated carbon-steel hooks (similar in design, but smaller than the no. 10 hooks examined here – Table 1) to fast decay and a weak point which facilitated breakage (often within three weeks) during ingestion by yellowfin bream. Other authors have noted similar impacts (e.g. Aalbers et al. 2004).

While changes to wire material and diameter would have the most benefit in promoting temporal hook decay, there were also significant effects of the length of the bend, gape and front. The impacts of the bend and gape were restricted to the percentage of weight remaining and presented as a trend of greater oxidation among narrower lengths. Such a result is difficult to explain, although it might reflect a greater curvature of the wire
and the associated slightly increased outside surface area. A similar increase in surface area associated with longer shaft lengths up to 25 mm and front lengths up to 12 mm might also explain the slightly greater relative oxidation, although hooks with front and shaft lengths beyond these estimates were oxidised at a lower rate. More data across a wider range of hook sizes and types would be required to further test the relationship between the bend, gape and front of hooks and their oxidation.

In addition to oxidation, the shaft and front lengths also affected compression strength. As the shaft length increased, hooks generally became weaker owing to the load deflection (causing the shaft to bend - Case et al. 1999). However, any potential benefits associated with a longer weak shaft during ingestion might be negated since McGrath et al (2009) indicated that such hooks could not easily rotate in the digestive tract, minimising their ejection from sand whiting (Sillago ciliata). Hooks with long front lengths might be similarly difficult to eject. Also, according to the results observed here, and unlike for shaft length, longer front lengths had significantly greater compression strengths – although this may reflect the experimental design since such hooks were held more securely in the compression cylinders.

The latter result highlights one of the limitations of this study and reiterates the conditional interpretation of the results. Each of the four response variables were chosen to assess temporal hook degradation which, based on the observations of Broadhurst et al (2007) was assumed to be the same in seawater as that in the digestive tract of a fish. However, in some cases, hooks can be imbedded in internal tissue, resulting in lower oxidation rates (Aalbers et al. 2004), and therefore a greater maintenance of strength than that observed here. Also, while the tensile force measured at $T_0$ might correlate with the force imposed by a fish during capture (Edappazham et al. 2008), the data collected at $T_8$ and $T_{28}$ are unlikely to represent the same forces on hooks after ingestion (although these can be quite extreme – Broadhurst et al. 2007). Such differences mean that the results from this study should not be extrapolated beyond simple indices of degradation.

3.4.3 Conclusion

Notwithstanding the above, this study still supports recommendations for ongoing research into hook construction and provides direction for anglers that wish to minimise impacts to fish using conventional hook
designs. For example, future research might warrant examining designs made from composite materials that comprise a sufficiently resilient wire for the shaft, bend and front, but a weak point and barb that oxidise very quickly. It might also be feasible to incorporate weaker points on the wire, so that the hook breaks down quickly after ingestion. In the interim, anglers should be encouraged to avoid stainless-steel hooks and chose carbon-steel designs with the narrowest wire diameter. Based on the results here, hooks with similar absolute sizes, but made from a wire diameter of < ~0.9 mm should still provide sufficient tensile strength, while promoting rapid oxidation and subsequent breakage. Existing hooks (including stainless-steel designs) with thicker wire could be easily modified to make them weaker, by incorporating notches or bait-holder barbs. Such modifications could help to reduce unaccounted fishing mortality and further validate catch-and-release as an appropriate management tool for conserving stocks.

Acknowledgements

The experiment was funded by Industry and Investment New South Wales (NSW) and the NSW Recreational Fishing Trusts. Thanks to Richard Faulkner for extensive discussions, and Chris Dowling, Hester Bushell, Lachlan Roberts and Milan Duwenhogger for their technical assistance.
Chapter 4: Fate of three Australian teleosts after ingesting conventional and modified stainless- and carbon-steel hooks

Publication

ICES Journal of Marine Science (published)


This paper was a collaborative work by S. P. McGrath, M. K. Broadhurst, P. A. Butcher and S. C. Cairns. Shane McGrath contributed 50% of the research design, 50% of the data analyses and 40% of the interpretation of the data.
Abstract

Concerns over the fate of three coastal marine teleosts (mulloway, *Argyrosomus japonicas*, yellowfin bream, *Acanthopagrus australis* and snapper, *Pagrus auratus*) released by recreational anglers with ingested hooks has led to three experiments. These experiments aimed to determine if the wire material (stainless-steel and carbon steel) and/or design modifications to hooks affected mortality, hook breakage and ejection. Each of the experiments involved between 108 and 114 individuals of each species, that were allowed to ingest either conventional or modified (with small notches) J-hooks (absolute size ~250 mm²) made from three materials (stainless steel and nickel-plated and red-lacquer carbon steel). Once fish had ingested their hooks, they were removed and released into tanks and monitored for up to 61 days (along with 18 control fish). The total mortality among angled mulloway (over 61 days), bream (over 35 days), and snapper (over 41 days) was 35, 24 and 25%, respectively. There were no mortalities in the control groups. Of the survivors, 30, 61 and 77% subsequently ejected their hooks, respectively. Hook ejection was the primary factor that effected mortality in all three species with only one death among fish that ejected their hooks. The research supports the use of hooks that oxidise more readily and subsequently become weaker and are ejected at faster rates. However, there were some indications that nickel-plated hooks could be more detrimental to fish health as they resulted in more deaths among yellowfin bream and mulloway that ingested them when compared to the other two hook types.
4.1 Introduction

With a participation rate greater than 19% of the population, recreational fishing is one of the most popular sports in Australia (West and Gordon 1994; Henry and Lyle 2003). While there are no current definitive estimates of catches, a 12-month survey done in 2000/01 estimated that > 260 species of elasmobranchs, teleosts, crustaceans and cephalopods were targeted for a total harvest of 135 million individuals. The majority of teleosts were caught by anglers (85%); mostly inshore (Henry and Lyle 2003).

Like in other developed nations, large numbers of angled teleosts are released in Australia; traditionally in response to mandated legal sizes and personal quotas but also, more recently, due to voluntary non-consumptive angling (Arlinghaus et al. 2002; Henry and Lyle 2003; Arlinghaus et al. 2007a). Three important and recreationally popular coastal species are yellowfin bream (Acanthopagrus australis), snapper (Pagrus auratus) and mulloway (Argyrosomus japonicus), which have a combined estimated recreational catch of >17 million individuals per annum and are released at rates exceeding 62, 66 and 46%, respectively (Henry and Lyle 2003).

Concerns over potential mortalities and/or negative sub lethal impacts to angled-and-released yellowfin bream, snapper and mulloway have lead to several quantitative studies (Broadhurst et al. 1999; Broadhurst and Barker 2000; Broadhurst et al. 2005; Broadhurst et al. 2007; Butcher et al. 2007; Grixti et al. 2010). This work has estimated short-term (<10 days) fatalities of 3–38% for yellowfin bream (Broadhurst et al. 2005; Butcher et al. 2007), 8-33% for snapper (Chapter 2; Broadhurst et al., 2005) and up to 23% for mulloway (Butcher et al. 2007). While many of the observed deaths were attributed to the cumulative impacts of several biological, technical and environmental factors, anatomical hook location had a strong recurring presence, manifesting as proportionally more deaths among hook-ingested individuals than those caught in the mouth (e.g. Butcher et al. 2006; Butcher et al. 2007; Grixti et al. 2010). Further, for yellowfin bream, hook-ingested fatalities increased from 8% when fish were released with their lines cut, to 88% when the hooks were forcefully removed (Butcher et al. 2007). Similar mortality trends have also been observed for mulloway and snapper (Butcher et al. 2007; Grixti et al. 2010; Chapter 2).
Based on the above association between hook removal and mortality, and in accordance with overseas studies (e.g. Jordan and Woodward 1992; Aalbers et al. 2004), a recommendation was made in New South Wales (NSW) for hook-ingested fish to be released with their lines cut, rather than attempting to remove hooks (Broadhurst et al. 2007; Butcher et al. 2007). The long-term utility of this approach to minimising negative impacts has been validated for yellowfin bream (Broadhurst et al. 2007; Butcher et al. 2010). Specifically, Broadhurst et al (2007) observed a non-significant mortality rate of 15% for line-cut, hook-ingested individuals over more than three months, with 76% of survivors ejecting their hooks after an average of ~20 days while maintaining their overall condition. No similar data are available for snapper or mulloway, although during three days of monitoring, Grixti et al (2010) recorded a hook ejection rate of 13% among similarly treated snapper.

While the ability of many hook-ingested teleosts to survive and eject their hooks is clear (reviewed by Hall et al. 2009), little is known about the mechanisms underlying this process. For some species, it is likely that their morphology and feeding strategies have an important influence (e.g. Aalbers et al. 2004; Broadhurst et al. 2007; McGrath et al. 2009). For example, yellowfin bream and snapper typically consume molluscs and crustaceans and are presumably accustomed to ejecting or passing hard structures (Russell 1983; Kailola et al. 1993). Like many marine teleosts, these sparids have pharyngeal teeth which help to digest hard and sharp materials by breaking the indigestible parts into smaller pieces (Alexander 1970).

In addition to biological traits, both the mortality of fish and their hook ejection are strongly influenced by hook decay, presumably because this dictates how long the point and barb remain sharp (and the potential for associated internal damage) and the overall structural integrity is maintained (Aalbers et al. 2004; Broadhurst et al. 2007; Grixti et al. 2008). Recognition of the importance of hook decay precipitated a recent study (Chapter 3) to isolate some of the key technical factors contributing to this process. This work revealed that following submersion in seawater for up to 28 days, the material and diameter of the wire used to manufacture hooks strongly influenced the percentage weight and point remaining (due to oxidation) and the subsequent compression and tensile strengths of selected hooks.
More specifically, after a month of submersion, carbon steel hooks were 19% weaker than stainless steel hooks. The relationship between the diameter of the wire and hook decay was less clear, likely being influenced by several other interacting variables. In particular, the presence of bait-holder barbs and other similar modifications (notches) along the wire length negated a consistent, significant positive relationship between wire diameter and tensile and compression strengths for a range of hooks. It was hypothesised that, irrespective of the wire diameter, the bait holders and notches provided weak points and, for the carbon steel hooks in particular, facilitated deterioration of the protective coating and exposed the metal (Chapter 3). Broadhurst et al (2007) argued that such modifications would encourage corrosion and increase the chance of the hooks breaking into smaller pieces and being ejected.

The strong influence of hook material on decay is intuitive, with several agencies in North America and Europe recommending against using stainless steel. Such a strategy could be extended to include other hooks that have resilient anti-corrosive coatings, although this would have obvious economic impacts and would be difficult to enforce. An alternative approach to this issue, might involve modifying existing hook designs (irrespective of their material) to either encourage their decay and/or reduce their strength to promote breakage during ingestion (Chapter 3). The present study sought to test the potential for such effects among hook-ingested yellowfin bream, snapper, and mulloway over a period of up to 61 days. In addition to providing the first information on the longer-term post release-fate of the last two species, the specific aims were to determine whether wire material (stainless-steel and carbon steel with different coatings) and/or modifications comprising of notches along the shaft, bend and under the point of the hook (Chapter 3 - Figure 5) influenced mortality, hook breakage and ejection.

4.2 Materials and methods

Three experiments were completed at the National Marine Science Centre (NMSC) Coffs Harbour, Australia, between March and December 2008. The facilities included five 3000 L covered holding tanks located in an open area, and 63 experimental tanks of 110 L arranged in an enclosed room with a regulated photoperiod (light-to-dark ratio of 12:12 h). The tanks and aquaria were supplied with seawater (at ambient temperature – between 16.7 and 24.1 °C) at a rate of 30 L min⁻¹ and aerated using stone diffusers.
4.2.1 Collection of fish

Up to three months before starting each experiment, \( \approx 300 \) juveniles of the three species were collected and transferred to the five holding tanks (\( \approx 60 \) individuals tank\(^{-1} \)) at the NMSC. Snapper (119–329 mm, total length – TL) and yellowfin bream (150–280 mm TL) were caught by commercial fishers (using traps) and researchers (using cast nets and hook-and-line), respectively while mulloway (241–298 mm TL; first generation reared) were purchased from a local aquaculture facility. All fish were handled according to the methods described by Butcher et al. (2007) and fed commercially available 4 mm fish pellets and Australian sardine (Sardinops neopilchardus) at a rate of \( \approx 1\% \) biomass day\(^{-1} \) until two days before starting the experiments.

4.2.2 Hooks used

The hooks were chosen based on their almost identical design (J) and shape (absolute size \( \approx 250.4 \) mm\(^2 \) – See Chapter 3– Figure 5), but different wire materials: stainless steel and red-lacquer coated and nickel-plated carbon steel (Table 6). These materials represent varying levels of corrosion resistance, estimated by the manufacturer as evidence of oxidation across 3, 30 and 52%, respectively, of the total surface area after several hours of exposure to salt spray. Using a rotary tool (24-mm disc), half of the hooks (termed ‘modified’) made from each material had three small notches (similar to bait-holder barbs - Broadhurst et al., 2007) cut into the shaft, bend and point (to \( \approx 80\% \) of the wire diameter; See Chapter 3-Figure 5). The notches were designed to reduce hook strength and, for the carbon-steel designs, increase the surface area and subsequent oxidation. The remaining hooks made from each material were left unmodified (and termed ‘conventional’). All hooks were weighed to the nearest 0.0001g using an electronic balance (Ohaus adventurer analytical balance, AR2140). Modified hooks were only used if they weighed at least 99% of their original mass.
Table 6: Initial technical specifications (mean ± s.e.) of the six treatment hooks investigated for their rates of ejection and decay and influences on the mortality of mulloway, yellowfin bream and snapper after being ingested. Lengths ($n = 12$), weight ($n = 54–56$) and absolute size are in mm, mg and mm², respectively.

