Injury, physiological stress and mortality of recreationally discarded crustaceans in New South Wales, Australia

Jesse Carl Leland

Southern Cross University

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Injury, physiological stress and mortality of recreationally discarded crustaceans in New South Wales, Australia

JESSE C. LELAND
Bachelor of Applied Science (1st Class Honours)

A thesis submitted to the School of Environment, Science and Engineering, in fulfilment of the requirements for the degree of Doctor of Philosophy

SOUTHERN CROSS UNIVERSITY
OCTOBER 2014
ORIGINALITY DECLARATION AND CERTIFICATION

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Daniel J. Bucher (Principal supervisor)
ABSTRACT

In Australia, decapod crustaceans are trapped and hand collected by recreational fishers each year in large amounts, particularly freshwater crayfish (e.g. Euastacus spp.), portunid crabs and palinurid rock lobsters (including S. serrata, P. pelagicus and S. verreauxi). Recreational fishers are regulated by gear restrictions which, combined with input control restrictions, result in large proportions of their catches of these groups being discarded (i.e. from 40–70%). However, formal data supporting such mandatory discarding are lacking and the fate of recreationally discarded crustaceans is unknown. Similarly, information on the relative selectivities and efficiencies of common recreational traps are needed to justify substantial regulatory differences.

This thesis is presented as a series of published papers. The above issues were investigated by firstly identifying cost-effective and field-deployable crustacean haemolymph assessment methods that are useful for measuring the physiological responses (e.g. related to interactions with fishing gears) of exploited species and those of conservation concern (e.g. Euastacus spp.). Secondly, haemolymph indices were used in separate experiments to assess physiological stress and injury and discard mortality among recreationally discarded S. serrata, S. verreauxi and P. pelagicus and the immediate impacts to fish bycatch.

Scylla serrata and P. pelagicus were caught with commonly used traps ("round", "rectangular" and “wire” pots and “hoop nets”) deployed for 3, 6 or 24 h. Sagmariasus verreauxi were both trapped (with “rectangular” and “round” pots deployed for 24 h) and hand-collected by free divers. For all species, injury was quantified, before the discarded individuals and controls (i.e. previously caught animals) were placed into cages for mortality monitoring. Haemolymph samples were taken from S. serrata, S. verreauxi and P. pelagicus either immediately after capture or after some monitoring (i.e. 3–7 d). Growth was assessed for S. verreauxi only.
For both portunids, round pots caught significantly more crabs and catches were positively correlated with deployment duration. For *S. serrata*, hoop nets were the least efficient. Most trapped (64%) and hand-collected (79%) *S. verreauxi* were undersized. For *P. pelagicus*, undersized and ovigerous individuals comprised 22% and 4% of the total catch. Round traps targeting *S. serrata* and *P. pelagicus* caught significantly more fish bycatch. All fish bycatch from *P. pelagicus* and *S. verreauxi* traps survived, but some *Acanthopagrus australis* died in *S. serrata* traps (3%).

All species studied showed quantifiable physiological responses, but these were generally transitory. Injury was common for *Scylla serrata* (18% – biased towards hoop nets), while being relatively rare for *P. pelagicus* (5%) and trapped *S. verreauxi* (4%). Compared with trapping, *S. verreauxi* hand collection was frequently injurious (53%) and caught wider size and moult stage ranges. Most injured *S. verreauxi* regenerated some missing appendages, but their mean carapace lengths were less than those from intact conspecifics. No *S. serrata* and few *P. pelagicus* (1.1%) died and the only *S. verreauxi* mortalities (3.3%) occurred from within-trap predation by *Octopus tetricus*.

For all traps, simple approaches including installing escape gaps and/or increasing mesh sizes could reduce unwanted catches, while reducing their ghost fishing potential. Injury and the potentially lethal consequences (e.g. subsequent predation mortality) might be mitigated through careful handling and release techniques. Despite some deleterious capture-related effects the results indicate the potential for high survival and support mandatory discarding to help control the exploitation of recreational crab and lobster stocks.
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PUBLICATIONS INCORPORATED INTO THESIS

I, Jesse C. Leland, state that Chapters 2, 3, 4 and 5 in this thesis were peer-reviewed prior to publication in international scientific journals (see links for evidence). Further, I affirm that where necessary I have obtained permission from the copyright owners to use any third-party copyright material reproduced in this thesis and to use any of my own published work in which the copyright is held by another party.

Jesse C. Leland


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CONTRIBUTION STATEMENT

Chapter 1 – The general introduction was written by J.C. Leland (100%). It was edited to incorporate some of the comments and suggestions made by his supervisors.

Chapter 2 – J.C. Leland’s original conference presentation at the ‘Conservation of Freshwater Crayfish’ symposium in Sapporo, Japan (January 2011) formed the conceptual basis for this paper. The specific authorship percentage contributions are agreed to be: Leland, J.C. (60%), Furse, J.M. (40%).

Chapter 3 – J.C. Leland participated in all stages of this paper’s development and contributed approximately equally (overall) to the first author (P.A. Butcher). The specific authorship percentage contributions are agreed to be: Butcher, P.A. (40%), Leland, J.C. (40%), Broadhurst, M.K. (15%), Paterson, B.D. (2.5%), Mayer, D.G. (2.5%).

Chapter 4 – J.C. Leland participated during all stages of this paper’s development and provided an overall contribution greater than that of any co-author. The specific authorship percentage contributions are agreed to be: Leland, J.C. (60%), Butcher, P.A. (20%), Broadhurst, M.K. (15%), Paterson B.D. (2.5%), Mayer D.G. (2.5%).

Chapter 5 – J.C. Leland participated during all stages of this paper’s development and provided an overall contribution greater than that of any co-author. The specific authorship percentage contributions are agreed to be: Leland, J.C. (55%), Butcher, P.A. (22.5%), Broadhurst, M.K. (17.5%), Paterson, B.D. (2.5%), Mayer, D.G. (2.5%).

Chapter 6 – The general discussion was written by J.C. Leland (100%). It was edited to incorporate some of the comments and suggestions made by his supervisors.
Jesse C. Leland

Daniel J. Bucher
ADDITIONAL RELEVANT PUBLICATIONS

List of additional peer-reviewed publications by the candidate that are relevant to the thesis, but not included in it (see links for evidence).


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http://ci.nii.ac.jp/search?q=Leland%2C+Furse%2C+Coughran&range=0&count=&sortorder=&typ e=0


http://www.bioone.org/doi/abs/10.1163/193724012X633405
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CHAPTER ONE
General Introduction
Chapter 1: General Introduction

Declining catches among harvested marine species are a global problem, but wild catches of decapod crustaceans are increasing (FAO, 2010). In some locations, crustacean catches are increasing as demersal fish catches decline (FAO, 2005). The majority of the annual global harvest comprises relatively few high-value decapod groups including shrimp and prawns (sub-order Natantia) and marine crabs and lobsters (Reptantia) (FAO, 2010; Johnston et al., 2012).

In Australia, reptant decapods are economically valuable (~AUD$460 million annually) (ABS, 2010) and many species are also ecologically threatened, particularly freshwater clawed lobsters (infra-order Astacidea) (Coughran and Furse, 2010), with most fisheries close to their maximum sustainable production limits (Flood et al., 2012). Fully exploited stock yields can be maximised through increased resource use efficiency, including minimising unaccounted fishing mortality (ICES, 2005; Cooke and Cowx, 2004; Baker and Schindler, 2009).

Decapods are subject to both anthropogenic and natural stressors and indicators for quantifying sub-lethal physiological stress would be valuable for both fisheries and conservation management (Baker and Schindler, 2009; Coughran and Furse, 2010; Coughran, 2013). This thesis presents a series of published manuscripts, the first identifying and describing the importance of sub-lethal physiological stress indices for endangered freshwater crayfish management (Leland and Furse, 2012), with the remaining three applying such techniques to recreationally and commercially important portunid crabs (Butcher et al., 2012; Leland et al., 2013a) and a palinurid rock lobster (Leland et al., 2013b).

1.1 Recreational crustacean fisheries

In the past, most research and management paradigms have largely ignored the potential for deleterious effects from the recreational fishing sector in marine fisheries (Murphy and Kruse, 1995;
Cooke and Cowx, 2004). Factors that have contributed to this attention deficit include the difficulties associated with detecting recreationally induced collapses, monitoring deficiencies and complex fisher behaviour (Post et al., 2002; Cooke and Cowx, 2004). Similar to other developed countries, recreational fishing in Australia is rapidly increasing in popularity, with a national fishing survey in 2000/2001 reporting an estimated 3.36 million participants (i.e. >19% of the population) (Henry and Lyle, 2003; Cooke and Cowx, 2004). Associated fishing pressure will probably continue to increase with population growth. Recognition of these issues has prompted discard mortality studies for some popular Australian recreational fish species (e.g. McGrath et al., 2009, 2011), but similar studies for crustaceans are lacking.

Recreational discarding can be extremely high for many crab and lobster species both within Australia (Henry and Lyle, 2003) and overseas (Parsons and Eggleston, 2005). For example, in the Florida Keys an annual two-day “mini-season” occurs during which recreational divers targeting spiny lobsters (Panulirus argus) cause substantial appendage loss among local populations (i.e. a >27% increase in the number of injured individuals) (Parsons and Eggleston, 2005). Increasingly, managers and scientists are acknowledging the need to quantify recreational fishing effects (Hancock, 1995; Cooke and Cowx, 2004; Barber and Cobb, 2007).

Australian recreational fishers catch substantial numbers of crabs and lobsters; of which approximately half (>5 million individuals) are discarded owing to various input controls (Henry and Lyle, 2003) including minimum legal sizes and prohibitions on taking ovigerous females. The basic assumption of this management approach is high survival among discards, particularly for undersized and ovigerous individuals, which are imperative to sustain spawning biomass and recruitment (King, 2007).

Within Australia there is substantial interstate variation in input control regulations. For example, fishers targeting blue swimmer crabs (Portunus pelagicus) in New South Wales (NSW) can use
entanglement devices that are illegal in other states (Table 1 – information sourced from the respective state fisheries websites). Ideally, input controls should minimise catches of illegal or undesirable individuals; thereby mitigating the potential for deleterious capture-related effects (Cochrane, 2002). However, there are no definitive studies on the basic relative selectivities and efficiencies of Australia’s legal recreational gears (or collection methods) to justify the differences in regulation including those for trap possession limits (Table 1).

1.2 Unaccounted fishing mortality

Traditional fishing mortality models did not consider the potential for unaccounted fatalities (Ricker, 1976); yet increasing attention is being directed towards investigating this issue (ICES, 2005; Baker and Schindler, 2009; Uhlmann and Broadhurst, 2013). For some exploited species, unaccounted fishing mortality contributes substantially to their total fishing mortality, thus precise estimation is required for effective stock management (ICES, 2005; Broadhurst et al., 2006a; Baker and Schindler, 2009). Like for other fishing methods, unaccounted fishing mortality in recreational crustacean fisheries can be categorised into mortality sub-components resulting from: (i) intentional (i.e. avoiding and escaping) or unintentional (i.e. dropping out) exclusion from catches, (ii) onboard discarding, (iii) ghost fishing (i.e. by derelict traps), (iv) habitat destruction, (v) related predation or infection or vi) depredation (ICES, 2005; Broadhurst et al., 2006a; Uhlmann and Broadhurst, 2013).