<table>
<thead>
<tr>
<th>Steel Hook</th>
<th>Manufacturer’s Wire coating type</th>
<th>Diameter (mm)</th>
<th>Shaft Length (mm ± s.e.)</th>
<th>Front Length (mm ± s.e.)</th>
<th>Bend Length (mm ± s.e.)</th>
<th>Gape Length (mm ± s.e.)</th>
<th>Absolute Size (mm² ± s.e.)</th>
<th>Weight (g ± s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>C Nickel</td>
<td>1.06</td>
<td>21.33 ± 0.01</td>
<td>11.21 ± 0.01</td>
<td>11.41 ± 0.01</td>
<td>9.96 ± 0.01</td>
<td>243.44 ± 0.16</td>
<td>279.41 ± 0.26</td>
</tr>
<tr>
<td>a#</td>
<td>C Nickel</td>
<td>1.06</td>
<td>21.34 ± 0.01</td>
<td>11.21 ± 0.01</td>
<td>11.42 ± 0.01</td>
<td>9.96 ± 0.01</td>
<td>243.65 ± 0.16</td>
<td>276.38 ± 0.20</td>
</tr>
<tr>
<td>b</td>
<td>C Red lacquer</td>
<td>1.06</td>
<td>21.00 ± 0.02</td>
<td>11.58 ± 0.01</td>
<td>11.82 ± 0.01</td>
<td>9.80 ± 0.01</td>
<td>248.24 ± 0.33</td>
<td>277.97 ± 0.22</td>
</tr>
<tr>
<td>b#</td>
<td>C Red lacquer</td>
<td>1.06</td>
<td>21.00 ± 0.02</td>
<td>11.58 ± 0.01</td>
<td>11.82 ± 0.01</td>
<td>9.81 ± 0.01</td>
<td>248.23 ± 0.33</td>
<td>274.66 ± 0.20</td>
</tr>
<tr>
<td>c</td>
<td>S Na</td>
<td>0.98</td>
<td>21.43 ± 0.02</td>
<td>11.47 ± 0.01</td>
<td>11.91 ± 0.01</td>
<td>10.44 ± 0.01</td>
<td>255.16 ± 0.24</td>
<td>229.44 ± 0.29</td>
</tr>
<tr>
<td>c#</td>
<td>S Na</td>
<td>0.98</td>
<td>21.42 ± 0.02</td>
<td>11.45 ± 0.01</td>
<td>11.90 ± 0.01</td>
<td>10.30 ± 0.04</td>
<td>254.92 ± 0.28</td>
<td>226.30 ± 0.31</td>
</tr>
</tbody>
</table>

#, modified with three notches on the shaft, bend and front (see Chapter 3 - Figure 5); C, carbon steel; S, stainless steel; Na, not applicable.

Replicates of each of the three unmodified hook types were analysed for their elemental constitutes by Spectrometer services (Coburg, Australia). Two replicate analyses were done, each involving 10 hooks ($\approx 2$ g of steel). Approximately 0.5 g of each sample was ground into particles ($<10 \mu$m in diameter) and the percentage of carbon and sulphur was determined using infrared absorption after combustion in an induction furnace (LECO combustion analyser, CS230) following Australian standards AS1050.16 and AS1050.32. The remaining sample was then dissolved using an acid digest process and an inductively coupled plasma atomic emission spectroscopy (ICP-AES) scan applied to determine the composition of all other elements.
4.2.3 Experimental procedure

On the first day of each experiment, 24 fish (termed controls) were randomly selected from two of the holding tanks, removed using knotless nets and measured and weighed. Within two minutes of capture, six of these fish were secured in a foam block and a 1-ml blood sample collected via caudal vascular puncture using 22 gauge needles and heparinised syringes (see Broadhurst et al. 2005, for details). The remaining 18 individuals were placed in pairs into nine, randomly allocated experimental tanks in the enclosed aquaria room. Where two similar-sized fish were placed into the same tank, one had the top caudal fin clipped to facilitate subsequent identification. Six individuals of each species were concurrently angled from the wild near the NMSC (using conventional hook-and-line) and also immediately sampled for blood (as described above) to eventually provide baseline estimates of plasma glucose as an index of stress (see below).

Once the controls were placed into their experimental tanks, hooks within each of the six categories (i.e. hooks made from the three materials and comprising modified and conventional designs—Table 4) were attached to either clear or yellow 8-kg polyvinylidene fluoride line. The different line colours were used to eventually facilitate the identification of replicate hooks between fish within tanks (see below). Each category \( n = 6 \) of hook was used to angle at least 18 fish (i.e. a minimum total of \( 108 \)) from the three remaining holding tanks over up to four days. All fish were allowed to ingest the hook before being removed from their holding tank, held in one hand while the line was cut \( \approx 5 \) cm from their mouth, measured \( (TL) \), weighed and released in pairs (caught with yellow and clear line) into 54 of the experimental tanks. Because there was some short-term mortality (i.e. within 120 mins of release) among angled mulloway, dead fish were replaced in the experimental tanks and with additional hook-ingested fish (up to two per treatment). All experimental fish were offered food (as above) and monitored daily for between 5−9 weeks. To maintain stocking densities in the experimental tanks and except for the short-term mortalities for mulloway (see above), any dead fish were removed and replaced with fish from the holding tanks (caudal fin clipped for identification).
4.2.4 Data collected and statistical analyses

The times of capture and release into the experimental tanks, type of treatment or control, initial TL (mm), monitoring period, daily mortality and resumption of feeding were recorded for all fish. The presence or absence of blood at the mouth during initial capture and the daily ejection of hooks was also noted for treatment fish. Any completely (whole) ejected hooks were cleaned and re-weighed to determine the percent loss of mass attributable to corrosion. Any hook breakages were noted. Water temperature (°C) and dissolved oxygen (DO - mg L⁻¹) were monitored daily using a water quality sensor (U-10 Horiba).

At the end of each monitoring period, appropriate numbers (see Results) of the surviving treatment and control fish were sampled for blood (as above). The remaining treatment fish were euthanised in a solution of benzocaine (ethyl-p-aminobenzoate; 100 mg L⁻¹ in seawater) before being dissected. The ingested hooks were then removed and weighed as above. All remaining fish were released.

The data were analysed separately within each experiment using several approaches. Generalized linear mixed models (GLMMs) were used to test for the independence of various continuous and categorical fixed effects and the random effect of experimental tanks on the survival of individuals at the end of the monitoring periods. The fixed factors included in this analyses were: the continuous factors of fish TL (mm), hook weight (g) and wire diameter (mm); the binary factors of hook breakage, hook ejection, steel type, hook modification, nickel plating, red lacquer coating and the presence of blood; and the nominal factor of researcher. All categorical effects were treated in a qualitative manner by converting them to dummy variables using reference cell coding (Quinn and Keough 2002). A second group of GLMMs were run for the surviving angled fish using the same factors as above, but with hook ejection as the response variable.

GLMMs were fitted using the lmer function in the lme4 package of the R statistical package, version 2.12.0 (R development Core Team 2009; Bates et al. 2011), following a procedure similar to that outlined by Zuur et al. (2007). This involved starting with what might be a “just beyond optimal” model that included all fixed components and the single random effect. Derivation of the optimal, most parsimonious model involved a stepwise selection process whereby redundant explanatory variables (fixed
effects) were progressively deleted from the model. The most parsimonious model was identified by the lowest value for a penalised log-likelihood in the form of Akaike's Information Criterion (AIC = \(-2 \times \log\)-likelihood + \(2[p + 1]\), where \(p\) is the number of parameters in the model; Burnham and Anderson 2002; Johnson and Omland 2004). Where the difference between any particular model and the top ranked model (i.e. the one that resulted in the smallest AIC) was <2, model adequacy was assessed using a likelihood ratio test (Zuur et al. 2007) and the significance of individual model coefficients assessed with the Wald statistic (Agresti 1996; Crawley 2005).

Variability in the rate of oxidation (defined as the arcsine-root transformed proportion of weight lost per day) of the whole ejected and dissected hooks for each of the species were analysed using a linear mixed model (LMM). The fixed factors in the analyses were the six hook treatment types, their location at the end of the experiment (either ejected or dissected from fish) and the interaction between these two parameters. The aquaria tanks were included as a random effect and the \(TL\) of fish was included as a covariant. Predicted means of interest were back-transformed and depicted graphically. All LMMs were fitted in ASReml-R (Butler et al. 2009).

Blood samples from the three experiments were analysed for concentrations of glucose (mmol L\(^{-1}\)) derived by means of colorimetric clinical kits (Roche Diagnostics, USA) using an enzymatic spectrophotometric assay according to the manufacturer’s instructions. These data were collected to assess whether fish were unusually stressed as a consequence of their treatment and/or confinement during the experiments. Specifically, the hypothesis of no difference in blood glucose among (i) immediately sampled wild caught fish (i.e. the baseline), (ii) confined fish in the holding tanks at the start of the experiment, and (iii) angled fish with both hooks ejected and still ingested, and (iv) control fish in tanks at the end of the experiments, was tested using analysis of variance (ANOVA). Where transformation was required, but still did not stabilize variances, the significance level for the ANOVA was set at \(p = 0.01\) to reduce the likelihood of committing a type I error.

4.3 Results
All of the treatment hooks had an almost identical shape and profile, with comparable shaft, front and bend lengths (Table 5). The gape lengths, absolute sizes, weights and wire diameters were slightly more
divergent (Table 6). Of these latter parameters, wire diameter and hook weight were considered the most important in terms of oxidation and were therefore included in the subsequent GLMMs (see below and Chapter 3).

Much greater differences were observed in the elemental constituents among hooks; especially between the stainless- and two carbon-steel designs (Table 7). In particular, stainless-steel hooks had proportionally less iron (mean concentration of <84.53 vs. <96.50%), carbon (0.40 vs. 0.74%), manganese (0.28 vs. 0.55%) and nickel (0.17 vs. 2.00%), but more silicon (0.39 vs. 0.14%), cobalt (0.01 vs. <0.01%), copper (0.11 vs. 0.04%), chromium (13.12 vs. 0.02%), vanadium (0.07 vs. <0.01%) and molybdenum (0.88 vs. <0.01%; Table 7). The main difference between the nickel-plated and red-lacquer coated hooks was the amount of nickel (2.52 vs. 1.4%). Among all hooks, 15 other elements were present in trace quantities (Table 7).

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Nickel-plated carbon steel</th>
<th>Red-lacquer carbon steel</th>
<th>Stainless steel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Fe</td>
<td>&lt;96.016 ± 0.280</td>
<td>&lt;96.976 ± 0.180</td>
<td>&lt;84.53 ± 0.045</td>
</tr>
<tr>
<td>Chromium</td>
<td>Cr</td>
<td>0.010 ± 0.000</td>
<td>0.025 ± 0.005</td>
<td>13.115 ± 0.050</td>
</tr>
<tr>
<td>Nickel</td>
<td>Ni</td>
<td>2.520 ± 0.280</td>
<td>1.475 ± 0.235</td>
<td>0.170 ± 0.010</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Mo</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
<td>0.880 ± 0.000</td>
</tr>
<tr>
<td>Carbon</td>
<td>C</td>
<td>0.735 ± 0.005</td>
<td>0.740 ± 0.010</td>
<td>0.404 ± 0.000</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
<td>0.490 ± 0.000</td>
<td>0.615 ± 0.035</td>
<td>0.280 ± 0.020</td>
</tr>
<tr>
<td>Silicon</td>
<td>Si</td>
<td>0.195 ± 0.005</td>
<td>0.075 ± 0.015</td>
<td>0.390 ± 0.030</td>
</tr>
<tr>
<td>Copper</td>
<td>Cu</td>
<td>0.020 ± 0.000</td>
<td>0.060 ± 0.010</td>
<td>0.110 ± 0.030</td>
</tr>
<tr>
<td>Vanadium</td>
<td>V</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
<td>0.065 ± 0.015</td>
</tr>
<tr>
<td>Sulphur</td>
<td>S</td>
<td>&lt;0.010 ± 0.000</td>
<td>0.020 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Co</td>
<td>0.004 ± 0.000</td>
<td>0.004 ± 0.000</td>
<td>0.014 ± 0.001</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>P</td>
<td>0.010 ± 0.000</td>
<td>0.010 ± 0.000</td>
<td>0.010 ± 0.000</td>
</tr>
<tr>
<td>Aluminium</td>
<td>Al</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
</tr>
<tr>
<td>Niobium</td>
<td>Nb</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
</tr>
<tr>
<td>Titanium</td>
<td>Ti</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
<td>&lt;0.010 ± 0.000</td>
</tr>
<tr>
<td>Arsenic</td>
<td>As</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Barium</td>
<td>Ba</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Beryllium</td>
<td>Be</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Bismuth</td>
<td>Bi</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Cd</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Lead</td>
<td>Pb</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Mercury</td>
<td>Hg</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Selenium</td>
<td>Se</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Silver</td>
<td>Ag</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
<tr>
<td>Tin</td>
<td>Sn</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
<td>&lt;0.005 ± 0.000</td>
</tr>
</tbody>
</table>
4.3.1 Fate of mulloway

Mulloway were assessed for up to 61 days across a mean (± s.e.) DO of 6.48 ± 0.04 mg l⁻¹ and water temperature of 21.38 ± 0.19°C. In addition to the 18 controls (mean size ± s.d. of 264.3 ± 15.0 mm TL), between 18 and 20 fish (114 in total) were monitored for each of the six hook-ingestion treatments (mean size ± s.d. of 265.9 ± 12.1 mm TL). The additional replicates for some treatments were due to deaths within 210 min of hooking (Figure 15a-c). None of the controls died, but the total mortalities among the treatment groups ranged between 17 and 47%, and mostly occurred within 21 d (Figure 15a-c). All treatment and control fish resumed feeding within a week of being placed in the experimental tanks and for fish that ejected hooks, it was not possible to determine whether the expulsion was via the mouth or anus.