Some studies have focused on highly visible (i.e. obvious) and relatively large volume vessel-based discarding of bycatch. Perhaps this is because such estimates can be the simplest to quantify and are often assumed to account for large proportions of unaccounted deaths (Uhlmann and Broadhurst, 2013), although growing awareness and concern over sustainability and welfare issues have also played some role (Bartholomew and Bohnsack, 2005; Arlinghaus et al., 2007). The potential for unaccounted fishing mortality, however, is often overlooked (Baker and Schindler, 2009;

<table>
<thead>
<tr>
<th>State</th>
<th>Nets</th>
<th>Type</th>
<th>Min. SMO (mm)</th>
<th>Max. size (m)</th>
<th>Pots</th>
<th>Min. SMO (mm)</th>
<th>Max. size (m)</th>
<th>Possession max. n</th>
<th>MLS (mm)</th>
<th>Quotas</th>
</tr>
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<tbody>
<tr>
<td>NSW</td>
<td>Hoop and drop</td>
<td>13</td>
<td>1.25 × 1.0</td>
<td>50</td>
<td>1.2 × 1.0 × 0.5 or 1.6 ø</td>
<td>5 nets and 1 pot</td>
<td>~131(60)</td>
<td>~131(60)a</td>
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<td>NT</td>
<td>Drop only</td>
<td>15</td>
<td>1.0 × 1.0 × 1.0</td>
<td>nr</td>
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<td>5b (combined)</td>
<td>None</td>
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<td>WA</td>
<td>Drop only</td>
<td>nr</td>
<td>1.5 × 1.5</td>
<td>Illegal</td>
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<td>10</td>
<td>127</td>
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<td>SA</td>
<td>Drop and lift</td>
<td>nr</td>
<td>1.07 × 0.92</td>
<td>Illegal</td>
<td>Illegal</td>
<td>10 or 6 (combined)</td>
<td>110</td>
<td>110a</td>
<td>40f</td>
<td></td>
</tr>
<tr>
<td>QLD</td>
<td>Drop only</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>4 (combined)</td>
<td>115</td>
<td>Illegal</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

CL — the distance from the frontal notch to the posterior margin of the carapace. The CW definition varies between states, WA fishers measure at the widest point (i.e. including the spines), but those in SA and QLD exclude the spines (i.e. measuring to the spine base). All states use CW for MLS except NSW (CL in parentheses). SA quotas can comprise combined catches of *P. pelagicus* and sand crab (*Ovalipes australiensis*). Scoop nets and hand collection are legal in all states. Hand-held wire hooks and crab rakes are permitted in WA and SA, respectively. ‘Hoop’ nets are entanglement devices. ‘Drop’ and ‘lift’ nets are actively fished containment traps.

a non-ovigerous *P. pelagicus* only
b boat limit of 10 when two or more fishers are present
c *P. pelagicus* quotas vary across regions
d boat quota per day of 20 or 40 *P. pelagicus* when two or more fishers are present
e boat quota per day of 120 *P. pelagicus*
Uhlmann and Broadhurst, 2013) and few studies have investigated the relationship between fishing-related injury and delayed mortality among recreationally discarded crustaceans (but see Powrie and Tempero, 2009; Frisch and Hobbs, 2011).

Previous studies have demonstrated considerably different discard mortality estimates for Australian crustaceans discarded from commercial fishing gear (e.g. treatment specific fatalities of 0–100%) (Wassenberg and Hill, 1989, 1993; Hill and Wassenberg, 1990; Kennelly et al., 1990; Uhlmann et al., 2009). However, a broad correlation exists between the extent of injury and mortality for many species (e.g. Kennelly et al., 1990; Uhlmann et al., 2009). Further, Powrie and Tempero (2009) and Frisch and Hobbs (2011) reported that hand collection of lobster (*Jasus edwardsii* and *Panulirus versicolour*, respectively) by recreational SCUBA divers caused substantial injuries to discards. However, the differences in collection methodologies (e.g. use of SCUBA and hand-held devices and gloves) in these studies reduces their relevance to recreational fishing practices in most Australian states.

1.3 Injury and physiological stress

In nearly all fisheries, certain individuals are discarded (Kirkwood and Brown, 1998; Cochrane, 2002; King, 2007). Information on the best practise catching and handling methods are essential to help mitigate sub-lethal stress and damage from these events (Bergmann et al., 2001; Ridgeway et al., 2006; Barber and Cobb, 2007). Of primary concern for crustaceans is their propensity to autotomise limbs (along pre-existing fracture planes) when threatened or physically damaged (Norman and Jones, 1991; Juanes and Smith, 1995; Wasson et al., 2002). Although appendage sacrifice is beneficial in terms of wound avoidance and escape, the immediate, short- and long-term effects vary with the type and number of limbs lost (Juanes and Smith, 1995; McGaw, 2006; Patterson et al., 2007; Uhlmann et al., 2009). Alterations in prey choice (ap Rheinallt and Hughes, 1985), growth and competitiveness (Juanes and Smith, 1995) are commonly cited deleterious effects of autotomy. Commercially discarded decapods with missing appendages often show increased short-term mortality (Kirkwood
and Brown, 1998; Barber and Cobb, 2007; Uhlmann et al., 2009) and limb loss can increase the possibility of infection (Barber and Cobb, 2007).

Injury and physiological stress during the discarding process can cause quantifiable alterations to crustacean haemolymph constituents (Jussila et al., 1997; Yildiz and Atar, 2002; Malev et al., 2010). Crustaceans have three different haemocyte types, which are generally identified as hyalinocytes, semi-granulocytes and granulocytes (Söderhäll and Smith, 1983; Johansson et al., 2000). Semi-granulocytes and granulocytes are vital to critical immune responses including phagocytosis and bacteria encapsulation (Hose et al., 1990; Clare and Lumb, 1994; Johansson et al., 2000). Quantifying the relative abundances of differing cell types can indicate immune potential (Jussila et al., 1997) and sub-lethal stress related to particular events (Poole et al., 2008; Malev et al., 2010).

Physiological stress can be also be quantified by changes in other haemolymph constituents including ionic compounds, glucose, lactate and total protein levels (Jussila et al., 1997; Paterson and Spanoghe, 1997; Bergmann et al., 2001; Yildiz and Atar, 2002; Ridgeway et al., 2006; Malev et al., 2010). In commercial fisheries, particular attention often focuses on changes in glucose and lactate concentrations, which can increase proportional to limb loss (Patterson et al., 2007), handling and emersion stress (Bergmann et al., 2001; Poole et al., 2008). Another useful indicator is haemolymph clotting capacity, with increased clotting times generally indicating amplified stress (Jussila et al., 1997; Uhlmann et al., 2009). However, the utility of haemolymph indices can be strongly influenced by other factors including environmental conditions (Johnson, 1980), sex (Sequeria et al., 1995) and moult stage (Bauchau, 1981).

1.4 Animals studied

1.4.1 Freshwater crayfish

Australia’s >130 native freshwater crayfish species (Coughran and Furse, 2010) comprise some common widely-distributed taxa (e.g. including some Cherax species – Kailola et al., 1993) and
others that are relatively rare with restricted ranges (e.g. *Euastacus bindal* – Furse et al., 2012). The genus *Euastacus* comprises 49 species (37% of all Australian freshwater crayfish species), of which 80% are threatened due to combinations of natural characteristics (e.g. slow growth and low fecundity) and anthropogenic pressures (e.g. overfishing, climate change and habitat fragmentation) (Coughran and Furse, 2010). Despite this, some large *Euastacus* species are popular among recreational fishers for consumption (e.g. the Murray crayfish, *E. armatus*, Henry and Lyle, 2003), while others are prized by aquarium enthusiasts for their morphological characteristics (e.g. spines and colourations) and long lifespan (e.g. >25 years for *E. sulcatus*) (Coughran, 2013).

Coughran and Furse (2010) reported recreational fishing as a threat to large *Euastacus* species, noting that the broad taxonomic groupings like “spiny crayfish” in fishing regulations can focus fishing pressure on particular species that reach the minimum legal size (i.e. *E. armatus*, *E. sulcatus*, *E. spinifer* and *E. valentulus*). The definitive work on recreational harvesting of Australian freshwater crayfish only reported catches for one broad group (freshwater crayfish – *Cherax* spp.) and a single species, *E. armatus* (Henry and Lyle, 2003). Most of the annual harvest (~7.5 million individuals) comprises *Cherax* species (Henry and Lyle, 2003). However, a considerable proportion (6%) consists of the rarer *E. armatus* (listed as data deficient – Coughran and Furse, 2010), of which approximately 70% (or 387,402 individuals) was discarded (Henry and Lyle, 2003).

1.4.2 *Scylla serrata*

Four portunid crabs of the genus *Scylla* (i.e. *S. tranquebarica*, *S. paramamosain*, *S. olivacea* and *S. serrata* – mud crabs) are distributed throughout estuarine and mangrove systems of the Indo-West Pacific (Kailola et al., 1993; Keenan et al., 1998; Gopurenko et al., 1999). In Australia, small quantities of *S. olivacea* are caught along the northern parts of the fishery (i.e. north Queensland), but the primary target species is the giant mud crab (*S. serrata*). *Scylla serrata* is distributed from the central coast of NSW, north through Qld, the Northern Territory and west to Exmouth Gulf, Western Australia (Kailola et al., 1993). These portunids can attain large carapace widths (up to 28 cm) and
weights (up to 3 kg). At maturity their large chelae comprise a substantial amount of their biomass (Kailola et al., 1993). Due to these characteristics *S. serrata* are commercially valuable (up to ~AU$44 kg⁻¹ – in season fisher price) and are highly prized by commercial and recreational fishers (Kailola et al., 1993; Henry and Lyle, 2003).

1.4.3 *Sagmariasus verreauxi*

The eastern rock lobster (*Sagmariasus verreauxi*; previously *Jasus verreauxi*) inhabits coastal reefs (<10–200 m depth) south from Tweed Heads, NSW to Port MacDonnell, South Australia (Montgomery, 1992; Kailola et al., 1993). Like other palinurids, *S. verreauxi* often attain large carapace lengths (>26 cm CL) and weights (up to >8 kg) and are a popular seafood species (Kailola et al., 1993). Their iconic status and desirable eating qualities make *S. verreauxi* highly sought after and in NSW, large quantities are harvested by recreational (~26 metric tonnes; Andrew et al., 1997) and commercial fishers (~130 metric tonnes; valued at AUD$ 7.8 million annually; G. Liggins pers. com.).

1.4.4 *Portunus pelagicus*

*Portunus pelagicus* is widely distributed throughout estuarine and coastal waters of the Indo-West Pacific (Kailola et al., 1993; Lai et al., 2010). Lai et al. (2010) revised *P. pelagicus* into four species (including *P. armatus*, *P. reticulatus* and *P. segnis*) based on combinations of genetic, morphological and morphometric characteristics. However, because Lai et al. (2010) was not conclusive (i.e. did not assess reproductive compatibility), and to maintain consistency with previous literature, in this thesis I report on *P. pelagicus* as per the original description. *Portunus pelagicus* occurs throughout all mainland Australian states (Kailola et al., 1993), where they are targeted by recreational and commercial fishers (Henry and Lyle, 2003; Johnston et al., 2012).
1.5 Capture methods and discarding

Crustaceans are generally opportunistic omnivores with varied diets that include bivalves, polychaetes, teleosts, other crustaceans (including conspecifics) and plant material (Hill, 1979; Williams 1982; Holdich and Lowery, 1988; de Lestang, 2000). Throughout Australian waters, recreational fishers exploit the predictable gustatory responses of freshwater and marine crustaceans to baited gears and most of the annual harvest is trapped, with the remainder caught using other methods (e.g. by hand – Table 2) (Henry and Lyle, 2003). For *S. serrata* and *P. pelagicus* the hand-caught harvest proportions are relatively low. However, approximately one third of the annual *S. verreauxi* catch is hand collected by recreational free divers (Table 2) (Henry and Lyle, 2003). Neither method is 100% selective, and substantial proportions of the total *S. serrata*, *S. verreauxi* and *P. pelagicus* annual catches are discarded (Table 2).

Table 2. Total annual catches and proportions of Australian mud crab, palinurid rock lobsters and blue swimmer crab that are recreationally trapped or hand caught, before being discarded (modified from Henry and Lyle, 2003).

<table>
<thead>
<tr>
<th>Species</th>
<th>Total catch (millions)</th>
<th>Trapped (%)</th>
<th>Hand caught (%)</th>
<th>Discarded (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mud crab (mostly <em>Scylla serrata</em>)</td>
<td>2.6</td>
<td>92</td>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>Lobster (including <em>Sagmariasus verreauxi</em>)</td>
<td>1.3</td>
<td>67</td>
<td>32</td>
<td>49</td>
</tr>
<tr>
<td>Blue swimmer crab (<em>Portunus pelagicus</em>)</td>
<td>6.6</td>
<td>78</td>
<td>1</td>
<td>40</td>
</tr>
</tbody>
</table>

Discarding in trap fisheries is related to gear selectivity and efficiency, which influence the catch composition in terms of species, size and number (Cochrane, 2002). Regulation by input controls is one option to minimise the unwanted catches of both target and non-target species (Cochrane, 2002; King, 2007), but detailed studies on the relative selectivities and efficiencies of Australian recreational traps are lacking. Bellchambers and de Lestang (2005) assessed selectivity for commercial *P. pelagicus* traps and reported high selectivity biased towards large males, but their findings are not directly relevant to recreational situations, because of substantial differences in terms of gear construction and deployment variables (e.g. duration and multiple-trap configurations).
1.6 Thesis aims, purpose and format

This thesis was undertaken in response to a lack of information on the: i) injury, physiological stress and short-term fate of recreationally discarded crustaceans, ii) relative selectivity and efficiency of common baited traps (and collection methods), and iii) immediate impacts to non-target discards in Australia. The overall thesis purpose was to assess the efficacy of current approaches to controlling stock exploitation and to identify simple ways to mitigate discarding and mortality, while reducing injury and minimising the potential for unwanted indirect effects. Chapter 2 comprises an overview of existing field deployable and cost-effective methods for determining physiological stress among endangered freshwater crayfish. Based on this work, a suite of haemolymph parameters was chosen as physiological stress indicators for the subsequent Chapters, which investigated aims i–iii above for three key recreational Australian species: 1) Scylla serrata (Chapter 3), 2) Sagmariasus verreauxi (Chapter 4) and 3) Portunus pelagicus (Chapter 5). Chapter 6 provides an integrated discussion of the individual chapters and presents the overall study conclusions and directions for further research.

Chapters 2–5 were peer reviewed and published as individual research articles, therefore some repetition was unavoidable (e.g. in the methodology). Throughout the thesis, small variations occur (e.g. in terminology – the use of soak time vs. deployment time), because of differing editorial preferences for publication. To make it clear, each published chapter begins with a ‘Prelude’ that gives the full citation and chapter purpose.
Chapter 2 – Prelude

Chapter 2 has been peer-reviewed and published in the international journal *Crustacean Research* (full citation details below). Chapter 2 is presented as it was published, except for where: i) small changes were required to maintain thesis coherence (e.g. in the Figure numbering) and ii) other minor textural changes were made to satisfy examiners comments.

The purpose of this Chapter was to provide an overview of decapod stress assessment methods from the primary literature, with particular attention to portable low-cost methods. This information was presented in a novel context (i.e. application to freshwater crayfish conservation) and the preferred methods identified were subsequently used to quantify physiological changes in recreationally exploited marine decapods (in Chapters 3, 4 and 5).

Please cite Chapter 2 as:

CHAPTER TWO

Haemolymph analysis
Chapter 2: Potential utility of haemolymph analysis in non-lethal conservation studies on threatened Australasian freshwater crayfish: portability and practicality

J.C. Leland\textsuperscript{1,2}, J.M. Furse\textsuperscript{2}

\textsuperscript{1} Marine Ecology Research Centre, School of Environmental Science and Management, Southern Cross University, Lismore, NSW 2480, Australia
\textsuperscript{2} Environmental Futures Centre and the Griffith School of Environment, Griffith University, Gold Coast campus, Queensland 4222, Australia.

ABSTRACT

Straightforward and inexpensive analysis of blood constituents can provide quantifiable information on sub-lethal stress in an animal and a measure of their overall physical fitness. Such methods have been widely used on a range of marine and terrestrial species, primarily those of commercial or recreational importance. Freshwater crayfish in many regions of the world face a common suite of threats and threatening processes that include: exotic species (including other freshwater crayfish, associated diseases and parasites), habitat fragmentation and destruction, anthropogenic pollution, overexploitation and increased environmental temperature. Although some studies have investigated the effects of these on freshwater crayfish in-part (i.e. measured by gross symptoms), the subtle, often asymptomatic physiological effects are poorly understood. The analysis of haemolymph provides a simple, inexpensive, high resolution, portable (i.e. suitable for field analysis and assessment in remote areas) and non-lethal method for the evaluation of sub-lethal stress and immunocompetence status in freshwater crayfish. There is considerable scope for application of these existing techniques in conservation initiatives for rare and endangered freshwater crayfish in Australasia, in particular by providing: i) non-lethal stress assessments, ii) quantification of compromised health and iii) increased understanding of the physiological impacts from key threats and threatening processes.
2.1 Introduction

Techniques for evaluating the stress-effects of threats and threatening processes on freshwater crayfish either do not exist, or those that are available only provide relatively coarse resolution data (i.e. usually simple mortality or survival). Typically, such techniques are laboratory based and also require the sacrifice of substantial numbers of animals (e.g. for competition trials, or dissections) and are therefore unsuitable for use at remote field sites, or on rare and/or endangered species. The resulting lack of understanding is an impediment to conducting and revising freshwater crayfish conservation assessments, such as the IUCN Red List of threatened species (see Coughran and Furse, 2010), and to the implementation of management plans or conservation measures (Furse and Coughran, 2011b).

The analysis of blood constituents has been used to evaluate physiological stress in numerous marine and terrestrial animals including fishes (Meka and McCormick, 2005), crustaceans (Uhlmann et al., 2009), snails (Renwrantz and Spielvogel, 2011), deer (Bateson and Bradshaw, 1997) and freshwater crayfish (Hamann, 1975; Malev et al., 2010). For example, in Australia, crustacean haemolymph is often analysed to assess sub-lethal stress or morbidity of commercially and recreationally important decapods. However, the aim of such studies is generally to maximise the post harvest (Evans et al., 1999a; Jussila et al., 1999; Poole et al., 2008) or discard survival of exploited species (Uhlmann et al., 2009; Butcher et al., 2012).

Rapid or chronic changes in extrinsic factors (e.g. environmental temperature or hypoxic conditions) are often inherent in capture and discard processes and can cause deviation from normal physiological function (Jussila et al., 1997, 1999; Poole et al., 2008; Uhlmann et al., 2009). The quantification of changes in haemolymph chemistry can identify periods of elevated stress, particular stressors (Evans et al., 1999b; Malev et al., 2010), reduced immunocompetence (Fotedar et al., 2001), and when homeostasis returns (Pascual et al., 2003).
Commonly used physiological stress indicators include total and differential haemocyte counts (THC and DHC) (Jussila et al., 1997), clotting capacity (Jussila et al., 2001), total protein (typically measured as refractive index (RI)) (Dall, 1975; Leavitt and Bayer, 1977), glucose and lactate (Malev et al., 2010) and ion concentrations (e.g. potassium, magnesium and calcium) (Uhlmann et al., 2009). However, other factors must be considered when analysing haemolymph parameters, including moult-stage, gender (Pascual et al., 2003; Malev et al., 2010) and size (Uhlmann et al., 2009), due to associations with haemolymph changes during the ecdysial cycle (Greenaway, 1993).

We see gaining an understanding of fine-scale haemolymph changes as essential to determine abiotic and biotic variables that impact on rare and endangered freshwater crayfish (e.g. increasing environmental temperature, effects of pollutants and exotic species interactions). These methods might offer substantial advantages over existing techniques, because only small volumes of haemolymph are required (e.g. ~25–300 µL per test) and the small-gauge needles used are minimally intrusive (e.g. 26G needle, 0.45 mm ø). Repetitive sampling over time is unlikely to be lastingly injurious or lethal to specimens (Hamann, 1975; Paterson et al., 1997), although the possibility for adverse side effects (i.e. infection) cannot be absolutely excluded. Furthermore, as haemolymph constitutes ~30% of a crayfishes biomass (marine species, see Belman, 1975) and total volume can be quickly adjusted through biological functions (Greco et al.,1986), these methods are suitable for use on rare and endangered species.

The objective of this paper is to outline examples of how existing methods of haemolymph constituent analysis might benefit freshwater crayfish conservation. The potential application and utility of such studies are discussed within the context of a previously identified knowledge gap for rare and endangered Australasian freshwater crayfish species.
2.2 Examples of existing field deployable and inexpensive methods

2.2.1 Haemolymph extraction

Haemolymph samples can be extracted using a 1 mL syringe (cost <US$1.00 each) from the ventral sinus at the basis of either fifth pereopods (Figure 1) (Shields et al., 2003; Uhlmann et al., 2009), or from under the abdomen (Stewart et al., 2004). Samples for haemocyte counts are withdrawn into a chilled syringe (on ice) pre-filled with a suitable anti-coagulant (e.g. 1% formalin, at 9 parts formalin to 1 part haemolymph) (Malev et al., 2010). Before storage, the actual volume of haemolymph extracted must be noted, for subsequent volume adjustment (to account for dilution).

![Figure 1](image1.png)

**Figure 1.** Extracting haemolymph through the ventral sinus at the basis of the fifth pereopod with a small-gauge needle (*Euastacus sulcatus* Riek).