The most parsimonious GLMM explaining the variation among mortalities was reduced to three significant fixed variables, including hook modification, nickel plating, and hook ejection (p < 0.05; Tables 8 and 9). Specifically, more fish died after ingesting modified than unmodified hooks (42 vs. 27%) and those that had nickel plating, than those that did not (43 vs. 31%; p < 0.01; Figure 15a-c; Tables 8 and 9). None of the fish (n = 22) that ejected their hooks died, compared to 43% that remained hook ingested (p < 0.001; Tables 8 and 9).

For the surviving mulloway, the most parsimonious GLMM explaining their hook ejection was reduced to hook modification, hook breakage, and nickel plating (p < 0.05; Tables 8 and 9). Of these factors, only the latter two were significant, with 41% of nickel-plated hooks ejected compared to only 9% of the remaining two steel types (p < 0.05; Figure 16a-c; Tables 8 and 9). Further, significantly more of the ejected hooks were broken than whole (56 vs. 13%; p < 0.05; Tables 8 and 9), and the majority of fish (78%) that ejected broken hook retained part of the hook until the conclusion of the monitoring period.
Figure 15: The cumulative percentage of mortality of (a, b & c) mulloway *Argyrosomus japonicus*, (d, e & f) yellowfin bream *Acanthopagrus australis*, and (g, h & i) snapper *Pagrus auratus* after ingesting the six treatment hooks during the experiment.
Figure 16: The cumulative percentage ejection of the ingested treatment hooks by (a, b & c) mulloway *Argyrosomus japonicus*, (d, e & f) yellowfin bream *Acanthopagrus australis*, and (g, h & i) snapper *Pagrus auratus*. 
There were significant differences in the mean predicted (back transformed) proportion of weight lost per day between all whole ejected and dissected hooks (LMM; Wald $F = 28.45, p < 0.01$), and among the six different hook treatments, irrespective of their location (LMM; Wald $F = 30.70, p < 0.01$; Figure 17a). More specifically, whole ejected hooks had a predicted proportion of weight lost per day that was approximately five times greater than those that were dissected from fish (Figure 17a). In addition, modified nickel-plated had the greatest proportion of weight lost per day and oxidised more than seven times faster than their modified red-lacquer carbon steel counterparts, whereas stainless-steel hooks had minimal oxidation (Figure 17a). There was no significant interaction between hook location and the hook treatment type, or any effect of fish $TL$ on the rate of hook oxidation (LMM; Wald $F = 0.18$ and $0.83, p > 0.05$, respectively).

ANOVA detected significant differences in the concentrations of ln($x+1$) transformed plasma glucose among the groups of sampled fish ($F_{4,42} = 3.49, p < 0.01$). In particular, fish that were immediately sampled from the wild (mean ± s.e. of $1.00 ± 0.00 \text{ mmol L}^{-1}$) had slightly lower glucose than those in the holding tanks ($2.03 ± 0.08 \text{ mmol L}^{-1}$) prior to the experiment, and the control ($2.37 ± 0.54 \text{ mmol L}^{-1}$), hook-ingested ($1.88 ± 0.18 \text{ mmol L}^{-1}$) and hook-ejected ($2.60 ± 0.23 \text{ mmol L}^{-1}$) fish at the end of the experiment. All plasma glucose levels were within previously assessed limits.

Table 8: Summary of fixed variables tested in parsimonious generalized linear mixed models for their independence on the mortality and hook ejection of hook-ingested mulloway, yellowfin bream and snapper over 61, 35 and 41 days, respectively.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mulloway Mortalities Hook ejection</th>
<th>Yellowfin Bream Mortalities Hook ejection</th>
<th>Snapper Mortalities Hook ejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hook ejection</td>
<td>*** Na</td>
<td>*** Na</td>
<td>*** Na</td>
</tr>
<tr>
<td>Hook modification</td>
<td>* ○</td>
<td>*** Na</td>
<td>*** Na</td>
</tr>
<tr>
<td>Nickel plated</td>
<td>** *</td>
<td>* ○</td>
<td>* ○</td>
</tr>
<tr>
<td>Hook broken</td>
<td>– *</td>
<td>– ○</td>
<td>– ○</td>
</tr>
<tr>
<td>Steel type</td>
<td>– –</td>
<td>– ○</td>
<td>– ○</td>
</tr>
<tr>
<td>$TL$</td>
<td>– –</td>
<td>– ○</td>
<td>– ○</td>
</tr>
<tr>
<td>Bleeding</td>
<td>– –</td>
<td>– ○</td>
<td>– ○</td>
</tr>
</tbody>
</table>

○ $P > 0.1$; • $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; −, term not included in the final parsimonious model; Na, not applicable.
Table 9: Significant categorical (counts) and continuous (mean ± s.d.) fixed effects ($p < 0.05$) identified in generalized linear mixed models affecting the mortality and hook ejection of mulloway, yellowfin bream and snapper over 61, 35 and 41 days, respectively.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mulloway Mortalities</th>
<th>Mulloway Hook ejection</th>
<th>Yellowfin Bream Mortalities</th>
<th>Yellowfin Bream Hook ejection</th>
<th>Snapper Mortalities</th>
<th>Snapper Hook ejection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Hook ejection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>0</td>
<td>Na</td>
<td>Na</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>52</td>
<td>40</td>
<td>Na</td>
<td>Na</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>Hook modification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34</td>
<td>25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No</td>
<td>40</td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nickel plated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>16</td>
<td>22</td>
<td>15</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>No</td>
<td>53</td>
<td>24</td>
<td>70</td>
<td>7</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>Hook broken</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td>9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No</td>
<td>–</td>
<td>–</td>
<td>85</td>
<td>13</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Steel type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stainless</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$TL$ (mm)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

–, Term not included in the final parsimonious model ($p < 0.05$); Na, not applicable.
Figure 17: The predicted proportion of weight loss per day of the ejected and dissected, and the six hook treatment groups (a & b) mulloway, *Argyrosomus japonicus* (c & d) yellowfin bream, *Acanthopagrus australis* and (e & f) snapper *Pagrus auratus*. 
4.3.2 Fate of yellowfin bream

Yellowfin bream were monitored for 35 days across a mean (± s.e.) DO and water temperature of 6.66 ± 0.03 mg l⁻¹ and 17.79 ± 0.11°C. The 18 control and 108 treatment fish were similar in size (mean ± s.d. of 196.9 ± 20.6 vs. 223.5 ± 20.4 mm TL). None of the controls died, but the mortalities among the treatment groups ranged between 0 and 44%, and mostly occurred within seven days (Figure 15d-f). All treatment and control fish resumed feeding within a week of being placed in the experimental tanks and for fish that ejected hooks, it was not possible to determine whether the expulsion was via the mouth or anus.

The most parsimonious GLMM explaining the deaths was reduced to the significant fixed effects of nickel plating and hook ejection, with 39% of deaths being among fish that ingested nickel-plated hooks compared to only 17% for the other steel types combined ($p < 0.05$; Tables 8 and 9). There were no deaths among any fish ($n = 50$) that ejected their hooks, but 45% of the remaining fish ($n = 58$) that retained their hooks died ($p < 0.05$; Tables 8 and 9). For the surviving fish, hook ejection varied between 50 and 67% among the six treatment groups (Figure 16d-f). The most parsimonious GLMM included TL and nickel plating, but only the former was significant ($p < 0.05$; Tables 8 and 9). Irrespective of the hook type, there was a clear positive relationship between the size of fish and their rates of ejection (Table 9 and Figure 18).

![Figure 18](Image)

**Figure 18:** The logistic regression and predicted line value for the effect of total length (mm) on hook ejection by yellowfin bream, *Acanthopagrus australis*. 
There was no significant difference in the mean predicted rate of oxidation between hooks that were ejected and dissected from yellowfin bream (LMM; Wald $F = 0.014$, $p > 0.05$), but irrespective of the hook location there was a significant difference among the six different hook treatments (LMM; Wald $F = 45.39$, $p < 0.01$). Specifically, modified nickel-plated had oxidised twice as fast as their unmodified nickel counterparts and more than four times faster than their modified red-lacquer carbon steel counterparts, while no stainless-steel hooks oxidised (Figure 17b). There was no significant interaction between hook location and the hook treatment type (LMM; Wald $F = 1.33$, $p > 0.05$), nor was there any effect of hook location or $TL$ on the rate of hook oxidation (LMM; Wald $F = 0.01$ and 0.00, $p > 0.05$, respectively). In addition, during dissection of nickel-plated hook-ingested fish it was noted that several had increases in melanin pigmentation (blacking) around the portal areas of the liver.

There were significant differences in the concentrations of plasma glucose of yellowfin bream that were immediately sampled from the wild (mean ± s.e. of $2.25 ± 0.24$ mmol L$^{-1}$) and the holding tanks ($2.18 ± 0.20$ mmol L$^{-1}$) prior to the experiment and control ($1.24 ± 0.14$ mmol L$^{-1}$) and hook-ingested ($1.41 ± 0.18$ mmol L$^{-1}$) and -ejected ($1.33 ± 0.08$ mmol L$^{-1}$) fish at the end of the experiment (ANOVA $F_{4, 72} = 6.75$; $p < 0.01$). However, all glucose levels were within the range of baseline estimates.

4.3.3 Fate of snapper

Eighteen control (mean size ± s.d. of $237.4 ± 35.1$ mm $TL$) and 108 hook-ingested (mean size ± s.d. of $248.2 ± 39.8$ mm $TL$) fish were monitored for up to 41 days across a mean (± s.e.) DO and water temperature of $6.34 ± 0.07$ mg l$^{-1}$ and $20.48 ± 0.21$ºC. None of the controls died, while the mortalities among the treatment groups ranged between 11 and 56% (Figure 15g-i), and all occurred within 5 d (most occurred within the first 24 h). All treatment and control fish resumed feeding within a week of being placed in the experimental tanks.

The most parsimonious GLMM to explain these deaths included, hook ejection, $TL$, hook modification and bleeding; of which only the first three were significant ($p < 0.05$; Tables 8 and 9). Hook ejection had a strong impact, with only one fatality among 63 fish that ejected their hook, but 26 deaths (58%) from the remaining 45 hook-ingested individuals ($p < 0.001$; Tables 8 and 9). Mortalities were also significantly biased towards fish that ingested conventional rather than modified hooks (39 vs. 11%) and towards those that were larger (Table 9).
Hook ejection varied between 50 and 94% among the six treatment groups, with a mean (± s.e.) of 9.3 ± 1.0 days and was mostly explained by steel type and modification (parsimonious GLMM, \( p < 0.05 \); Figure 16g-i; Tables 8 and 9). Carbon-steel hooks were ejected faster than the stainless-steel designs (69 vs. 36%; \( p < 0.01 \); Figure 16g-i; Tables 8 and 9). Similarly, modified designs were ejected more frequently than the conventional designs (69 vs. 48%; \( p < 0.05 \); Figure 16g-i; Tables 8 and 9). In addition, it was not possible to determine whether the expulsion was via the mouth or anus.

The LMM detected significant differences in the mean predicted (back transformed) proportion of weight lost per day between ejected and dissected hooks (LMM; Wald \( F = 18.70, p < 0.01 \)), and irrespective of hook location among the six different hook treatments (LMM; Wald \( F = 11.36, p < 0.01 \); Figure 17c). Specifically, ejected hooks had a predicted proportion of weight lost per day that was \( \approx 3.5 \) times faster than those that were dissected (Figure 17c). Also, nickel-plated hooks oxidised faster than red-lacquer hooks, while only one stainless-steel hook exhibited minimal oxidation (Figure 17c). There was no significant interaction between hook location and the hook treatment type, or any effect of fish TL on the rate of hook oxidation (LMM; Wald \( F = 1.27 \) and 1.20, \( p > 0.05 \), respectively).

The concentrations of plasma glucose were significantly different among wild fish (mean ± s.e. of 1.02 ± 0.02 mmol L\(^{-1}\)) and those in the holding tanks (2.90 ± 0.09 mmol L\(^{-1}\)) at the start of the experiment, and control (2.50 ± 0.36 mmol L\(^{-1}\)), hook-ingested (2.77 ± 0.49 mmol L\(^{-1}\)) and ejected (2.37 ± 0.15 mmol L\(^{-1}\)) fish at the end of the experiments (\( F_{4,25} = 6.65; p < 0.01 \)). However, like for the other species, all levels of glucose were within baseline estimates.