2.2.2 Total and differential haemocyte counts

Typically, THC and DHC are determined using smearing and staining techniques (e.g. May-Grünwald). However, some studies have differentiated between hyaline (agranular) and granular cells (granulocytes and semi-granulocytes) using differences in size, morphology and granular content (LeMoullac et al., 1997; Butcher et al., 2012). Butcher et al. (2012) validated the repeatability and
accuracy of this technique by comparing counts of stained and unstained cells. While this
differentiation technique may reduce fine-scale differences (i.e. between granular cell types) it can be
readily implemented to quantify broad-scale shifts in haemocyte proportions.

To obtain THC and DHC transfer 25 µL of fixed haemolymph into a NEUBAUER™
haemocytometer with a pipette (Jussila et al., 1997). The total number of cells in five (of 25) large
squares (dependant on total cell abundance) are counted (at 100× magnification) and recorded. The
total cell count gives the THC value and the number of granular cells observed comprises the DHC,
and both are multiplied by the haemocytometer conversion factor. Ideally haemocyte counts should
be performed within 1–5 days (with samples stored at 4°C) (e.g. Uhlmann et al., 2009) to avoid the
need for re-suspension.

2.2.3 Refractive index (for total protein)

Place ~25 µL of fluid haemolymph immediately onto a VetQuip™ (VQ5600) refractometer
(calibrated with distilled water) and record the RI (scale: 1.340–1.360 nD units) (Dall, 1975; Leavitt
and Bayer, 1977). This can be reported simply as refractive index (Poole et al., 2008), or converted to
total protein (Shields et al., 2003; Butcher et al., 2012). For the latter, take 20–30 haemolymph
samples (of known RI) and analyse for total protein (mg/mL). Once the correlation between RI and
total protein is known (e.g. Poole et al., 2008), the equation for the line of best fit can be used to
convert future RIs for that species (and congenerics) in the field.

2.2.4 Glucose, lactate and ion concentrations

Field measurements of glucose concentrations can be made with a hand-held reflectometer (e.g.
One Touch® II Meter, Lifescan, Milpitas, CA, USA, ~US$100.00) using commercial grade test strips
(Malev et al., 2010). Similarly, lactate can be quantified using a Accutrend plus™ meter (Roche
Diagnostics, Australia, ~US$250.00) (Wells and Pankhurst, 1999), with a ~25 µL sample of fluid
haemolymph. For samples that are below the minimum detectable limit, 50% of that value can be substituted.

If ion analyses are required (e.g. calcium, magnesium or potassium), haemolymph samples can be placed in Eppendorf™ vials and immediately frozen in liquid nitrogen. Subsequent laboratory analyses of these parameters (for <US$5.00 per sample) can then be performed concurrently with glucose and lactate concentrations using an OLYMPUS™ AU400 automated clinical analyser.

2.2.5 Clotting time

Extract ~25 µL of haemolymph and expel into a 1.5 ml vial. Immediately transfer 12–25 µL of haemolymph into a vertical glass micro-pippetor tube (1.2 mm internal ø) following Jussila et al. (2001). Unclotted haemolymph will move downward and reach the bottom, at this point the tube is inverted 180°. This process is repeated until the clotting ‘endpoint’ occurs; which is defined by the absence of downward movement (Figure 2). Record the time elapsed from the initial extraction until the endpoint is reached. Generally, clotting times do not exceed 2–3 minutes, and values exceeding this are recorded as “unclotted” (Jussila et al., 2001; Uhlmann et al., 2009).

![Figure 2](image.png)

**Figure 2.** The endpoint of an in-situ haemolymph clotting capacity test for *Euastacus sulcatus* Riek (clotted haemolymph indicated by the arrow).
2.3 Discussion

Recent global conservation assessments have: i) identified freshwater crayfish as among the world’s five most endangered animals groups (N. Dewhurst pers. com.), ii) emphasised the highly endangered status of many freshwater crayfish species, and iii) identified several key threatening processes affecting these crayfish (Coughran and Furse, 2010; Furse and Coughran, 2011a; IUCN, 2011b). For example, translocated species (i.e. *Cherax quadricarinatus* (von Martens) and *Cherax destructor* Clark) have the capacity to displace native populations (Horwitz, 1990; Coughran and Daly, 2012; Leland et al., 2012). Australia is home to 135 freshwater crayfish species (Coughran and Furse, 2012), of which 47% have been assessed as Critically Endangered, Endangered or Vulnerable vs. IUCN Red List criteria (IUCN, 2011b), indicating the highly endangered nature of the fauna, and likely increased conservation needs in the future.

In contrast, Japan has a single native freshwater crayfish species (*Cambaroides japonicus* (de Haan)), which is considered an Endangered species by the Japanese Fisheries and Environmental Agencies. *Cambaroides japonicus* is also listed by the IUCN as Data Deficient (DD), a classification that does not indicate whether or not the species is threatened, but rather that there is insufficient data to evaluate and place the taxon into any category (IUCN, 2011a). Such listing highlights there are specific knowledge gaps for the species that need addressing. *Cambaroides japonicus* is threatened by two exotic and highly invasive species (the American signal crayfish *Pacifastacus leniusculus* (Dana) and red swamp crayfish *Procambarus clarkii* (Girard)) (Nakata and Goshima, 2003; Kawai and Machino, 2010). These species have dispersed widely and pose serious threats to *C. japonicus* due to competitive advantages, and by acting as disease vectors of the fungal crayfish plague (*Aphanomyces astaci* Schikora) (Yamanaka et al., 1997; Evans and Edgerton, 2002; Nakata et al., 2002; Nakata and Goshima, 2003; Nakata et al., 2004; Nakata et al., 2006; IUCN, 2011b). While threats to the native Australian and Japanese freshwater crayfish have been identified and investigated in part, further research into the sub-lethal effects of these stressors is required.
We feel there is substantial scope to address previously identified knowledge gaps for rare and endangered freshwater crayfish species, refine the resolution at which sub-lethal stress is measured (e.g. compromised immunocompetence before physical exhaustion) and reduce the necessity for highly-invasive or lethal methods (e.g. LD/LC$_{50}$ tests). For example, thermal tolerance studies generally use observation and evaluation of gross symptoms that (i.e. righting response, Nakata et al., 2002), while unambiguous, do not evaluate sub-lethal effects (Bone et al., 2014).

The use of gross symptoms (i.e. righting response) in thermal tolerance studies indicates the point at which physiological stress renders a freshwater crayfish unable to right itself (see Nakata et al., 2002). Such tests are not benign, and are not suitable for studies on Critically Endangered freshwater crayfish species. However, the methods outlined in this paper should be suitable for use on rare and Critically Endangered species and confer appreciable benefits over some existing and often injurious or lethal methods. In a field of research which is chronically short of funding (Furse and Coughran, 2011b), these inexpensive methods offer an opportunity to address knowledge gaps that would otherwise remain unanswered. Such studies would facilitate conservation assessments and improve understanding of the requirements necessary to manage and conserve threatened species. We believe the Endangered *C. japonicus* and some Critically Endangered Australian *Euastacus* (e.g. *Euastacus bindal* Morgan, and *Euastacus dharawalus* Morgan) are ideal candidates for novel applications of haemolymph constituent analysis.

From an experimental design, execution and cost point of view, these methods are superior to some existing techniques, because of their capacity for large sample sizes (i.e. due to low cost), are easily replicated and provide almost immediately quantified information. Furthermore, the equipment for haemolymph extraction is standard and easily sourced throughout the world, most equipment can be obtained from local non-specialist suppliers (i.e. pharmacies), and is inexpensive (<US$1.00 per sample collection). The cost of equipment for haemolymph analysis is generally not prohibitive, for
example the haemocytometer required for THC and DHC range from US$35.00–250.00 and will last for many years.

Most haemolymph analysis is performed on standard laboratory equipment (e.g. microscopes), or can be done with an inexpensive portable field microscope. Samples can be easily collected (and preserved) in the field and stored for laboratory analysis. Haemolymph extraction and most analyses are uncomplicated and reasonably easy to perform; any competent person can be trained in basic analysis techniques in a few hours. The straightforward nature of the methods and minimal equipment requirements can enable inexpensive analysis, and therefore remove common problems including lack of specialist expertise, expensive equipment, high per-sample processing costs and remote field sites: all of which are often impediments to critical research on rare and endangered Australasian freshwater crayfish. Application of the existing methods outlined in this paper should prove useful in crayfish conservation studies in other regions of the world.

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Chapter 3 – Prelude

Chapter 3 was peer-reviewed and published in the *ICES Journal of Marine Science* (full citation details below). Chapter 3 is presented as it was published, except for where: i) small changes were required to maintain thesis coherence (e.g. in the Figure numbering) and ii) other minor textural changes were made to satisfy examiners comments.

The purpose of this Chapter was to quantify injury, physiological stress and mortality among recreationally discarded giant mud crab (*Scylla serrata*), and teleost bycatch, while assessing the relative selectivities and efficiencies of common Australian traps. This allowed for the identification and prioritisation of mitigation strategies for reducing unwanted effects, with a view towards increasing the effectiveness of exploitation control.

Please cite Chapter 3 as:

CHAPTER THREE

Giant mud crab
Chapter 3: Giant mud crab (*Scylla serrata*): relative efficiencies of common baited traps and impacts to discards

P.A. Butcher¹, J.C. Leland¹, M.K. Broadhurst¹, B.D. Paterson¹, D.G. Mayer⁴

¹NSW Department of Primary Industries, Fisheries Conservation Technology Unit, National Marine Science Centre, PO Box 4321, Coffs Harbour, New South Wales 2450, Australia
²Marine Ecology Research Centre and National Marine Science Centre, School of Environment, Science and Engineering, Southern Cross University, PO Box 157, Lismore, New South Wales 2480, Australia
³Department of Agriculture, Fisheries and Forestry, Bribie Island Aquaculture Research Centre, PO Box 2066, Woorim, Queensland 4507, Australia
⁴Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, GPO Box 267, Brisbane, Queensland 4001, Australia

**ABSTRACT**

This study was initiated in response to a scarcity of data on the efficiency, selectivity and discard mortality of baited traps to target *Scylla serrata*. Five replicates of four traps, including “hoop nets”, rigid “wire pots” and collapsible “round” and “rectangular” pots were deployed for 3, 6 and 24 h in two Australian estuaries. Trapped *S. serrata* were “discarded” into cages and monitored with controls over 3 d. All *S. serrata* were assessed for damage, while subsets of immediately caught and monitored individuals had haemolymph constituents quantified as stress indices. All traps retained similar-sized (8.1–19.1 cm carapace width) *S. serrata*, with catches positively correlated to deployment duration. Round pots were the most efficient for *S. serrata* and fish—mostly *Acanthopagrus australis* (3% mortality). Hoop nets were the least efficient and were often damaged. No *S. serrata* died, but 18% were wounded (biased towards hoop nets), typically involving a missing swimmeret. Physiological responses were mild and mostly affected by biological factors. The results validate discarding unwanted *S. serrata* for controlling exploitation, but larger mesh sizes or escape vents in pots and restrictions on hoop nets would minimise unnecessary catches, pollution and ghost fishing.
3.1 Introduction

The genus *Scylla* (“mud crabs”, Family: Portunidae) comprises four species (*S. tranquebarica*, *S. paramamosain*, *S. olivacea* and *S. serrata*); all of which are distributed throughout mangrove systems of the Indo-West Pacific (Keenan et al., 1998; Gopurenko et al., 1999). In Australia, *S. olivacea* occurs in small quantities, but *S. serrata* (giant mud crab), is the most abundant and widely distributed; predominantly north from central New South Wales (NSW) and west to Exmouth Gulf, Western Australia. Like its congeners, *S. serrata* can attain large sizes (up to 28 cm carapace width – CW and 3 kg) which, combined with their exceptional eating qualities, makes them commercially important (e.g. up to ~US$40 kg$^{-1}$ at point of first sale) and popular among recreational fishers (Kailola et al., 1993; Henry and Lyle, 2003).