4.4 Discussion

The total mortalities of mulloway, bream, and snapper (35, 24 and 25%) were within the ranges observed for line-cut hook-ingested conspecifics assessed in previous studies across mostly shorter periods (15 - 44%; Chapter 2; Broadhurst et al. 2007; Butcher et al. 2007; Grixti et al. 2010) and other species worldwide (reviewed by Hall et al. 2009). In addition, the mortality rates were also much lower than those observed for hook-ingested fish that had their hooks removed (42 - 100%; Chapter 2; Butcher et al. 2007; Grixti et al. 2010). The hook ejection rates for mulloway, bream, and snapper (30, 60 and 78%) were also comparable to
previously observed hook ejection rates over mostly shorter periods (3 - 56 d; Broadhurst et al. 2007; Butcher et al. 2007; Grixti et al. 2010). These results and the relatively short feeding cessation (within 1 week), combined with conformity among the mean plasma glucose between the controls and treatment groups at the conclusion of the experiment, provide further support for cutting the line on hook-ingested fish (Aalbers et al. 2004; Hall et al. 2009; Butcher et al. 2010).

While the mortality and hook ejection rates were comparable to the rates observed in other studies over mostly shorter time periods, there was however, an obvious difference in mortality and hook ejection rates for mulloway in comparison to snapper and yellowfin bream. Mulloway ejection rates were more than half of the other two species, and their mortality rate was 10% higher. These discrepancies between species are mainly attributed to the general physiology/morphology and longer monitoring period, respectively (Broadhurst et al. 2006a; McGrath et al. 2009). For example, previous research has suggested that there is a relationship between the rate of ejection and the morphology of the digestive tract (McGrath et al. 2009). This is also supported by the data for yellowfin bream in this study as there was positive correlation between fish TL and hook ejection; larger individuals more readily ejected their hooks than smaller fish (Figure 18). This is most likely due to the larger gut cavity size and the ability of the fish to move the hook within their digestive tract and subsequently eject their hook. However, the lower ejection and higher mortality rates for mulloway could also be attributed to inter-specific dietary preferences. For example, mulloway primarily feed on softer organisms, whereas snapper and yellowfin bream are accustomed to eating hard substances (Russell 1983; Kailola et al. 1993). However, despite the inter-specific differences among mortalities, the totals were still relatively low when compared to the mortality of other hook ingested species (reviewed by Hall et al. 2009).

There were a number of factors that significantly contributed towards mortalities, which included; nickel plating, fish TL, modification and hook ejection. Hook ejection was particularly important with all mortalities (except for one snapper) restricted to fish that retained their hooks. However, the majority of mortalities in yellowfin bream and snapper were relatively soon after hook ingestion so this could also be related to internal hooking location and damage. The damage from hooking was particularly important for snapper with larger fish being more likely to die once ingesting hooks. This positive relationship is probably due to larger fish being able to exerting more force when hooked and thus increasing the severity of internal damage during the capture and handling process (Fridman 1986).
Nickel plating and hook modification were also reoccurring factors that contributed to likeliness of mortalities. The latter factor significantly influenced mortality in snapper and mulloway, but the effects were divergent. Specifically, fewer snapper died that ingested modified than conventional hooks. It is difficult to determine the actual cause of these differences as there is minimal literature available on the effects of similar hook design modifications on snapper mortality. However, since all mortalities were within 5 days, it is hypothesised that there was a specific feeding interaction with modified hooks, that led to the hooks embedding differently in the digestive tract. Whereas, nearly twice as many mulloway died after ingesting modified compared to conventional hooks and it was attributed to hook oxidation (carbon steel hooks only).

The consensus is that oxidising ingested hooks have few negative effects on fish health (Aalbers et al. 2004; Broadhurst et al. 2007; Hall et al. 2009). However, the results from this study indicated that mortality was significantly affected by hook type. More specifically, 43 and 39% of mulloway and yellowfin bream that ingested nickel-plated hooks died, compared to only 31 and 17% of fish that ingested the remaining hook types, respectively. The nickel plating could have detrimental effects on fish health as nickel is classified as a heavy metal and is considered harmful if in concentrations over 0.02 mg/L\(^1\) in seawater (Roberts 2001; Berkowitz et al. 2008). With nickel composing \(\approx 2.5\%\) of nickel-plated hooks (Tables 6 and 7) it could be assumed that each hook would contain \(\approx 7\) mg of nickel. In addition, there are also several other elements in hooks that would be released as the hook oxidises (Co, Cr, CU, Fe and Mn; Table 7). Some of these are essential in trace levels to all organisms to aid specific catalytic functions, but most are only required in low concentration. However, if metal levels exceed the biological concentration required, they can cause severe damage and result in deactivation of important enzyme reactions, damage cellular structures and contributed to DNA modification (Berkowitz et al. 2008). But, DNA modification or the carcinogenesis capabilities of metals are generally due to continued and longer-term exposure (Ottolenghi et al. 1975; Kasprzak 1995).

There was some indication of the sub-lethal effects of ingested nickel-plated hooks in several of the yellowfin bream. For example, there were four individuals that had visible increases in melanin or changes in pigmentation in portal areas of the liver. Although none of these fish died, previous studies have attributed similar liver damage to pollution and increased metal ion absorption (Hartley et al. 1996). Further, changes in pigmentation have been associated with increases in melenomacrophage centres (MMC) within the liver which...
have been previously used as biomarkers for environmental degradation and pollution (Hartley et al. 1996; Manera et al. 2000; Stentiford et al. 2003). The above observation is possible evidence of the effects of the nickel-plated hooks, but the changes in MMC could also be associated with stress (Hartley et al. 1996; Rabitto et al. 2005). Further, there could be some other underling factors contributing to mortality that were not recorded in this study. For example, it is most likely that the nickel was a cumulative factor affecting mortality rather than being solely responsible for the increased mortalities. Excessive nickel levels in rats have been linked to reduced antibody and lymphocytes counts within blood and in turn lead to immunosuppression, which increase susceptibility to pathogens and disease (Mehrmofakham and Treagan 1981; Harkin et al. 2003).

However, while the above speculates the ingestion of nickel-plated hooks possibly being detrimental to the health and contributes to increased mortalities of yellowfin bream and mulloway, the use of nickel-plated hooks is also positive. For example, ejection of nickel-plated hooks from mulloway was four times greater than the ejection in the remaining two hook types. This high ejection rate is most likely owing to chemical properties of nickel-plated hooks and their susceptibility to oxidation. Therefore it would be beneficial to encourage recreational anglers to use hooks that are more susceptible to oxidation and thus promoting hook ejection.

Similarly, hook oxidation was also related to hook breakage (Chapter 3) and this significantly contributed to hook ejection in mulloway, with more mulloway ejecting broken than whole hooks. While it is obvious that hooks breaking into smaller pieces would facilitate faster ejections, it could have long-term effects on fish health as part of the hook may still remain in the digestive tract. For example, only 22% of the surviving mulloway ejected their broken hooks whole and the remaining fish retained part of their hook in the digestive tract until the end of the 61 day monitoring period. Broadhurst et al (2007) observed comparable trends for yellowfin bream with more than half of the ingested hooks breaking into smaller pieces and being ejected. The remaining piece of hook could then oxidise further within the digestive tract, and it may even pierce vital organs.

The above suggested that there is a relationship between hook oxidation and ejection, and this was further supported by the analysis of the predicted proportion of weight lost day$^{-1}$. The analysis identified hooks that were ejected by mulloway and snapper had significantly more oxidation loss per day than the hooks retained by fish. However, this was not the case with yellowfin bream, and this was mainly attributed to the
high ejection rate of stainless-steel hooks compared to the other two species. The variability in oxidation between the two hook materials was primarily attributed to their chemical composition.

Similar to above, the hook modification was another factor related to increased oxidation that significantly influenced hook ejection in snapper, with more fish ejecting modified than conventional hooks. However, the increased rate of oxidation in modified hooks is only likely in carbon steel hooks due to stainless steel hooks being more resilient to oxidation. In addition, steel type also significantly affected hook ejection in snapper with more fish ejecting carbon-steel than stainless-steel hooks. The increased oxidation rate for carbon steel hooks could contribute towards their higher ejection rate in snapper, as the increased oxidation evokes a foreign body response (Coleman et al. 1974; Welch et al. 2007). While the rate of stainless-steel hook ejection in snapper and mulloway was relatively low compared to the two remaining hook types (about a third) and had no effect on mortality in any of the species, they have the potential to negatively impact health of released hook-ingested fish. For example, stainless-steel hooks remain sharp, which could consequently, prevent feeding, cause irritation in the digestive tract and could eventually pierce vital organs. This could lead to internal infection, osmoregulatory dysfunction and subsequently increases the probability of death (Figure 19).

Figure 19: Infection caused by the irritation of a stainless-steel hook that could not be passed by a snapper.
The results from this study may be positive and have the potential to reduce the negative impacts that are associated with the catch-and-release of hook-ingested mulloway, yellowfin bream and snapper. However, they were also done under controlled conditions and therefore, the long-term post-release survival in the wild could be different. This is due to a range of other factors, including a greater susceptibility to disease and predation and/or a reduced ability to acquire food. Nevertheless, in the meantime, it would seem appropriate to use hooks with minimal oxidation resistance, due to them becoming weaker and are subsequently ejected more frequently than their more resilient counterpart (Chapter 3). However, nickel-plated hooks also resulted in slightly greater mortalities and this indicates that the metal composition of hooks could possibly contribute to further negative effects on the health of hook-ingested fish. Therefore, further research is required to assess whether the metal composition in hooks could result in long-term implications for fish health.

Acknowledgements

This study was funded by Industry and Investment NSW (I&I NSW), the NSW Recreational Fishing Trusts and University of New England, Armidale. Research was approved by the I&I NSW Animal Care and Ethics Authority (reference 05/02) and the University of New England Animal Research Authority (reference 07/165).
Chapter 5: Absorption of metals in mulloway (Argyrosomus japonicus) after ingesting nickel-plated carbon-steel hooks.

Publication

Aquatic toxicology (In prep)


This paper was a collaborative work by S. P. McGrath, A. J. Reichelt-Brushett and P. A. Butcher. Shane McGrath contributed 70% of the research design, 90% of the data analyses and 70% of the interpretation of the data.
Abstract

The absorption of metals by mulloway during the decay of ingested nickel-plated hooks was quantified in this study. Twenty-five treatment fish (obtained from a single aquaculture cohort) were allowed to ingest nickel-plated hooks and were monitored along with 25 controls (fish held in aquaria with no ingested hooks) for up to 42 days for hook ejection and mortality. Treatment fish that ejected their hooks were euthanised (along with control fish) before being removed from their tanks for blood, liver and muscle sample collection. The samples were prepared and analysed to determine the concentrations of six metals (Co, Cr, Cu, Fe, Mn and Ni). Metal concentrations in experimental mulloway were also compared with 14 fish sampled from the wild. The results showed that increased oxidation influenced hook ejection, and that hook-ingested mulloway had significantly elevated concentrations of nickel in their liver and blood samples, but not muscle. Specifically, mean concentration of nickel was > 7.7 and 18.7 times higher in the blood and liver samples of the treatment mulloway, respectively, when compared to the wild and control mulloway. Further, liver samples had excessively high concentrations of copper and nickel likely to cause severe health problems for humans that consume the liver. This research has shown that there is an avenue for metal absorption from ingested hooks; however, further research is required to determine if these concentrations have any sub-lethal effects.
5.1 Introduction

Mulloway are part of the Sciaenidae family; comprising >270 species worldwide, with 20 species found in Australian waters (Paxton and Eschmeyer 1994; Kuiter 2006; Froese and Pauly 2009). Mulloway are highly sought after by both commercial and recreational fishers for their eating qualities and sport by the latter group (Starling 1992; Scandol et al. 2008). In 2000/01, a national recreational fishing survey estimated that of the >0.6 million mulloway caught nearly half of these were subsequently released (Henry and Lyle 2003). This large release rate is most likely due to juvenile mulloway (below the LML) inhabiting coastal waters, where >85% of recreational fishing effort is exerted (Griffiths and Heemstra 1995; Griffiths 1996; Henry and Lyle 2003). Consequently, these high release rates resulted in concerns over their eventual fate, which has led to studies investigating their post-release fate (Broadhurst and Barker 2000; Butcher et al. 2007; Chapter 4).

In aquaria experiments Butcher et al. (2007), observed that mortalities among mulloway were primarily due to anatomical hook location. More specifically, hook ingestion resulted in more deaths (up to 16 times greater) than those recorded for mouth-hooked individuals. This research also showed that mortalities from hook ingestion were reduced from 87 to 8% when fish were released with their lines cut, rather than forcefully removing the ingested hook (Butcher et al. 2007). Increased mortality rates have also been recorded in other fish species that have had their ingested hooks removed after capture (Jordan and Woodward 1992; Aalbers et al. 2004; Butcher et al. 2006; Grixti et al. 2010; Chapter 2). These results have led to the recommendation that hook-ingested fish should be released with their lines cut instead of physically removing the hook (Broadhurst et al. 2007; Butcher et al. 2007; Grixti et al. 2010).

Many marine fish that have been observed to survive with ingested hooks do eventually eject them (Hall et al. 2009; Chapter 4), however, limited information is available on the actual mechanisms that contribute to the expulsion of the hook and the possibility that this process can be promoted by using specific hook designs and materials. It is most likely that the ability of some fish species to cope with ingested hooks is due to their morphology and feeding traits (e.g. Aalbers et al. 2004; Broadhurst et al. 2007; McGrath et al. 2009; Chapter 4). For example, the oesophagus in fish is very muscular
and can be contracted voluntarily, resulting in a higher capacity to regurgitate food than other vertebrates (Guillaume et al. 2001).

The general consensus from the few available studies is that hook removal is influenced by hook decay (Aalbers et al. 2004; Broadhurst et al. 2007; Grixti et al. 2008; Chapter 3 and 4). This is supported by the results in Chapter 4, which showed that ejected hooks oxidised more readily than retained hooks, and only one out of 133 fish that ejected their hook subsequently died. Therefore, an appropriate strategy to mitigate mortalities from hook ingestion could be through the promotion of hook ejection. This could include using hooks that have a low resistance to oxidation and incorporate areas that encourage corrosion.

The majority of long-term research on hook ingestion has mainly focused on obvious and specific factors that are likely to reduce mortality, rather than investigating the potential sub-lethal effects associated with angling and releasing fish (Aalbers et al. 2004; Broadhurst et al. 2007; McGrath et al. 2009; Grixti et al. 2010). For example, Broadhurst et al (2007) observed hook-ingested bream for up to 105 days and only recorded mortalities, hook ejections and stress levels (cortisol and glucose) at the conclusion of the experiment. Similarly, McGrath et al (2009) and Aalbers et al (2004) monitored hook-ingested fish for similar response variables for up to 21 and 90 days respectively. While the above hook-ingestion studies reported some oxidation of the ingested hooks, they only speculated that increased oxidation would be more beneficial for fish health without determining if there were any sub-lethal effects from the oxidising hook (Broadhurst et al. 2007; McGrath et al. 2009; Grixti et al. 2010).

Previous research has shown that the ingestion of nickel-plated hooks in yellowfin bream can lead to an increase in melanin pigmentation (or blackening) around the portal areas of the liver. While these abnormalities were not lethal, it was suggested that hook composition could be responsible for this observation (Chapter 4). Similar abnormalities in fish livers and higher mortality rates have also been associated with increased metal exposure in studies investigating the effects of environmental degradation and pollution on other teleosts (e.g. Hartley et al. 1996; Manera et al. 2000; Stentiford et al. 2003). Consistent with these findings, Chapter 4 observed more deaths among yellowfin bream
and mulloway that ingested nickel-plated hooks compared to those that ingested stainless-steel and red-lacquer coated carbon-steel hooks. However, this only suggests that the metals absorbed from the hooks may have an adverse effect on the overall health of the fish.

Most metals are essential to all organisms in trace levels, because they aid specific catalytic functions (Berkowitz et al. 2008). However, if levels exceed the requirements, they can adversely affect growth, reproduction, bioaccumulation, immune defence, ion balance, essential enzyme reactions, and cause DNA modification and/or liver degeneration (e.g. Larsson et al. 1985; Atchison et al. 1987; McLusky and Hagerman 1987; Mohamed and Saleh 1996; Yoshitomi et al. 1998; Berkowitz et al. 2008; Vinodhini and Narayanan 2008; Crafford and Avenant-Oldewage 2010). Some hooks contain potentially harmful metals (i.e. cadmium, chromium, copper, nickel) in the wire or anti-corrosive coating (Kasprzak 1995). Nickel is exceptionally harmful due to the redox biochemistry. The several oxidative states result in nickel being highly carcinogenic because of the increased chance of cell absorption (Kasprzak 1995). It is also theorised that metal absorption is higher in the digestive system due to the lower acidity of digestive fluids increasing the availability of free ions (e.g. McConchie 1988; MunillaMoran and SaboridoRey 1996). As some metals impair vital functions (such as nerve and muscle function, respiration, circulation, and hormonal regulation), increased metal absorption from hooks may cause irreversible damage to integral functions associated with reproduction, potentially resulting in death. This could cause large changes in aquatic fish populations and affect long-term sustainability (Larsson et al. 1985).

Saltwater accelerates the electro-chemical reaction that causes metals to corrode, thus hooks tend to incorporate anti-corrosion layers to limit corrosion (Shu et al. 1999). However, like the hooks themselves, these coatings often include potentially toxic metals (i.e. nickel, tin, zinc and cadmium). Therefore, it is imperative to establish if metals from anti-corrosive coatings have the potential to accumulate in the tissues of exposed fish, before coherent recommendations and mitigation strategies can be implemented. The present study sought to investigate the accumulation of metals in the blood, liver and muscle of mulloway following the ingestion of nickel-plated carbon-steel hooks for up to 42 days.
5.2 Materials and methods

The experiment was completed at the National Marine Science Centre (NMSC) Coffs Harbour, Australia, between November 2010 and January 2011. The facilities used included four 3000 L covered holding tanks located in an open air area, and fifty 110 L experimental plastic tanks arranged in an enclosed room with a regulated photoperiod (light-to-dark ratio of 12:12 h). All tanks were supplied with flow through seawater (2 L min\(^{-1}\)) and aerated using stone diffusers. The water quality parameters of salinity, dissolved oxygen and water temperature were monitored daily for the duration of the study.

5.2.1 Collection of fish

Four weeks before starting the experiment, 100 first generation mulloway (195−523 mm total length, \(TL\)) were purchased from a local aquaculture facility and transferred to four holding tanks (25 individuals tank\(^{-1}\)) at the NMSC. All fish were transported and handled according to the methods described by Butcher \textit{et al.} (2007) and fed commercially available 4 mm fish pellets at a rate of \(\approx 1\%\) biomass day\(^{-1}\) until two days before commencing the experiment.

5.2.2 Experimental procedure

On the first day of the experiment, twenty-five fish were anaesthetised and removed using knotless nets from two of the 3000 L tanks. These fish were measured and weighed, then individually placed (randomly) into small experimental tanks (110 L) in the enclosed aquaria room (termed ‘controls’). Twenty-five mulloway from the two remaining 3000 L were allowed to ingest hooks consisting of a J-hook design (nickel-plated carbon steel; absolute size of 250.4 mm\(^2\); see Chapter 3 - Figure 5) that were attached to 8 kg monofilament line, baited with school prawns (\textit{Metapenaeus macleayi}) and termed ‘treatments’. All hooks were modified following the description outlined in Chapter 3. Once fish ingested a single hook, they were retrieved (within 5 seconds) and removed via a knotless landing net (having their weight fully supported) from the tank. While being held with one hand, the line was cut approximately 5 cm from the mouth. The fish were then measured and weighed (as above) before being released into individual small 110 L experimental tanks. Three hook replicates were analysed for metal concentrations (see below).
The 50 treatment and control mulloway were fed pellets only (see above) and monitored daily over 42 days for any mortalities and hook ejections (treatment). Ejected hooks were removed from tanks, cleaned and weighed to determine oxidation rates. In addition, fish that ejected their hooks were euthanised (along with a replicate control fish) before being removed from their tanks and having blood and tissue samples taken. Blood samples (≈1 mL) were collected using heparinised syringes (22-gauge needles) via caudal vascular puncture (see Broadhurst et al. 2005). The tissue samples (muscle and liver) were taken during the dissection following the methods described by Brooks and Rumsey (1974). In addition, a pH measurement was taken from the digestive fluids in the stomach and intestines using a calibrated micro pH probe (3mm ø probe tip) by inserting the pH bulb through a small incision (5 mm) made in the stomach and intestinal lining. The blood, liver and muscle samples were analysed (see below) to determine the concentration of metals (see below).

Any dead fish were removed from the tanks and sampled along with their hooks in a similar way to the euthanised fish described above. Random temporal sampling of a subset of live hook-ingested and control mulloway was also undertaken 14 and 28 days after release. This included euthanising and sampling (as above) three mulloway from each of the control and treatment groups. Any remaining treatment and control fish at the conclusion of the experiment (42 days) were also euthanised and sampled as above. To determine changes in metal concentrations during the controlled experiment, baseline estimates were obtained by concurrently sampling mulloway from the local aquaculture facility (n = 3) and from the wild (n = 14; using conventional hook and line) near the NMSC.

5.2.3 Metal analyses

In addition to the blood, liver and muscle samples from each fish, four (2 g) samples of commercially available 4 mm fish pellets were also analysed for concentrations of metals. Samples were also taken from the ponds where the mulloway were harvested from and the experimental tanks at the NMSC. Specifically, three water (50 mL) and sediment (25 g) samples from each of the two ponds and three water (50 mL) samples from the experimental tanks were analysed for concentrations of metals.
Sediment, food, muscle, and liver samples were placed separately into petri dishes and dried in ovens at 80 °C for 48 hours. After drying, all samples were weighed and transferred into digestion flasks. Blood samples were left whole (as it provides a good index for metal exposure - see Sigel and Sigel 1988) and weighed before being transferred into flasks for digestion. For digestion of organic samples (food, blood, muscle and liver), hydrochloric acid (10 mL) was added to the flasks, while nitric acid (7.5 mL) and hydrochloric acid (2.5 mL) were added to flasks that contained inorganic samples (sediment and hooks). All the digestion flasks were then heated to 120 °C, and left until fully digested. Each of the homogenously digested samples was then decanted into a 50 mL flask and diluted with milli-Q water to exactly 25 mL, in reference to standard solutions. Water samples (25 mL) were filtered through 0.45 µm cellulose acetate and had approximately 2 mL of nitric acid added. Metal concentrations were measured using an inductively coupled plasma mass spectrometer (ELAN DRC-e ICPMS). All metal concentrations in the food, liver, muscle and inorganic samples are presented as mg kg\(^{-1}\) dry weight. Water and blood metal concentrations are presented as mg L\(^{-1}\).

Accuracy and validity of the ICPMS results was tested with a reference material (TORT 2, Lobster Hepatopancreas, National Research Council, Canada). The certified reference material values and the values for the present work for Co, Cr, Cu, Fe, Mn and Ni are displayed in Table 10.

**Table 10:** The percentage recovery for Cobalt, Chromium, Copper, Iron, Manganese and Nickel concentrations (mg kg\(^{-1}\)) in Lobster Hepatopancreas (TORT 2), (National Research Council, Canada), for the present work and the certified values.

<table>
<thead>
<tr>
<th></th>
<th>Co</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Ni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present work</td>
<td>0.55 ± 0.06</td>
<td>0.71 ± 0.06</td>
<td>93.0 ± 6.4</td>
<td>79 ± 8</td>
<td>12.30 ± 0.66</td>
<td>2.65 ± 0.16</td>
</tr>
<tr>
<td>Certified values</td>
<td>0.51 ± 0.09</td>
<td>0.77 ± 0.15</td>
<td>106.0 ± 10.0</td>
<td>105 ± 13</td>
<td>13.60 ± 1.20</td>
<td>2.50 ± 0.19</td>
</tr>
<tr>
<td>% recovery</td>
<td>108</td>
<td>92</td>
<td>88</td>
<td>75</td>
<td>90</td>
<td>106</td>
</tr>
</tbody>
</table>

5.2.4  **Data collected and statistical analyses**

Data comprising the date of release into the experimental tanks, treatment or control, initial TL (to the nearest mm) and weight (to the nearest 0.1 g), monitoring period, and daily mortality were recorded for all fish. The daily ejection of hooks was also noted for treatment fish. Any ejected and dissected hooks were cleaned and re-weighed to determine the percentage of mass lost due to corrosion. Water salinity (psu), temperature (°C) and dissolved oxygen (DO - mg L\(^{-1}\)) were monitored daily using a
water quality sensor (U-10 Horiba). Sizes (TL) of treatments, control and wild mulloway groups were compared using analysis of variance (ANOVA) to test for any differences in mean size among the three groups.

All data were analysed separately within each experiment using generalized linear mixed models (GLMMs) with a normal error term. Logit-link function was used to test for the independence of various continuous and categorical fixed effects and the random effect of experimental tanks (included in all models) on the concentrations of metals in mulloway blood, liver and muscle at their sampling times (Zuur et al. 2007). The fixed variables included in this analysis were: the continuous variables of fish TL (mm); stomach acid pH and intestinal fluid pH; the binary factor of food present in stomach; and the nominal factor of sample group (treatment, control and wild). All categorical (binary and nominal) effects were treated in a qualitative manner by converting them to dummy variables using reference cell coding (Quinn and Keough 2002). GLMMs were performed on each of the three sample types (blood, muscle and liver) and for each of the six metals (Ni, Fe, Cr, Mn, CU and Co) using the same variables as above, but with sample metal concentrations as the response variable. In the case where GLMMs revealed that the treatment group (hook ingestion) was significantly contributing to the differences in concentrations of metal in the samples, then a second group of GLMMs was run for the respective samples, excluding control and wild fish. The continuous variables of percentage of hook weight loss, the binary variables of hook breakage, hook ejection, mortality, and the organ the hook pierced or stomach wall were also included in the second group of GLMMs.

GLMMs were fitted using the lmer function in the lme4 package of the R statistical package, version 2.12.0 (R development Core Team 2009; Bates et al. 2011). In order to fit the GLMMs, a procedure similar to that outlined by Zuur et al (2007) was followed. This involved starting with what might be a “just beyond optimal” model that included all fixed components and the single random effect. Derivation of the optimal, most parsimonious model involved a stepwise selection process whereby redundant explanatory variables (fixed effects) were progressively deleted from the model. The most parsimonious model was identified by the lowest value for a penalised log-likelihood in the form of Akaike's Information Criterion (AIC = -2 x log-likelihood + 2[p + 1], where p is the number of
parameters in the model; Burnham and Anderson 2002; Johnson and Omland 2004). Where the difference between any particular model and the top ranked model (i.e. the one that resulted in the smallest AIC) was < 2, model adequacy was assessed using a likelihood ratio test (Zuur et al. 2007) and the significance of individual model coefficients assessed with the Wald statistic (Agresti 1996; Crawley 2005).