Various fishing methods and gears are used to catch *S. serrata* (Motoh, 1983). Traditionally, Australian recreational techniques have involved: (i) “active” methods using bare hands, spears, scoop nets and pole/crab hooks; and (ii) “passive” baited traps, including “hoop” and “drop/lift” nets, “rigid-wire” pots and, more recently, “collapsible-netted” pots (Table 3 – information sourced from the respective state fisheries websites). Active methods are considered more selective because the fisher makes a conscious decision about targeting an individual. By comparison, traps are deployed (typically for up to 24 h), with their selectivity and efficiency varying according to numerous design- and deployment-specific factors. While there is anecdotal information suggesting that some *Scylla* traps are more efficient (i.e. retain more crabs) than others, there are few scientific data, and certainly none to support the substantial interstate variation among the legal Australian designs (Table 3).

It is known that, like all fishing gears (Miller, 1990), none of the Australian traps are 100% selective for just *Scylla* spp. (with some also catching teleosts that are subsequently discarded) or their various size and/or sex restrictions (e.g. only males in Queensland; Table 3). Combined with various input controls, including personal quotas (5–10 crabs day$^{-1}$; Table 3), this results in up to 1.8 million *Scylla* spp. (or ~68% of the total recreational catch) being discarded each year (Henry and Lyle,

<table>
<thead>
<tr>
<th>Location</th>
<th>MLS (cm)</th>
<th>Quota</th>
<th>Pots</th>
<th>Nets</th>
<th>Possession limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>n</td>
<td>Max. size (m)</td>
<td>Min. mesh size (mm)</td>
</tr>
<tr>
<td>NSW</td>
<td>~12.5(8.5)</td>
<td>~12.5(8.5)</td>
<td>5</td>
<td>1.2 × 1.0 × 0.5 or 1.6 ø</td>
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<tr>
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<td>15.0</td>
<td>Illegal</td>
<td>10</td>
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<td>10&lt;br&gt; b</td>
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<tr>
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<td>15.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Illegal</td>
<td>Illegal</td>
</tr>
</tbody>
</table>

CL — the distance from the frontal notch to the posterior margin of the carapace. CW — defined as the distance between the ninth antero-lateral spines. Minimum legal sizes (MLS) are CW in all states except NSW (CL in parentheses). Female *Scylla* spp. in QLD must be released immediately. Scoop nets and hand collection are allowed in all states, but pole hooks are restricted to WA. ø, diameter; max., maximum; min., minimum; n, number.

<sup>a</sup> stretched mesh opening
<sup>b</sup> boat quota day<sup>1</sup> of 30 *Scylla* spp. when there are three or more fishers on board
<sup>c</sup> trap capacity not to exceed 0.5 m<sup>3</sup>
<sup>d</sup> a smaller MLS of 12.0 cm CW applies to *S. olivacea*
<sup>e</sup> boat quota day<sup>1</sup> of 10 *S. serrata*
Such mandatory discarding assumes low unaccounted fishing mortality and minimal long-term impacts to survivors (King, 2007), but few studies (none in the primary literature) have been done to validate these prerequisites for any *Scylla* spp.

The only relevant work includes an unpublished honours thesis on the extent and effects of limb shedding (Penny, 2001), and an internal report on minimising the post-harvest mortality of commercially fished *S. serrata* (Poole et al., 2008). However, discard-mortality studies of other crustaceans indicate that even minor damage during capture can cause significant mortality (Kennelly et al., 1990; Kirkwood and Brown 1998; Bergmann and Moore, 2001). For example, mortality among netted *Ranina ranina* (monitored for 8–21 d) ranged from 5–12% for undamaged crabs, to 90–100% for those missing one cheliped or two pereopods (Kennelly et al., 1990; Kirkwood and Brown, 1998).

It is also apparent that immediate and short-term (e.g. < 3 d – Pollock and Pine, 2007) discard mortality assessments are fairly easily determined, often via confinement studies (for reviews see Murphy and Kruse, 1995; Broadhurst et al., 2006a). By contrast, quantitative estimates of protracted impacts, including other fatalities (i.e. due to predation or morbidity) are more difficult to acquire.

One indirect method of assessing the potential for protracted impacts is to quantify physical damage and/or physiology, and use these as indicators (Broadhurst et al., 2006a; Poole et al., 2008). Often, short-term, negative effects are correlated with limb loss during capture or handling (Onizuka, 1972; Kirkwood and Brown, 1998), which can then have longer-term deleterious costs. Examples of such outcomes include alterations in prey choice (Juanes and Hartwick, 1990), impaired growth (Norman and Jones, 1991), susceptibility to infection (Barber and Cobb, 2007), reduced reproductive output (Smith, 1992) and competiveness (Juanes and Smith, 1995), and even mortality (Barber and Cobb, 2007).
In addition to physical damage, variation in haemolymph constituents such as glucose, lactate, total protein and circulating haemocytes are commonly used to quantify stress (Jussila et al., 1997; Paterson and Spanoghe, 1997; Bergmann et al., 2001; Malev et al., 2010). For example, catching-and-discarding processes can influence normal physiological function (Jussila et al., 1997; Malev et al., 2010), and for many species, including *S. serrata*, there can be significant relationships between haemolymph constituents and morbidity and mortality (Poole et al., 2008). However, like for physical impacts, the difficulty remains in assessing which constituents are most appropriate. In any preliminary study, it would therefore seem appropriate to collect as much information as possible.

Considering the above, there are two broad uncertainties facing the current management of Australian recreational *Scylla* spp. fisheries: (i) the relative size and species selectivities and efficiencies (i.e. ability to retain crabs) of the most common traps; and (ii) any associated lethal and sub-lethal impacts to discards. The aims of this study were to provide the first steps towards addressing these issues and then prioritizing strategies for minimising unaccounted fishing mortality within a broader objective of facilitating more effective control over stock exploitation. For logistical reasons, the research was done in NSW, but the results have implications for all fisheries targeting *S. serrata* and its congeners.

### 3.2 Materials and methods

The study was done in the Corindi (29°58’S 153°13’E) and Wooli (29°50’S 153°13’E) estuaries, in the Solitary Islands Marine Park between February and June 2010. The sampled areas had been closed to all fishing effort for eight years and were closely monitored by government compliance officers. The areas were chosen to minimise the possibility of traps and/or catches being stolen, and to ensure sufficient numbers and size ranges of *S. serrata*. 
3.2.1 Treatment traps

Boat-based researchers deployed replicates of four traps which were chosen so that their specifications complied with the existing legal requirements in most relevant states and represented a broad range of those most commonly used (Figure 3). The treatments included: (i) hoop nets [0.75 m Ø (diameter) × 0.65 m made from 150 mm (stretched mesh opening – SMO), 0.5 mm Ø monofilament polyamide mesh, 10 mm Ø galvanised steel frame and a 50 mm Ø polystyrene (PS) buoy at the apex of the mesh – Figure 3a]; (ii) rectangular collapsible knotted polyethylene (PE) mesh pots (0.88 × 0.55 × 0.20 m and a 8 mm Ø galvanised steel frame), 80 mm mesh (1 mm Ø twine) hung on the bar with two 0.55 × 0.28 m open “V” shaped entrances – termed “rectangular pots” (Figure 3b); (iii) rectangular “wire pots” [0.90 × 0.60 × 0.30 m and made from 50 × 75 mm wire mesh (2 mm Ø wire) with two 0.30 × 0.15 m open funnel entrances – Figure 3c]; and (iv) round, collapsible knotted PE mesh pots (0.90 m Ø × 0.27 m and a 9 mm Ø galvanised steel frame), 50 mm mesh (SMO; 1 mm Ø twine) with four 0.30 × 0.20 m semi-closed funnel entrances which were held tightly together at one end and required some force to open – termed “round pots” (Figure 3d). Both pots and hoop nets are used during the day and night for varying durations (usually less than 24 h), but their mechanisms for catching *S. serrata* differ. Hoop nets are designed to entangle crabs as they try to access the bait, while pots rely on individuals gaining access to bait through different-shaped entrances. All traps were measured for SMO, entrance size, length, width and height to ensure uniformity, and submerged in seawater for 24 h prior to first use.

Six days of fishing were done across three periods (~4 weeks apart) in each estuary starting in March 2010. On each fishing day, 15 replicates of the four traps were baited with ~0.8 kg of chopped sea mullet (*Mugil cephalus*; in a 0.25 × 0.20 m wire-mesh bait bag – 10 × 10 mm mesh) and individually deployed (with a rope and 100 mm Ø PS buoy) 50–100 m apart to ensure independence (validated by Williams and Hill, 1982). All traps were deployed at dawn into mangrove-lined mud and sand channels, 5–50 m wide and 0.3–3.5 m deep. Five replicates of each trap were retrieved after
Figure 3. Lateral view of the (a) hoop net, and (b) rectangular, (c) wire and (d) round pots.
3, 6, or 24 h. The 3 and 6 h deployments were done during daylight following typical fisher behaviour.

3.2.2 Relative trap efficiencies and immediate impacts to all discards

After each trap was retrieved, the time taken for each *S. serrata* to be removed and the method used (i.e. extracted by hand or emptied into the boat) were recorded. Each individual was sexed, measured with vernier calipers (to the nearest 0.1 cm) for CL and CW and assessed for moult stage following Hay et al. (2005): (i) post-moult – clean and highly flexible shell, no wear on chelae; (ii) early inter-moult – moderately flexible shell and some wear on chelae; or (iii) late inter-moult – little or no flex in shell, and/or large, significant wear on chelae. The locations and numbers of missing and/or damaged limbs (cheliped, pereopod or swimmeret) and/or any carapace damage were quantified and each wound was categorised as “sealed” or “unsealed” following Ulhmann et al. (2009). A total of 652 *S. serrata* were “discarded” into cages for short-term mortality assessments (described below), while the remainder (*n* = 38) were marked (for method see Hill, 1975) with 55 mm green t-bar tags (Hallprint Ltd. Adelaide) to differentiate between subsequent catches (i.e. “new” and previously caught *S. serrata*) and those that were immediately released.

All teleosts were identified to species, counted and measured for total length (*L_T* – nearest 0.1 cm) before being assessed for fin damage and mortality prior to release. After each deployment, the total number of broken meshes was recorded and the trap replaced if damaged.

3.2.3 Short-term mortality of discarded *S. serrata*

Most trapped *S. serrata* (irrespective of size) were individually placed into 20 l polyvinyl chloride cages (0.3 m ø × 0.4 m depth) fabricated from buckets with 18 mm ø holes in the lids (*n* = 5) and sides (*n* = 15) to facilitate water exchange. The cages were designed to hold 10 l of water while each individual was being transferred to a 200 m bottom-set line (12 mm ø polypropylene rope) between
two anchors (termed the “monitoring site”), where they were attached ~1 m apart and submerged in ~1.5 m of water.

Up to ten similar-sized “control” *S. serrata* were also caged and attached to the long line on the same day as the trapped discards. The control crabs (*n* = 60) were originally trapped with wire pots (25 mm mesh) up to one month earlier in estuaries near Coffs Harbour (30°18’S, 153°09’E). During their initial collection, only individuals with intact limbs and no visible damage were placed individually into water-filled buckets (0.3 m diameter – ø × 0.4 m depth) and transported to the National Marine Science Centre (NMSC), for distribution among four aerated, flow-through (5 l min⁻¹) 3800 l tanks containing bricks and cement tiles for habitat. The confined *S. serrata* were offered dead school prawns (*Metapenaeus macleayi*) at a rate of ~10% of their body mass d⁻¹ and monitored before eventually being transferred as required for monitoring in the field.

Two Greenspan data loggers recorded water temperature (°C), salinity (PSU) and dissolved oxygen (mg l⁻¹) at the monitoring site (every 30 min). After three days, all *S. serrata* were checked for mortalities, before some damaged and undamaged individuals were sampled for haemolymph (described below). All survivors were tagged and immediately released back into the estuaries.

3.2.4 Physiological responses of discarded *S. serrata*

Non-repetitive haemolymph samples were taken from subsets of damaged and undamaged *S. serrata* after (i) capture and (ii) monitoring (all within 30 s of trap/cage retrieval). All controls were sampled for haemolymph at the end of monitoring. For each individual, two consecutive samples were extracted through the membrane of either swimmeret. The first syringe (1 ml, 25-gauge needle) was chilled at 4°C and prefilled with 0.20 ml of Na-cacodylate anti-coagulant before 0.20 ml of haemolymph was extracted and expelled into a 1.5 ml Eppendorf™ vial (Jussila et al., 2001). Samples were refrigerated at 4°C for up to five days before the total number of circulating haemocytes (THC) was recorded (×10⁶ cells ml⁻¹).
Typically, differential cell counts are performed on haemolymph smears using staining techniques (e.g. Clare and Lumb, 1994), and/or differences in size, morphology, granular content and nuclear/cytoplasmic ratios (e.g. Hose et al., 1990; Clare and Lumb, 1994; Matozzo and Marin, 2010). However, *S. serrata* semi-granulocytes and granulocytes were clearly distinguishable from hyalinocytes from the same cell suspension under compound light microscopy (at 100×). For this reason, differential haemocyte counts (DHC) of semi-granulocytes and granulocytes combined were ascertained and are presented as a percentage of the THC. To verify our DHC method we did three replicate counts from independent haemolymph samples using smeared and stained (May-Grünsfeld) and unstained haemocytes. A total of 7572 haemocytes was counted and the mean proportional values of hyalinocytes (n = 6319) and granular cells (n = 1253) were calculated. The standard error of the proportional amounts of the two groups for each method was calculated at ± 1.08 haemocytes, indicating that our method was precise and yielded similar counts.

The second syringe (2 ml, 22-gauge needle) was used to extract 1–2 ml of haemolymph to quantify clotting time (s), refractive index (nD units), glucose (mM) and lactate (mM). To assess clotting time, two plastic micropipette tips mounted on forceps were held horizontally and ~10 µl of haemolymph suspended between the tips. The forceps were opened and closed to alternately separate fluid haemolymph until the droplets became fixed in shape and repelled each other (i.e. clotting had occurred). The refractive index (scale: 1.340–1.360 nD units) was recorded by placing ~0.05 ml of haemolymph onto a temperature-compensated refractometer (VetQuip™ VQ5600). Refractive indices were converted to total protein using the equation calculated by Poole et al. (2008). Glucose (range of 1.1–33.3 mM) and lactate (range of 0.8–22.0 mM) concentrations were measured with a hand-held meter (Accutrend plus™ Roche Diagnostics, Australia) (see Wells and Pankhurst, 1999). Samples below the minimum detectable limits for glucose and lactate were recorded as 50% of the lowest value.
3.2.5 Data analyses

The data were analysed with generalised linear mixed models (GLMM; McCullagh and Nelder, 1989), using the GLMM procedure in GenStat (2010). The fixed effects included “trap type”, “deployment duration”, “month” and “location”, and other covariates (as appropriate). The random effects were replicate traps spatially distributed within days. A normal model (with transformation if required) was appropriate for most parameters, but the Poisson model with a log-link was used for the catch composition and the physical damage analyses. The binomial distribution with a logit-link was used for binary data, which are presented as percentages.

Where significant Wald values were detected, relevant means were separated by post-hoc t-tests, using the procedure RPAIR in GenStat (2010). Contingency tables of counts were analysed via Pearson's Chi-square. Adjusted means (± s.e. for normal models only) are presented. Means from Poisson and binomial models are back-transformed (to the original scale).

3.3 Results

3.3.1 Water quality

Water temperatures temporally declined at both Corindi and Wooli, with means (± s.d.) greater during March (24.3 ± 2.0 and 25.9 ± 0.6 °C) than in April (22.9 ± 1.3 and 23.4 ± 0.4 °C) and May (20.8 ± 1.4 and 19.0 ± 0.6 °C). Conversely, owing to flooding during February 2010, salinities were lower during March (9.8 ± 4.6 and 6.1 ± 3.1 PSU) than April (25.7 ± 2.8 and 15.2 ± 1.6 PSU) and May (25.6 ± 5.0 and 25.5 ± 1.2 PSU). Dissolved oxygen remained fairly constant (March – 6.2 ± 2.0 and 4.8 ± 0.9 mg l⁻¹; April – 6.7 ± 1.2 and 6.1 ± 0.3 mg l⁻¹; May – 6.9 ± 0.9 and 6.5 ± 0.3 mg l⁻¹).

3.3.2 Relative trap efficiencies and catch-clearance rates

Six-hundred-and-ninety S. serrata (477 males and 213 females) 8.1–19.1 cm CW (mean ± s.d. of 13.7 ± 2.2 cm, n = 627) were caught, including 63 recaptures of 57 individuals. Approximately half of the S. serrata were post-moults (49.1%), followed by early (27.2%) and late (23.6%) inter-moults.
The majority were removed without handling from the rectangular (96.8%), round (95.6%) and wire (93.8%) pots, but not from hoop nets (3.2%), where they were untangled using bare hands.

The time (√ transformed) required to remove trapped individuals was significantly influenced by the main effects of trap type, moult stage, sex and CW (GLMM, $p < 0.001$). Post-hoc $t$-tests for these main effects revealed that the time taken to remove $S$. serrata from hoop nets (adjusted mean of 35.2 s) was significantly longer than for rectangular pots (18.1 s); both of which took significantly longer to clear than wire (9.7 s) and round (6.8 s) pots ($t$-test, $p < 0.05$). Early inter-moult (9.9 s) took significantly longer to remove than late inter-moult (3.7 s) ($t$-test, $p < 0.05$), but there was no significant difference between either of these two groups and post-moult (8.9 s) ($t$-test, $p > 0.05$). Similarly, males took almost twice as long to remove than females (10.1 vs. 5.7 s) (GLMM, $p < 0.05$). Irrespective of sex, removal time was also significantly and positively correlated with CW (slope of $y = 0.022x – 0.087$) with adjusted means of 3.2–14.9 s for crabs 9.0–18.0 cm CW (GLMM, $p < 0.05$).

Generalised linear mixed models detected significant main effects of trap type and deployment duration for the numbers of undersized, legal and total $S$. serrata and a significant interaction for the latter two variables ($p < 0.01$; Table 4, Figure 4). Specifically, for all traps except rectangular pots, there was a positive relationship between catches and deployment duration; especially for round pots which caught the most $S$. serrata (adjusted means of 1.1–3.3 and 0.7–2.3 for total and legal crabs trap$^{-1}$, Figure 4a, b). Although no significant interaction was detected between trap type and deployment duration for undersized crabs, the adjusted means followed the same trend as above for round traps (0.4–1.0 crabs trap$^{-1}$, Figure 4c), but were similar among deployment durations for the remaining designs.

Thirty percent of all $S$. serrata were undersized. The percentage of undersized $S$. serrata was not significantly affected by deployment duration or trap type, indicating similar size selectivities (GLMM, $p > 0.05$; Table 4). There was a significant effect of location on the percentage of
undersized *S. serrata* (Wooli – 40.7% and Corindi – 23.3%) and the numbers of legal (0.7 and 0.3 crabs trap\(^{-1}\) at Corindi and Wooli) and total (0.9 and 0.6 crabs trap\(^{-1}\) at Corindi and Wooli) individuals (GLMM, \(p < 0.05\); Table 4). The total and undersized catches were also significantly greater in April (0.9 and 0.2 crabs trap\(^{-1}\)) and March (0.8 and 0.3 crabs trap\(^{-1}\)) than in May (0.6 and 0.1 crabs trap\(^{-1}\)) (GLMM and post-hoc \(t\)-tests; \(p < 0.05\)).

Three-hundred-and-six teleosts comprising six species were caught, and significantly more by round pots (97.4%; GLMM, \(p < 0.01\)), including 257 yellowfin bream, *Acanthopagrus australis* (mean \(L_T\) ± s.d. of 14.9 ± 3.4 cm), 20 diamond fish, *Monodactylus argenteus* (7.6 ± 1.5 cm \(L_T\)), seven long-finned eel, *Anguilla reinhardtii* (77.9 ± 21.6 cm \(L_T\)), two striped catfish, *Plotosus lineatus* (24.5 ± 3.5 mm \(L_T\)) and one 7.0 cm \(L_T\) sand mullet, *Myxus elongatus*. Bycatches in hoop nets (three yellowfin bream – 16.8 ± 2.8 cm \(L_T\)), wire (one dusky flathead, *Platycephalus fuscus* – 60.0 cm \(L_T\) and four yellowfin bream – 19.7 ± 5.8 cm \(L_T\)) and rectangular (one dusky flathead – 32.0 cm \(L_T\) and eight yellow bream, 23.4 ± 4.9 cm \(L_T\)) traps were comparatively rare. A GLMM exploring just the factors affecting teleost catches in round pots (1.2 ± 0.3 fish trap\(^{-1}\)) detected significant influences of deployment duration and sampling month (\(p < 0.01\); Table 4). Specifically, adjusted mean catches were significantly greater during 24 h (1.6 fish trap\(^{-1}\)) than 6 h (1.0 fish trap\(^{-1}\)) deployments (post-hoc \(t\)-test, \(p < 0.05\)), but there was no difference between catches from either of these groups and those from 3 h deployments (1.2 fish trap\(^{-1}\)) (post-hoc \(t\)-test, \(p > 0.05\)). Significantly more fish were caught during May (2.6 fish trap\(^{-1}\)), than in March (0.6 fish trap\(^{-1}\)), but both groups were not different to catches in April (1.2 fish trap\(^{-1}\)) (post-hoc \(t\)-tests, \(p < 0.05\)). Most teleosts had some damage to their fins, but other than seven *A. australis* that were killed by *S. serrata* whilst the traps were being emptied (providing a total immediate mortality of 3%), all vigorously swam away after release.