Variability in the rate of oxidation (defined as proportion of weight loss per day) of the ejected and dissected hooks were analysed using a GLMM. The fixed factor in the analyses was the hook location at the end of the experiment (either ejected or dissected from fish). The experimental tanks were included as a random effect and the $TL$ of fish was included as a co-variant. All percentage data were $\sin^{-1}(\sqrt{x})$ transformed.

5.3 Results

Daily readings of DO, temperature and salinity remained consistent over the 42 days with means ($\pm$ s.e.) of 7.99 ± 0.04 mg L$^{-1}$, 22.91 ± 0.11ºC and 35.1 ± 0.05, respectively. Twenty-five control (mean size ± s.d. of 314.44 ± 57.26 mm $TL$; Table 11), and 25 treatment (mean size ± s.d. of 312.52 ± 79.63 mm $TL$; Table 11) mulloway were used during the experiment, and a further 14 were sampled from the wild (mean size ± s.d. of 312.52 ± 79.63 mm $TL$; Table 11) to obtain baseline estimates. ANOVA failed to detect any significant differences in the mean $TL$ among the control, treatment and wild groups of sampled fish ($F_{2,61} = 0.01, p > 0.05$).

<table>
<thead>
<tr>
<th>Table 11: Ranges and means (± s.d.) for total length ($TL$ - mm), stomach (S) and intestinal (I) pH ranges of the treatment, control and wild fish used in the experiment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$.</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Wild</td>
</tr>
</tbody>
</table>

None of the control fish died during the experiment. Conversely, of the 25 hook-ingested fish that were released into the 110 L tanks, four died within two days of release (hook pierced vital organ in all four individuals; three in the heart and one in the liver), eight fish ejected their hooks between 4 and 41 days, and 13 were sampled with their hooks still ingested. Due to control and treatment fish
being removed for temporal sampling during the experiment, it was not possible to determine the actual hook ejection or mortality rates.

There were significant differences in the mean predicted proportion of weight loss per day between hooks that were ejected and those that were dissected (GLMM, \(p < 0.01\)). More specifically, ejected hooks had a predicted proportion of weight loss per day that was approximately 2.5 times greater than those that were dissected from fish (Figure 20a and b). There was no significant effect of fish TL on the rate of hook oxidation (GLMM, \(p > 0.05\)).

5.3.1 Metal analyses

Analyses of the metal content of the nickel-plated hooks revealed that iron and nickel were the primary constituents of the hooks (Table 12), with a mean (± s.d.) percentage composition of 61.63 ± 5.95% and 1.95 ± 0.23%, respectively. Other minor constituents included manganese (0.36 ± 0.02%), chromium (0.02 ± 0.00%), copper (0.02 ± 0.00%) and cobalt (0.01 ± 0.00%; Table 12).

Table 12: The percentage metal composition (mean ± s.d.) of the size 2 nickel-plated hooks (n = 3) used.

<table>
<thead>
<tr>
<th></th>
<th>Nickel-plated carbon-steel hook</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>61.63 ± 5.95</td>
</tr>
<tr>
<td>Ni</td>
<td>1.95 ± 0.23</td>
</tr>
<tr>
<td>Mn</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>Cr</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Cu</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Co</td>
<td>0.01 ± 0.00</td>
</tr>
</tbody>
</table>
Figure 20: Scatter plot of the temporal differences in the percentage of weight loss (a) and the mean predicted proportion of weight loss per day (b) of the ejected and dissected hooks from mulloway, *Argyrosomus japonicus*.

Analyses of the fish feed and pond sediment revealed iron and manganese to be the dominant metals in both samples (Table 13). The remaining four metals (Cu, Cr, Ni and Co) were found in lower concentrations in the food and pond sediment samples (Table 13). Analyses of the metal concentrations in water samples from the aquaculture ponds and the NMSC revealed very low levels of all tested metals (Table 13).
Table 13: Metal concentrations (± s.d.) in the aquaculture food (n = 4), pond water and sediment (n = 3), and seawater at the NMSC (n = 3).

<table>
<thead>
<tr>
<th></th>
<th>Food (mg kg⁻¹)</th>
<th>Pond sediment (mg kg⁻¹)</th>
<th>Pond seawater (mg L⁻¹)</th>
<th>Seawater (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>3.11 ± 0.34</td>
<td>1.12 ± 0.29</td>
<td>0.00 ± 0.00</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Fe</td>
<td>631.62 ± 38.63</td>
<td>1678.60 ± 643.01</td>
<td>&lt; 0.01*</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Cr</td>
<td>3.15 ± 0.35</td>
<td>2.91 ± 0.74</td>
<td>0.00 ± 0.00</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Cu</td>
<td>18.62 ± 2.23</td>
<td>2.91 ± 0.18</td>
<td>0.00 ± 0.00</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Mn</td>
<td>206.11 ± 18.39</td>
<td>10.53 ± 1.64</td>
<td>0.03 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Co</td>
<td>1.59 ± 0.17</td>
<td>0.51 ± 0.10</td>
<td>0.44 ± 0.08</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

* Below detection limits

5.3.1.1 Nickel

The most parsimonious GLMM explaining the variation among nickel concentrations in the blood was reduced to three fixed variables, including food present, TL and treatment (Table 14). However, only the latter was significant, with mean blood nickel concentration being > 7.7 times higher than the control and treatment groups (p < 0.001; Table 14; Figure 21a). GLMM of the nickel concentrations in the blood of treatment fish revealed that variability was significantly influenced by the hook piercing the stomach wall (p < 0.05; Table 15), whereby, mean concentrations of nickel in the blood was higher if the hook pierced the stomach wall (Table 16). The variation of nickel concentrations found in the liver was explained by treatment, TL, stomach pH and food present (Table 14). Treatment group was the only significant variable, with mean nickel concentrations in the liver being >18.7 times higher in treatment fish compared to the control and wild fish (p < 0.01; Table 14; Figure 21b). GLMM of the nickel concentrations in the liver of treatment fish revealed that the variability was significantly influenced by TL, intestinal pH and whether the hook pierced internal organs. All three variables were significant (p < 0.05; Table 15), but only the latter is discussed further because it was found to be highly significant (p < 0.001; Table 15). More specifically, the mean nickel concentrations in the liver were higher if the hook pierced the stomach wall (Table 16).

Analyses of the nickel concentrations in the muscle showed that the differences in samples were explained by the fixed variables treatment group, TL and food present. Only the latter two variables significantly influenced nickel concentration, with the amount of nickel declining in the
muscle with increasing TL, and lower concentrations in mulloway that had no food present in their stomach ($p < 0.01$; Table 17; Figure 22a).

5.3.1.2 Iron

The most parsimonious GLMM for the iron concentrations in the blood and muscle were reduced to four fixed variables. None of these variables were significant in explaining the variation in iron concentrations. However, concentrations of iron in the liver were explained by four fixed variables including TL, intestinal pH, control group, and stomach pH. Intestinal pH significantly influenced the concentrations of iron in the liver ($p < 0.05$; Table 14) and the latter two variables were highly significant ($p < 0.001$; Table 14; Figure 21c). Mean concentrations of iron in the liver of treatment and wild mulloway were >3.7 times higher than the control group and iron concentrations declined with increasing pH.

5.3.1.3 Chromium

The most parsimonious GLMM explaining the chromium concentrations in the blood was reduced to three fixed variables that included the TL, intestinal pH, and control group. The latter was the only significant fixed variable with the mean chromium concentration in the blood being > 1.9 lower in the control group when compared to the treatment and wild groups ($p < 0.05$; Tables 14; Figure 21d). The most parsimonious GLMM explaining the variation among chromium liver concentrations was reduced to three significant fixed variables that included wild mulloway, TL and intestinal pH. The former two variables were highly significant ($p < 0.001$; Tables 14; Figure 21e) with the mean chromium liver levels in wild mulloway > 3.5 times higher compared to the other two groups. Additionally, the amount of chromium in the liver declined with increasing TL. The concentration of chromium in the muscle was explained by the wild group, TL and presence of food in the stomach. However, only the wild group was significant with mean chromium concentrations in the muscle > 2.9 times higher than the treatment and control groups (Tables 14; Figure 21f).
Figure 21: The differences in mean (+ s.d.) metal concentrations in treatment, control and wild mulloway *Argyrosomus japonicus* samples for: (a) liver nickel, (b) blood nickel, (c) liver iron, (d) blood chromium, (e) liver chromium, (f) muscle chromium and (g) blood copper samples. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
Table 14: Summary of fixed variables tested in parsimonious generalized linear mixed models for their independence on the concentrations of metals in the blood (B), liver (L) and muscle (M) of treatment, control and wild mulloway over 42 days.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Nickel</th>
<th>Iron</th>
<th>Chromium</th>
<th>Copper</th>
<th>Manganese</th>
<th>Cobalt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>L</td>
<td>M</td>
<td>B</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td>Treatment</td>
<td>***</td>
<td>**</td>
<td>○</td>
<td>○</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>Wild</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TL</td>
<td>•</td>
<td>○</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>Stomach pH</td>
<td>-</td>
<td>○</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intestinal pH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Food present</td>
<td>•</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

○ p > 0.1; • p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; -, term not included in the final parsimonious model.

Table 15: Summary of fixed variables tested in parsimonious generalized linear mixed models for their independence on the concentrations of metals in the blood (B) and liver (L) of treatment mulloway.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Nickel</th>
<th>Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>L</td>
</tr>
<tr>
<td>Hook broken</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Hook ejected</td>
<td>•</td>
<td>-</td>
</tr>
<tr>
<td>Pierced stomach wall</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>Pierced organ</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>TL</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Intestinal pH</td>
<td>○</td>
<td>*</td>
</tr>
<tr>
<td>Hook weight</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

○ p > 0.1; • p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; -, term not included in the final parsimonious model.
Table 16: The significant interaction \( (p < 0.05) \) between pierced stomach wall and organ in relation to metal concentrations (mean ± s.d. in mg kg\(^{-1}\)) in the liver and blood of treatment mulloway.

<table>
<thead>
<tr>
<th></th>
<th>Nickel</th>
<th>Copper</th>
<th>Manganese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td>Liver</td>
<td>Muscle</td>
</tr>
<tr>
<td>Pierced stomach wall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.43 ± 0.46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No</td>
<td>0.30 ± 0.33</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- term not included in the final parsimonious model.

Table 17: The significant interaction \( (p < 0.05) \) between the presence of food in the stomach and metal concentrations (mean ± s.d. in mg kg\(^{-1}\)) in the liver and muscle of mulloway.

<table>
<thead>
<tr>
<th></th>
<th>Nickel</th>
<th>Copper</th>
<th>Manganese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td>Muscle</td>
</tr>
<tr>
<td>Food present</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.36 ± 0.33</td>
<td>30.61 ± 48.34</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>No</td>
<td>0.22 ± 0.15</td>
<td>118.33 ± 177.91</td>
<td>0.06 ± 0.13</td>
</tr>
</tbody>
</table>

5.3.1.4 Copper

The most parsimonious GLMM explaining the variation in copper concentration in the blood was reduced to three fixed variables that included treatment mulloway, \( TL \), and stomach pH. The former was the only significant fixed variable with mean copper concentration in the blood being >2.2 times higher in treatment mulloway than the control and wild groups \( (p < 0.05) \); Table 14; Figure 21g).

GLMM analyses of the copper concentrations in the blood of treatment fish only failed to reveal any significant fixed variables that accounted for the differences \( (p > 0.1) \); Table 15). However, variation of copper concentration in the liver was explained by the control group, intestinal pH, \( TL \), stomach pH, and presence of food. However, only the latter three variables were significant \( (p < 0.05) \); Table 14). Concentration of copper in the liver decreased with \( TL \) and lower stomach pH. Mean concentration of copper in the liver of mulloway that had food present in their stomach during dissection was >3.8 times lower than fish that had no food present in their stomach (Table 17). The most parsimonious GLMM for the copper concentrations in the muscle was reduced to three fixed.
variables. However, none of these variables were significant in explaining the variation in concentrations of copper between the groups ($p > 0.1$; Table 14).

![Figure 22](image)

**Figure 22**: Regression analyses of the relationship between TL (mm) and metal concentration in mulloway, *Argyrosomus japonicas* for (a) nickel muscle, (b) cobalt muscle, (c) manganese blood samples, and (d) stomach pH and manganese liver concentration.

5.3.1.5 Manganese

The most parsimonious GLMM explaining the variation of manganese concentrations in the blood was reduced to three fixed variables that included treatment mulloway, food present, and TL. However, the latter was the only significant fixed variable, with the amount of manganese in the blood decreasing with increasing TL ($p < 0.05$; Table 14; Figure 22b). Variation in manganese levels
in the liver was explained by three fixed variables that included TL, intestinal and stomach pH. However, only stomach pH was significant \((p < 0.01; \text{Table 14; Figure 22d})\), with the amount of manganese in the liver increasing with increasing acidity. The variation in manganese concentrations in the muscle were explained by the treatment mulloway group, intestinal pH, TL and presence of food. Only the latter two fixed variables were significant and the amount of manganese in the muscle increased with TL \((p < 0.05; \text{Table 14})\). However, the mean concentration of manganese in the muscle was lower in mulloway that had no food present in the stomach \((p < 0.05; \text{Table 17})\).