### 3.3.3 *Scylla serrata* wounds and trap damage

Approximately 10% \((n = 71)\) of all *S. serrata* had old wounds as indicated by the presence of sclerotized or regenerated tissue, and mainly one (65%) or two (7%) missing chelipeds. Several
Figure 4. Adjusted mean numbers of the (a) total, (b) legal (≥ 8.5 cm CL), and (c) undersized (< 8.5 cm CL) Scylla serrata caught in hoop nets and rectangular, wire and round pots during 3, 6 and 24 h deployments.
Table 4. Summaries of the fixed effects in generalised linear mixed models for their independence on the numbers of total, legal (≥ 8.5 cm CL) and undersized (< 8.5 cm CL) *Scylla serrata*, and the proportion of the latter, and the total number of teleosts in round pots only.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>Total no.</th>
<th>No. legal</th>
<th>No. undersized</th>
<th>Proportion undersized</th>
<th>Teleosts</th>
<th>Total no.</th>
</tr>
</thead>
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<tr>
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<td>-</td>
<td>*</td>
<td>-</td>
<td>-</td>
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<td>Month</td>
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<td>-</td>
<td>*</td>
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<td>-</td>
<td>**</td>
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<tr>
<td>Trap type (T)</td>
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<tr>
<td>Deployment duration (D)</td>
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<td>***</td>
<td>-</td>
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<td>**</td>
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<tr>
<td>T × D</td>
<td>***</td>
<td>**</td>
<td>-</td>
<td>-</td>
<td>Na</td>
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The significance of Wald values are represented by - *p* > 0.1, ● *p* < 0.1, * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001. All interactions except T x D were non-significant (*p* > 0.05) and therefore removed from the final model. Na, term not used in the model.
individuals were also missing one cheliped, and up to three pereopods. Fifteen *S. serrata* (23.8% of the tagged individuals) with old wounds were tagged recaptures.

Eighteen percent (*n* = 124) of all *S. serrata* were wounded during capture or handling (Table 5). Approximately 14% of wounds were unsealed and primarily comprised torn coxa at the base of missing swimmerets (nine crabs – caused by entanglement in meshes), or carapace punctures that exposed the branchial chamber (seven crabs – caused by conspecifics chelipeds) (Table 5). A greater percentage of *S. serrata* lost swimmerets (8.7% of the total catch), than pereopods or chelipeds (both 5.4%, Table 5). New damage frequently included one (83.0%) or two (14.5%) missing limbs (Table 5). There was no influence of trap type (*χ^2^ = 7.39, *df* = 6; *p* > 0.05) or deployment duration (*χ^2^ = 5.72, *df* = 4; *p* > 0.05) on when new damage occurred (i.e. throughout the deployment, or during removal or handling), but this was mostly during their removal from all traps (range of 58.9–71.2%), and throughout the deployment of hoop nets (Figure 5).

The damage to *S. serrata* was significantly influenced by trap type, deployment duration, moult stage, sex and water depth (GLMMs, *p* < 0.05; Table 4; Figure 6). Irrespective of all other factors, total limb loss was clearly greatest in *S. serrata* that were caught in hoop nets (21.7% of the total catch for that trap type) and rectangular pots (22.7%) than the other designs (13–17.1%; post-hoc *t*-test, *p* < 0.05; Table 5; Figure 6a). Hoop-netted *S. serrata* also lost significantly more swimmerets than those caught in wire pots, but there was no difference between these individuals and those caught in rectangular and round pots (post-hoc *t*-test, *p* < 0.05; Figure 6b). Crabs caught in hoop nets also lost significantly more pereopods than those caught in round pots, but neither group was significantly different from those trapped in wire or rectangular pots (post-hoc *t*-test, *p* < 0.05; Figure 6c). Significantly more chelipeds were lost in rectangular pots than wire pots, although no differences existed between either of these two traps and hoop nets or round pots (post-hoc *t*-test, *p* < 0.05; Figure 6d).
Table 5. Numbers of *Scylla serrata* (*n* = 124) that had missing limbs (swimmerets, pereopods, or chelipeds) or a damaged carapace after capture from hoop nets (*n* = 92) or rectangular (*n* = 128), wire (*n* = 131) and round (*n* = 339) pots. Total limb loss is all limbs combined for each *S. serrata*. Percentage of total catch damaged given in parentheses for each trap type.

<table>
<thead>
<tr>
<th>Limb</th>
<th>Hoop net</th>
<th>Rectangular pot</th>
<th>Wire pot</th>
<th>Round pot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of <em>S. serrata</em> damaged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Swimmerets</td>
<td>1</td>
<td>10 (10.9%)</td>
<td>10 (7.8%)</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3 (3.3%)</td>
<td>0</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Pereopods</td>
<td>1</td>
<td>4 (4.3%)</td>
<td>7 (5.5%)</td>
<td>7 (5.3%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3 (3.3%)</td>
<td>1 (0.8%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1 (1.1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4–6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chelipeds</td>
<td>1</td>
<td>7 (7.6%)</td>
<td>12 (9.4%)</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1 (1.1%)</td>
<td>1 (0.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Carapace damage</td>
<td>3 (3.3%)</td>
<td>0</td>
<td>0</td>
<td>4 (1.2%)</td>
</tr>
<tr>
<td>Total limb loss</td>
<td>1</td>
<td>10 (10.9%)</td>
<td>25 (19.5%)</td>
<td>15 (11.5%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6 (6.5%)</td>
<td>3 (2.3%)</td>
<td>2 (1.5%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>1 (0.8%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4 (4.3%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 5. Percentage of *Scylla serrata* that were damaged during different stages of the fishing process, pooled across deployment durations. Total numbers for each group are provided above each histogram.

Table 6. Summaries of the variables tested in generalised linear mixed models for their independence on the various damage parameters for all *Scylla serrata* during trapping, removal and handling.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>Limbs lost</th>
<th>Swimmeret</th>
<th>Pereopod</th>
<th>Cheliped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Month</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trap type</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Deployment duration</td>
<td>***</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Water depth</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>Total no. of <em>S. serrata</em> in trap</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>**</td>
<td>***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moult stage</td>
<td>**</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>CW</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The significance of Wald values are represented by - $p > 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. All interactions were non-significant ($p > 0.05$) and were removed from the final model.
Total limb and cheliped loss were both significantly greater among post-moult than late inter-moult *S. serrata*, but neither was different from early inter-moult (Figure 6e, f). Males lost significantly more total limbs and swimmerets than females (GLMM, \( p < 0.05 \); Table 6; Figure 6g, h). *Scylla serrata* from 24 h deployments lost significantly more total limbs and chelipeds than those removed after 3 h, but neither was different from 6 h deployments (post-hoc \( t \)-test, \( p < 0.05 \); Figure 6i, j). Pereopod loss was also negatively correlated with water depth (GLMM, \( p < 0.05 \); Table 6).

None of the pots were damaged, but 7.9 ± 0.7, 7.0 ± 0.7, and 12.9 ± 1.2 meshes trap\(^{-1}\) were broken during the 3, 6 and 24 h deployments of hoop nets, respectively. No hoop nets had >20 meshes broken (i.e. unusable) after 3 h, but 3.3 and 58.8% did after 6 and 24 h deployments, respectively.

### 3.3.4 Survival and physiology of *S. serrata*

There were no fatalities among 652 trapped or 60 control (mean ± s.d. of 9.2 ± 1.7 cm CW; range of 8.2–18.2 cm CW) *S. serrata* that were caged and monitored for three days. At release after monitoring, all *S. serrata* were highly alert (as indicated by raising their chelipeds or moving their pereopods) during tagging and swam or walked away vigorously.

Haemolymph samples were more strongly affected by environmental and/or biological characteristics than the technical factors of interest (i.e. trap type or deployment duration), and in all cases restricted to main effects (Table 7). The only technical factor to significantly influence haemolymph was trap type for lactate (GLMM, \( p < 0.05 \); Table 7). The post-hoc \( t \)-test detected differences among means, with *S. serrata* caught in rectangular pots having significantly greater lactate concentrations (adjusted mean ± s.e. of 2.6 ± 0.3 mM) than those in hoop nets (1.9 ± 0.8 mM) and round (2.0 ± 0.3 mM), and wire (1.6 ± 0.3 mM) pots; all of which were not significantly different from each other (\( p < 0.05 \); Table 7).
Figure 6. Adjusted mean number loss per Scylla serrata of (a) total limbs, (b) swimmerets, (c) pereopods and (d) chelipeds among the four trap types, (e) total limbs and (f) chelipeds for moult stage, (g) total limbs and (h) swimmerets for sex, and (i) total limbs and (j) chelipeds for deployment durations. Dissimilar letters are used to separate significantly different treatments detected in post-hoc t-tests ($p < 0.05$), following generalized linear mixed models.
When treatment and control *S. serrata* were considered together, GLMMs did detect significant differences for lactate and THC \((p < 0.001\); Table 7). Specifically, lactate concentrations were significantly greater in damaged and undamaged *S. serrata* sampled immediately after landing, than damaged, undamaged and control individuals sampled at the end of 3 d monitoring (post-hoc \(t\)-test; \(p < 0.05\); Table 8). Likewise, although damaged *S. serrata* sampled immediately after landing (day 0) did not have significantly different THC to any other group, undamaged individuals had significantly greater THC than their control, damaged and undamaged conspecifics on day 3; all of which were equal (post-hoc \(t\)-test \(p < 0.05\); Table 8).
Table 7. Summaries of the technical and environmental/biological fixed factors tested for their independence on haemolymph parameters of *Scylla serrata* in generalised linear mixed models with (full – F) and without (reduced – R) controls.