5.3.1.6 Cobalt

The most parsimonious GLMMs for the concentration of cobalt in the blood and liver were both reduced to three fixed variables. Yet none of these were significant in explaining the variation in concentrations of cobalt in either blood or liver samples \((p > 0.1; \text{Table 14})\). Conversely, the analyses of the concentration of cobalt in the muscle revealed that the variation was explained by three fixed variables that included the control mulloway group, food present and TL. The latter was the only significant factor with the amount of cobalt in the muscle increasing with TL \((p < 0.05; \text{Table 14; Figure 22c})\).

5.4 Discussion

Irrespective of group (control and treatment), the concentration of metals in the muscle samples were generally lower than the blood and liver samples and were within ranges previously reported for other marine teleosts (Brooks and Rumsey 1974; Plaskett and Potter 1979; Canli and Atli 2003; Carvalho et al. 2005; Sivaperumal et al. 2007). Similarly, the wild mulloway had accumulations of the tested metals in the tissue and blood that were within ranges previously observed in other wild species (Brooks and Rumsey 1974; Canli and Atli 2003; Schmitt et al. 2009). Even the high concentrations of chromium observed in the wild mulloway were within the ranges of other wild teleosts (Brooks and Rumsey 1974; Canli and Atli 2003; Schmitt et al. 2009). Conversely, concentrations of copper and iron in the livers of the control and treatment mulloway had higher variability than other marine teleosts (Brooks and Rumsey 1974; Canli and Atli 2003). Similarly, the nickel concentrations in the
blood and liver of treatment mulloway were beyond the upper limits recorded for other fish species (Brooks and Rumsey 1974; Canli and Atli 2003; Schmitt et al. 2009).

The results of this study support previous research (Chapter 4), which found that the rate of hook ejection was influenced by corrosion. Specifically, the present study demonstrated that the rate of hook oxidation was more than double for ejected hooks than hooks dissected from mulloway (Figure 20b). Similarly, Broadhurst et al. (2007) suggested that the rate of oxidation will most likely influence the rate of hook ejection, however, it was also theorised that increased oxidation rates may result in other negative health issues for fish. Further support for this theory is evident in Chapter 4, as nickel-plated hooks resulted in more deaths among hook-ingested mulloway. There are numerous reasons for these mortalities, but the two most likely scenarios are that the sharp edges of oxidised hooks cause internal damage to the fish or the metals released during hook decay could influence mortality. Metal analyses of the hooks showed that nickel was one of the primary constitutes of the hooks (Chapter 4). This is particularly important because nickel is considered harmful to fish if in concentrations over 0.02 mg L\(^{-1}\) in seawater (Roberts 2001; Berkowitz et al. 2008). Further, with the nickel-plated hooks containing 5-7 mg of nickel (Table 12 and Chapter 4), it is highly feasible that as a result of electro-chemical reactions nickel will be released and absorbed by the fish via the gastrointestinal tissue (Kasprzak 1995). While, on average, only a small percentage of the ingested hook oxidises, the anti-corrosive coating is primarily composed of nickel and therefore the probability of nickel ion absorption by the fish increases (Chapter 3 and 4). Nickel concentrations in water associated with fish kills are generally > 4.5 mg L\(^{-1}\), which is well below the estimated nickel exposure observed in this study (Noga 2010), however, the pathway of absorption and mode of actions is likely to be different.

In addition to nickel, there are also several other metals in the hooks that could potentially be harmful (Co, Cr, Cu, Fe and Mn; Table 12). While most metals present in the hooks are essential in trace levels to all organisms, they can have negative effects on fish health if the concentrations exceed the biological requirements (Berkowitz et al. 2008). The exposure level could not be standardised due
to the varying rates of oxidation and the amount of nickel present in the hooks; which probably contributed to the high variability observed in metal concentrations in the present study (Figures 20 and 23). However, other studies have observed similar high variability both within and among species that have had standardised metal exposure. In these studies variations have been partly attributable to the ability of different tissues to absorb and accumulate metals, collection depth, age, sex and metal tolerance (Bryan et al. 1977; Bryan and Hummerstone 1978; Jenkins 1980; Eisler 1981; Chau and Kulikovsky-Cordeiro 1995; Eisler 1998; Al-Yousuf et al. 2000; Wong et al. 2001). Variations in samples could also be explained by the ability of fish to regulate their internal metal concentrations, as excess metals can be expelled via excretion through the gills or digestive tract (Bryan 1976; Klaassen 1976; Carvalho et al. 2005; Crafford and Avenant-Oldewage 2010).

The results from this present study also suggest that there is an avenue for metal absorption from the decaying hook. In addition, the metal concentrations observed in the present study were similar to the levels observed in fish exposed to heavily polluted water (Abou-Hadeed et al. 2008; Vinodhini and Narayanan 2008). The high nickel concentrations in the liver could potentially be extremely hazardous to fish health. This is because these high nickel concentrations could induce biochemical changes, that could lead to severe liver damage, changes in metabolism, and result in excessive stress (Vinodhini and Narayanan 2008; Chapter 4). Other research has also shown that increased levels of nickel can cause immunosuppression and changes in the reproductive system (Mehrmofakham and Treagan 1981; Larsson et al. 1985; Nath and Kumar 1990; Abou-Hadeed et al. 2008). Further, these responses due to increased metal loading could inevitably result in death.
Figure 23: Schematic representation of metal concentrations (µg g⁻¹) in (a) blood samples and (b) edible muscle samples of the treatment (■), control (◆) and wild (▲) mulloway. The unbroken black lines (—) show the range of samples and the placement of the shape depict the mean, the broken grey lines (…) acceptable limits for human consumption (i.e. 10.0 and 1.0 µg g⁻¹ for copper and nickel, respectively) set by the Australian National Health and Medical Research Council.

Moreover, the above trends of increased nickel concentrations in the liver and blood of treatment fish was not evident in the muscle samples, but this could be due to the exposure time. Larsson et al (1985) and Abou-Hadeed et al (2008) suggested that nickel tended not to accumulate in the muscle of fish compared to other tissue. More specifically, Abou-Hadeed et al (2008) observed that concentrations of nickel in the muscle of Nile tilapia (*Oreochromis niloticus*) after sub-chronic exposure (3.6 mg L⁻¹) was more than double the concentrations evident in the control fish after 56
days. Thus the exposure period of 42 days could explain the discrepancy observed between the concentrations of nickel in muscle samples compared to the blood and liver samples.

The majority of significant relationships between metal concentrations and fish size were negative. The relationship between concentrations of Cr in the liver and TL had a highly significant \((p < 0.001)\) negative relationship. Similarly, Canli and Atli (2003) observed the same negative relationships between Cr and Cu concentrations in the liver of *Mugil cephalus* and *Scomberesox saurus*. A possible explanation for the observed relationship with fish size in the present study is due to the differences in metabolic activity. Previous research has suggested that metabolic activity is relative to age, with younger individuals accumulating metals more readily than older fish (Douben 1989; Elder and Collins 1991; Canli and Furness 1993; Nussey *et al.* 2000; Widianarko *et al.* 2000).

The increased concentrations of nickel in treatment fish was also correlated to hooks piercing the stomach wall and organs, suggesting that metal absorption is linked to exposure to the stomach and subsequent stomach environment. The acidity of the digestive fluids appeared to influence some metal concentrations in the liver, with the majority showing a positive relationship with decreasing pH. This is most likely due to the stomach acids that are present in the gastrointestinal track enhancing the solubility and absorption of metals (Schade *et al.* 1968; Kapoor *et al.* 1976; Whitehead *et al.* 1996).

Conversely, the concentrations of copper in the liver appeared to increase with an increase in pH and this was primarily attributed to copper becoming more available for absorption at lower acidities. Other studies have suggested that copper is actively regulated by fish, resulting in excess copper being expelled (Widianarko *et al.* 2000). The low digestive acidity recorded in some mulloway might be the result of reduced feeding activity, because starved teleosts have been shown to maintain low pH values for several months (Barrington 1957; Kapoor *et al.* 1976; Ho *et al.* 1999).
The concentrations of iron in the livers of the treatment and wild mulloway were significantly greater (> 3.7 times) than the control fish, suggesting that farmed fish may have been iron deficient and that treatment fish could be absorbing some of the iron released from the decaying ingested hook. However, the food contained > 630 mg kg\(^{-1}\) of iron (Table 13) which should have been a sufficient supply of iron for the fish to assimilate. This explanation is also supported by the concentrations of iron in blood, due to the large variability in the treatment fish compared to the control fish. Previous studies have also shown similar differences between farmed and wild marine teleosts. Specifically, wild fish had greater concentrations of iron than farmed individuals (Carpene et al. 1998; Alasalvar et al. 2002). Carpene et al (1998) suggested the increased concentration of iron could be attributable to greater exercise in wild fish compared to farmed individuals. Nevertheless, it is difficult to determine if the iron absorbed from oxidising hooks is actually beneficial, as excessive iron intake can cause the destruction of red blood cells (Hartley et al. 1996). However, there is little research to support the trends in blood metal (i.e. nickel) concentrations observed in the present investigation, as most aquatic toxicology studies focus on tissue bioaccumulation. Although the concentrations of metals in the blood are the initial sign of excessive metal consumption, once metals enter the lumen of fish they can then be absorbed into gastrointestinal tissue where the metal ions then bond with proteins in the blood. The metal ions can then be distributed to organs, such as the liver and kidney and then gradually into the muscle (Dallinger et al. 1987; Edwards et al. 2001).

Even though direct mortalities from hook ingestion were more apparent than from metal absorption, this research has shown that metal absorption from the decaying ingested hooks can occur. Metal absorption in this experiment was evident in the liver and blood of treatment mulloway and these findings further support the suggestion proposed in Chapter 4, that nickel from ingested nickel-plated hooks could be influencing mortalities. Further research is required to determine sub-lethal effects of excessive nickel concentrations on mulloway, as research with other teleosts observed changes in blood cell composition and severe impacts on reproduction (Larsson et al. 1985; Nath and Kumar 1990). For example, when adult giant gourami, *Colisa fasciata*, were exposed to high levels of nickel, testicular degeneration occurred in males and oocyte re-absorption occurred in the ovaries.
of females (Nath and Kumar 1990). Future research would also benefit from investigations that include hook designs that incorporate organic non-toxic coating and metals with low resistance to oxidation. Such changes could minimise negative health concerns for fauna that ingest them. While the above presumes that the use of nickel-plated hooks is more detrimental to fish health, their use is not completely negative, because they result in more hook ejections than hooks with a high resilience to corrosion.

Although only a few edible muscle samples had concentrations of trace elements (Figure 23) that were above the recommended metal levels considered safe for human consumption, there were numerous liver samples that had excessively high concentrations of metals likely to cause health problems for humans if they were consumed. Of the six elements investigated, the most concerning was concentrations of copper, which were observed up to 70 times higher in the liver than the recommended safe consumption level. In addition, concentrations of nickel were also above desirable levels in the liver (>1 mg kg⁻¹). However, normally the liver is not consumed by humans and thus minimal or no hazard would arise (Brusted et al. 2006). In summary, it is probably not advisable to consume internal organs, such as the liver and even the gonad because they can accumulate high concentrations of metals.

Acknowledgements

This study was funded by NSW Department of Primary Industries, the NSW Recreational Fishing Trust and Southern Cross University. Research was approved by the Southern Cross University Animal Ethics Committee Research Authority (permit number 10/37).
Chapter 6: Conclusions and future directions

6.1 Conclusions

The objective of this thesis was to identify and quantify the prevalence of factors known to cause mortality in snapper, mulloway and yellowfin bream caught by recreational anglers, and to suggest simple methods to help mitigate the factors contributing to mortality. Overall, hook ingestion and the retention of ingested hooks produced significantly greater mortalities than mouth hooking and fish that ejected hooks, respectively. These findings are in agreement with previous studies and suggest that the severity of damage induced by recreational angling has a traceable relationship with mortality.

Considerable effort has been undertaken to investigate the ways in which hook ingestion can be minimised, very little research has been conducted on hooks once they are ingested. In the short-term, hook ingestion can cause extensive internal injuries which are exacerbated by physically removing the hook. This can be prevented by simply cutting the line on hook-ingested fish.

In Chapter 2, two field experiments were completed to investigate the immediate and short-term mortality of angled snapper. Hook ingestion was found to result in more deaths when compared to mouth hooking, and the removal of ingested hooks exacerbated mortality rates. Results from experiment 2 showed that lure fishing is a possible avenue to reduce the occurrence of hook ingestion, as relatively few fish ingested their lures. More specifically, bait fishing resulted in a hook ingestion rate that was more than eight times higher when compared with the rate of hook ingestion for lure caught snapper. Nevertheless, despite all the evidence supporting lures as a means of reducing hook ingestion, fish will still inevitably ingest lures. Therefore, it is essential that further investigation is undertaken on hook designs that minimise mortalities once the hooks/lures are ingested.

Previous research suggested that hook ejection reduces the risk of mortality in hook-ingested fish and that ejection could be promoted through increased hook decay. But forcefully allowing a large number of fish to ingest hooks as part of a research program presents several logistical and
ethical issues. The use of appropriate indices of decay that could serve as proxies would be ideal, as it would alleviate the exposure of large numbers of fish to the deleterious process of hook ingestion. **Chapter 3** investigated the effectiveness of 23 different hook designs and their structural degradation after up to 28 days of exposure to seawater. Hooks made from materials with a low resistance to oxidation and wire diameters of < 0.9 mm were found to provide sufficient tensile strength to capture the majority of fish targeted by recreational anglers, whilst promoting rapid oxidation and subsequent breakage. The inclusions of barbs or notches on the shaft were also found to improve the rate of oxidation and reduce the residual strength of the hooks. These modifications acted as points of corrosion due to an increase in surface area exposed to seawater and the removal of the anti-corrosive coating (modified hooks).

While the tensile force measured prior to seawater exposure ($T_0$) may reflect the force imposed by a fish during capture, the data collected at days eight ($T_8$) and twenty-eight ($T_{28}$) are unlikely to represent the same forces imposed on hooks in the digestive-tracts of fish. Such differences mean that the results from **Chapter 3** should not be extrapolated beyond simple indices of degradation. Therefore, the plausibility of increased hook ejection enhancing survival needs to be further investigated with different fish species in order to obtain support for the above results.

In **Chapter 4** the accuracy of the oxidation indices or technical parameters in enhancing ejection rates was demonstrated. The results of this chapter revealed that hook ejection was the primary factor influencing mortality in all three species, with only one death recorded among fish that ejected their hooks compared to nearly half of the individuals that retained them. This research supports the use of hooks that are more susceptible to oxidation, as they become weaker and subsequently ejected more readily. However, ingested nickel-plated hooks caused more deaths among yellowfin bream and mulloway when compared with the red lacquer coated and stainless steel hooks. This result warrants further investigation into the consequence of releasing fish with ingested nickel-plated hooks and the specific need to quantify why these fish died (i.e. possibly the absorption of metals following the ingestion of hooks).
In Chapter 5, mulloway were allowed to ingest nickel-plated hooks as they had the lowest overall protracted hook ejection rate as demonstrated in Chapter 4. The ingestion of nickel-plated hooks resulted in elevated concentrations of nickel in the liver and blood compared to wild and control fish. Furthermore, muscle samples from hook-ingested fish used for human consumption had concentrations of nickel that were above the recommended daily intake for humans (Figure 23). Liver samples also had excessively high concentrations of some metals likely to cause severe health problems. More specifically, concentrations of copper were observed to be up to 70 times higher than levels considered safe for consumption. However, it is unlikely that a hazard would arise for either of these sample types as the majority of mulloway used in the experiment were below the legal minimum length (LML) and concentrations of copper and nickel appear to decline with increasing fish size. Nevertheless, it is not advisable to consume internal organs, such as the liver and gonads, as they can accumulate high concentrations of metals. The results of this study must be interpreted with caution as significant variations in metal concentrations were observed among individual mulloway. Further research on metal accumulation and depuration with lifespan would provide valuable information related to possible human health concerns.

While the results from these studies have the potential to help reduce negative impacts associated with the catch-and-release of hook-ingested mulloway, yellowfin bream and snapper, they were performed under controlled conditions. Therefore, the long-term post-release survival of these species in the wild could be different. This is due to a range of other factors including a greater susceptibility to disease and predation and/or a reduced ability to acquire food. As the stress from capture can suppress the immune system and the ingested hooks could physically reduce the ability of individuals to ingest food. In addition, further research is required to determine the sub-lethal effects of hook ingestion and whether increased metal concentrations from oxidising hooks have negative implications for fish health. Also re-assessment of hook materials may also be required, as the hooks currently used by recreational anglers contain various metals and materials that pose potential health problems for organisms that ingest them. For example, some of the metals contained in hooks can
result in a suppressed immune system. However, the use of these materials are generally used due to their availability, resilience to corrosion and lower associated construction costs. More expensive hook designs would have obvious economic impacts and the use of such hooks may be difficult to enforce. However, if recreational fishers collectively move toward the aforementioned sustainable and less detrimental tackle practices, the long-term benefits would be great as organic coatings would have minimal effects on the environment. Additionally, the impacts of hooks still imbedded in fish would be relatively short-term.

6.2 Future directions and management implications

Despite lure fishing providing lower hook-ingestion rates in comparison to bait fishing for snapper, there is anecdotal evidence to suggest that lure fishing could have adverse effects on the sustainability of this particular species (Chapter 2). Anglers have suggested that fishing with newly developed lures increases the ability of anglers to catch more and larger individuals compared to conventional bait fishing practices. This is of particular concern as larger individuals produce better quality and more offspring than their smaller counterparts. Further research is required to determine the effectiveness of different hook designs (i.e. circle hooks) and hook sizes to limit hook ingestion, as feeding mechanisms vary between species. For example, some fish like tarpon *Megalops atlanticus* attack their prey with an explosive strike that characterized by suction and ram feeding, which mostly results in the prey being deeply ingested immediately (Wainwright and Bellwood 2002). Therefore, it could be assumed that this would result in an increased probability of hook ingestion during angling. In the meantime, lures should be used rather than bait for catch-and-release fishing practices in order to minimise hook ingestion.

The results from Chapters 3, 4 and 5 demonstrated a significant relationship between hook ejection and decay. This suggests that decay is the primary factor influencing hook ejection, but other factors may also influence ejection rate, for example, hook ejection could also be influenced by a foreign body response in fish. Future research could benefit from investigating and developing an organic coating to evoke foreign body responses once the hook is embedded in the stomach lining.
For example, the development of vegetable oil or beeswax based coating that would dissolve with seawater exposure. Further, methods should also be developed to limit internal damage once the fish are hooked in order to improve survival. A simple way of achieving this could be by supporting the weight of the fish via a net when lifting them from the water, to prevent the full weight of the fish being exerted on the hook point.

Despite the best efforts of numerous research projects investigating ways to limit hook ingestion, there is still the premise that fish will continue to ingest hooks. Therefore, the next step would be to investigate and develop hook designs that degrade quickly once immersed in seawater, which would inevitably increase the rate of hook ejection and reduce fish mortalities. This could be achieved through the implementation of simple design modifications, for example, small notches on the shaft and bend of the hook. Theoretically, these notches should not affect hooking efficiency and capture rate, and should be readily accepted by anglers. While there was a significant relationship between hook composition and ejection rate, with hooks that had a lower resistance (nickel-plated) to oxidation being more readily ejected than other hooks, nickel-plated hooks also resulted in more mortalities. Despite being unable to clearly elicit that increased metal concentrations influenced mortality, the results showed that there was a significantly higher nickel concentration within the treatment fish compared to wild and control fish. Therefore, this study shows that metal ions released from the oxidizing hooks can be absorbed by fish and stored in various tissues. As many metals are more soluble at higher temperatures, further research may be needed to assess metal toxicity in fish in warmer waters (Roberts 2001).

The results of this thesis raise several questions for further investigation, such as: what are the short-term effects of increased concentrations of metals? and, is there any long-term effects of the absorbed metals on the overall health of hook-ingested fish? Ideally, chemical and cell composition analysis of the blood from hook-ingested fish would help to identify any negative effects on their immune or reproductive systems. Haematological techniques could also be used to reveal sub-lethal metal accumulations and the response of fish to toxicants, as metals bond with proteins in the blood.
and result in changes in chemical composition of the blood (Edwards et al. 2001). Haematological sampling approach will enable the development of coherent mitigation strategies that can be implemented as an alternative to invasive techniques which require the fish to be euthanized (Larsson et al. 1985). Thus promoting a more ethically acceptable practice of determining the effects of ingested hook would be using haematological analysis methods rather than more destructive sampling methods (i.e. liver, flesh and gill samples). Further, it is imperative that the best practices based on the evolving science of hook ingestion technology stipulated above are accepted by the wider recreational fishing community as it has implications and benefits for global fish stocks. However, this would entail educating and publicising the benefits of the improved practices to fisheries sustainability as a whole before it would be accepted by the broader recreational fishing community.
References


doi:10.1002/jbm.820080503


Edgar, G. J. (1997). 'Australian Marine Life: the Plants and Animals of Temperate Waters.' (Reed Books Melbourne.)


Grant, E. (2002). 'Grant’s guide to fishes ' 9th Edn. (Grant Pty limited: Queensland.)


generalized linear models of biometric data: A case study of two estuarine finfish from New
South Wales, Australia. *Fisheries Research* 90, 187-197. doi:10.1016/j.fishres.2007.10.007


Inhalation of studies of nickel sulphide in pulmonary carcinogenesis of rats. *Journal of the
National Cancer Institute* 54, 1165-1172.

*Hoplostethus atlanticus*. *New Zealand Journal of Marine and Freshwater Research* 21, 295-
299. doi:10.1080/00288330.1987.9516225

concentrations in the snapper, *Pagrus auratus*. *Australian Journal of Marine and Freshwater
Research* 43, 345-356.


Wales Press: Sydney.)

Effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry* 7, 253-258.
doi:10.1007/bf00004714

Plaskett, D., and Potter, I. C. (1979). Heavy-metal concentrations in the muscle-tissue of 12 species of
teleosts from Cockburn sound, Western Australia. *Australian Journal of Marine and
Freshwater Research* 30, 607-616.

(Gunther), in Moreton bay, Queensland as determined by tag recoveries. *Journal of Fish


Starling, S. (1992). The fisherman's handbook: how to find, identify and catch the top Australian angling fish.' (Sandpiper Press  Pennant Hills.)


Sumpton, W., and Jackson, S. (2005). The effects of incidental trawl capture of juvenile snapper 
(Pagrus auratus) on yield of a sub-tropical line fishery in Australia: an assessment examining 
habitat preference and early life history characteristics. Fisheries Research 71, 335-347. 
doi:10.1016/j.fisheries.2004.07.003

Swimmer, Y., Suter, J., Arauz, R., Bigelow, K., López, A., Zanela, I., Bolaños, A., Ballestero, J., 
doi:10.1007/s00227-010-1604-4


Trnski, T. (2002). Behaviour of settlement-stage larvae of fishes with an estuarine juvenile phase: in 
situ observations in a warm-temperate estuary. Marine Ecology-Progress Series 242, 205-214. 
doi:10.3354/meps242205

8675-17.3.807

from two Australian penaeid fishing gears. Diseases of Aquatic Organisms 76, 173-186. 
doi:10.3354/dao076173

fish Cyprinus earpio (Common carp). International Journal of Environmental Science and 
Technology 5, 179-182.

Coral reef fishes. Dynamics and diversity in a complex ecosystem. (Academic Press; Orlando, 
FL).

Wedemeyer, G., and McLeay, D. J. (1981). 'Methods for determining the tolerance of fishes to 


Appendix 1: Southern Cross University, animal research authority

Approval to conduct research ‘Survival of hook-ingested mulloway’ by Southern Cross University Animal Care and Ethics Committee. Authority number 10/37.
ANIMAL RESEARCH AUTHORITY

10/3/

Shane McGrath

has been authorised by
Southern Cross University Animal Care and Ethics Committee
to conduct the following research:

Physical Impacts of Terminal Fishing Rigs in NSW, Australia

in NSW

This authority remains in force until 29/10/2011
unless suspended, cancelled or surrendered.

Professor Bill Boyd
Chair, Animal Care and Ethics Committee
Appendix 2: NSW Department of Primary Industries, animal research authority

Approval to conduct research ‘Using recreational anglers to estimate and maximise the survival of released line-caught fish’ by NSW DPI Animal Care and Ethics Committee. ACEC reference 03/12-Coffs Harbour.
Appendix 3: University of New England, animal research authority

Approval to conduct research ‘Physical impacts of terminal fishing rigs in NSW, Australia’ by University of New England Animal Ethics Committee. Authority number 09/037.
THE UNIVERSITY OF NEW ENGLAND
ANIMAL ETHICS COMMITTEE

ANIMAL RESEARCH AUTHORITY
And Approval for Animal Experimentation

RESEARCH TEAM: A/Prof S Smith, A/Prof R Faulkner, Mr S McGrath, Mr C Brand, Dr M Broadhurst & Dr P Butcher

EMERGENCY CONTACT: 6548 3908
6548 3919

Are authorized to conduct the following research:

TITLE: Physical impacts of terminal fishing rigs in NSW, Australia

ANIMALS (number): Snapper, Yellow Fin bream & Mulloway (200, 200 & 100)

LOCATION(s): National Marine Science Centre

PROCEDURES: 6, 7 & 10

This authority remains in force from 06/03/2009 - 06/03/2010 unless suspended, cancelled or surrendered.

CONDITIONS: Nil

This statement must be read in conjunction with the Conditions for Animal Experimentation at UNE as stated on the reverse.

06/05/2009
Jo-Anne Sozeu
Secretary, UNE AEC
Appendix 4: University of New England, animal research authority

Approval to conduct research ‘Physical impacts of terminal fishing rigs in NSW, Australia’ by University of New England Animal Ethics Committee. Authority number 07/165.
THE UNIVERSITY OF NEW ENGLAND
ANIMAL ETHICS COMMITTEE

ANIMAL RESEARCH AUTHORITY
And Approval for Animal Experimentation

RESEARCH TEAM: Dr S Smith, Dr M Rula, Dr R Fulkner, Mr S McGrath, Mr C Brand, Dr M Broadhurst & Dr P Batteler

EMERGENCY CONTACT: 6588 3908

Are authorised to conduct the following research:

TITLE: Physical impacts of terminal fishing rigs in NSW, Australia

ANIMALS (number): Snapper, Yellowfin, Bream, Mackerel (400)

LOCATION(S): National Marine Science Centre

PROCEDURES: 1/7

This authority remains in force from 19/12/2007 - 19/12/2008 unless suspended, cancelled or amended.

CONDITIONS: Nil

This statement must be read in conjunction with the Conditions for Animal Experimentation at UNE as stated on the reverse.

19/12/2007

Jo-Anne Szou
Secretary, UNE AEC