<table>
<thead>
<tr>
<th></th>
<th>THC (10^6 cells ml^-1)</th>
<th>DHC (%)</th>
<th>Clot time (s)</th>
<th>Total protein (mg ml^-1)</th>
<th>Glucose (mM)</th>
<th>Lactate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F  R</td>
<td>F  R</td>
<td>F  R</td>
<td>F  R</td>
<td>F  R</td>
<td>F  R</td>
</tr>
<tr>
<td>Technical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Damaged/day</td>
<td>**</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
</tr>
<tr>
<td>Trap type</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>●</td>
</tr>
<tr>
<td>Removal method</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>●</td>
</tr>
<tr>
<td>Time to remove from trap</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>Na</td>
</tr>
<tr>
<td>Total no. of <em>S. serrata</em> in trap</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
</tr>
<tr>
<td>Environmental/biological</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>●</td>
</tr>
<tr>
<td>Month</td>
<td>-</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>*</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moult stage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CW</td>
<td>-</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Deployment duration</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>Na</td>
</tr>
<tr>
<td>Water depth</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>Na</td>
<td>●</td>
</tr>
</tbody>
</table>

Damage/day includes damaged and undamaged *S. serrata* caught in traps on the day of capture (day 0) and the same groups plus the control *S. serrata* sampled after 3 d in cages. The significance of Wald F-values are represented by - *p > 0.1*, ● *p < 0.1*, * *p < 0.05, ** *p < 0.01, *** *p < 0.001. Na, term not considered. Carapace width was fitted as a fixed covariate in each model. All interactions were non-significant at *p > 0.05 and therefore removed from the final model.
Table 8. Adjusted mean (± s.e.) haemolymph parameters for undamaged and damaged trapped *Scylla serrata* sampled on day 0 (immediately after capture) and day 3 (from their cages); pooled across all traps and deployment durations. Control *S. serrata* were also sampled on day 3.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>THC (10^6 cells ml⁻¹)</th>
<th>DHC (%)</th>
<th>Clot time (s)</th>
<th>Total protein (mg ml⁻¹)</th>
<th>Glucose (mM)</th>
<th>Lactate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undamaged</td>
<td>72</td>
<td>10.0 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.3 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.1 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.7 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Damaged (during deployment)</td>
<td>6</td>
<td>7.9 ± 1.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.8 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.5 ± 13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.0 ± 7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undamaged</td>
<td>72</td>
<td>6.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.8 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.9 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.6 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Damaged (all)</td>
<td>53</td>
<td>8.1 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.4 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.9 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.7 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>7.1 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.0 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.0 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.0 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*n* = number of individuals sampled. Within columns, dissimilar superscript letters are used to separate significantly different treatments detected in generalized linear mixed models (post-hoc *t*-tests, *p* < 0.05). ‘Damaged (all)’ includes *S. serrata* injured during the deployment, removal and measurement.
3.4 Discussion

This study has quantified, for the first time, the relative efficiencies and selectivities of four common Australian traps used to target *Scylla* spp., and the immediate and short-term condition of confined crabs after capture, handling and discarding. Much of the variation among the observed performances of the traps can be discussed in terms of their specific fishing mechanisms, and this information ultimately used to support strategies by which potentially negative impacts can be mitigated; not only to the discards, but also the marine environment.

Of the designs tested, round pots were the most efficient, catching the most *S. serrata* and bycatch across all deployments. The relatively greater efficiency (i.e. more crabs) of round pots can probably be attributed to their entrance shape and number (four vs. two in the other two pots), which affect their ability to facilitate the entry and retention of organisms (i.e. semi-closed funnel vs. open funnel vs. open V-shaped entrance vs. entanglement). For example, the four semi-closed narrow-funnel entrances encompassed almost half of the entire circumference of the round trap, (compared with ~37 and ~20% for rectangular and wire pots), which effectively increased bait accessibility. Similar differences in trap entry efficiencies have been reported for many crustaceans (Vazquez Archdale and Kuwahara, 2005; Vazquez Archdale et al., 2006; Vazquez Archdale et al., 2010). For example, during a study on the shore swimming crab (*Charybdis japonica*), Vazquez Archdale et al. (2006) observed that traps containing funnels caught 100% of crabs that made contact, compared to only 66% by “V”-shaped designs.

Hoop nets provided greater bait accessibility than the pots, but the frequency of damaged meshes indicated that they were considerably less efficient at retaining *S. serrata* (or any other species across the same deployment durations). None of the pots had damaged meshes, but some rectangular and wire designs that contained no *S. serrata*, had ripped bait bags, probably because unlike the round pots, their entrances were wider (and rigid for wire pots) and allowed at least some individuals to escape or access the bait without entering the trap.
The escape of trapped *S. serrata* warrants consideration because this was probably associated with at least some unaccounted physical damage, especially from hoop nets; up to five of which can be set by a single fisher in NSW. In a heavily-fished environment where multiple recaptures occur, the number of physically damaged *S. serrata* could be substantial. In other fisheries, physical damage has been positively correlated with trapping effort, with up to 90% of *S. serrata* missing limbs in many heavily fished Queensland rivers (Penny, 2001). Studies of another crustacean have shown similar impacts (Krouse, 1976).

For the captured-and-discarded *S. serrata* in the present study, physical damage was mostly restricted to sealed wounds as a result of autotomy, and mainly occurred during their removal from the trap. Possibly, *S. serrata* were threatened by their capture and handling, and autotomized limbs as a survival mechanism (Vermeij, 1982). Autotomy most frequently occurred in netted crabs, with virtually all limbs severely tangled in meshes which took considerable time to remove. Cutting meshes would improve release times, and possibly autotomy, but with an obvious cost to netting materials. This latter point also raises an important consideration concerning the pollution of netting from damaged traps. The entanglement or ingestion of netting or lines has been recorded for fish (Possatto et al., 2011), birds (Franson et al., 2003), turtles (Moore, 2008) and whales (Jacobsen et al., 2010). The potential impacts of lost hoop nets were identified more than 20 years ago by Bartleet et al. (1993) and along with the results here, support their prohibition or restricted use in NSW.

Owing to their more resilient construction and different catching mechanisms, pots were not similarly damaged, although they also wounded *S. serrata*. For the round and rectangular pots, this mostly involved the propodus and dactylus segments of the swimmeret as they were restrained in the meshes. Also, when all pots were inverted during removal, some restrained limbs autotomised, although swimmerets were also torn at their coxa and were unsealed. Most of the limb loss by *S. serrata* in the wire pots occurred during inversion, as individuals collided with the wire mesh. Trap designs which open up completely would facilitate the quick removal of crabs, without having to
invert the trap or possibly handle them. However, this would only be effective if the bottom meshes of the trap remained tight when open, so that individuals could be released without entangling (especially swimmerets).

Irrespective of the type of trap, the physical damage to *S. serrata* also varied according to the deployment duration and depth. One of the simplest mechanisms by which damage might be limited would be to fish deeper (and therefore cooler and darker conditions) water and restrict deployments to 3 h, although conceivably, the latter could increase effort. For example, the relationship between catch and deployment duration was not proportional for any trap, with maximum efficiencies probably achieved in <3 h. Because *S. serrata* are more active at night, crabs caught during the 3 and 6 h deployments were probably already close to the trap and had opportunistically foraged on the presented bait (Hill, 1976). This means that multiple, shorter deployments at different locations would result in substantially greater catches than a single deployment of the same duration.

In addition to the technical factors affecting damage to *S. serrata*, were their moult stage and sex. Limb loss was more prevalent among post- and early inter-moults and males; all of which took the longest to remove from traps. Possible reasons for such damage were that the exoskeletons of post- and early inter-moults were softer after moulting, while males had more vigorous agonistic responses and were therefore more difficult to secure.

Although there were no associated mortalities, the observed injuries could have longer-term consequences. For example, crustaceans with missing chelipeds can alter their prey choice (ap Rheinallt and Hughes, 1985; Juanes and Hartwick, 1990) and while *S. serrata* with one cheliped may be able to crush prey, those missing both would be unable to defend themselves and less able to feed on molluscs, which are a major component of their diet (Hill, 1976). Damaged pereopods would also affect foraging because the dactyls are used to locate food via chemoreception (Hill, 1979). Physical damage to swimmerets might also impact on an individual’s ability to burrow and swim. This could
influence their ability to evade predation or manifest as some reproductive impact to females which migrate offshore to spawn (Hill, 1994). Further research is required to elucidate the long-term consequences of physical damage on growth, limb regeneration, reproductive output, competiveness, infection and mortality.

Unlike the technical factors that predominantly influenced wounding, spatial and temporal variation in environmental and biological factors had the greatest effect on *S. serrata* physiology. Similar observations have been made for other crustaceans (Johnson, 1980). However, there were significantly elevated THC and lactate concentrations among *S. serrata* sampled immediately after trap retrieval which could indicate some confinement stress (Huntingford et al., 1995; Elwood et al. 2009). For example, there would be an energy cost associated with multiple attempts at trap entry and particularly escape and/or being in the presence of aggressive conspecifics. Although lactate levels in some individuals during the current study were similar to those from morbid individuals collected from commercial fishers (i.e. > 5.0 mM – Poole et al., 2008) none died and, like THC, these had returned to similar concentrations as controls after 3 d.

The lack of any mortality, combined with limited physical and physiological impacts reflects the robustness of *S. serrata* and the harsh environment in which it lives. But, given the importance of environmental and biological conditions on physiology above, one consideration is the effects of stressors not examined in the present study. For example, physiological impacts to discarded *S. serrata* could be more severe across their northern distribution where average air and water temperatures exceed 30°C. Several studies have shown a positive correlation between temperature and the stress/mortality of crustaceans (Bergmann et al., 2001; Poole et al., 2008).

Irrespective of the fate of discarded *S. serrata*, there seems little point in catching undersized conspecifics and/or fish; all of which could be simply excluded via minor gear modifications. Given the similar poor size selectivity among all gears, a coherent starting point would be to ensure that the
The minimum legal mesh size for all gears corresponds to the minimum legal size (MLS) of *S. serrata* (e.g. 12.5 to 15 mm CW) in each state. Further research is required to quantify the impacts of any such changes, but it is likely that increasing SMO in pots to at least 100–120 mm in NSW would not only improve size selectivity for *S. serrata*, but also allow large numbers of *A. australis* to escape—since the largest of those typically caught here (i.e. 30 cm *L*_T) would have maximum girth of < 24 cm and less than the maximum perimeter of a 120 mm diamond mesh (Broadhurst et al., 2006b). However, one consideration for any mesh-size increase in NSW is that the same traps are used to target blue swimmer crabs (*Portunus pelagicus*); the adults of which are somewhat smaller than *S. serrata*. It might also be possible to reduce both teleost and *S. serrata* discards via so-called “escape vents”. Such openings can be inserted into existing traps, and have been used to improve species- and size-selectivity in several crustacean (including *Scylla* spp. Jirapunpipat et al., 2008) and teleost (Johnson, 2010) trap fisheries overseas.

This study has revealed considerable variation in efficiencies among *S. serrata* traps, which requires consideration in terms of regulating effort, not only in NSW, but throughout the entire distribution of the genus. Irrespective of relative efficiencies, and beyond the clear need to improve selectivity of all designs discussed above, the results support the prohibition of hoop nets or at least perhaps temporal restrictions on their deployment. Further research is also required to determine the effects of ghost fishing on *S. serrata* and fish (particularly by round pots). Ultimately, such strategies will help to more closely regulate the exploitation of *S. serrata* while minimising unwanted impacts to the marine environment.

Acknowledgements

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M. Grubert, D. Rotherham, H. Malcolm, D. Reynolds and two anonymous reviewers are thanked for their comments on the manuscript. Ethics approval was granted by the NSW DPI (Ref. 03/12) and SCU (Ref. 10/12) Animal Care and Ethics Committees.
Chapter 4 – Prelude

Chapter 4 has been peer-reviewed and published in the international journal *Fisheries Research* (full citation details below). Chapter 4 is presented as it was published, except for where: i) small changes were required to maintain thesis coherence (e.g. in the Figure numbering) and ii) other minor textural changes were made to satisfy examiners comments.

The objective of this Chapter was to investigate the differences between eastern rock lobster (*Sagmariasus verreauxi*) collection methods in terms of selectivity, physical damage, physiological stress, mortality and growth among recreationally discarded individuals. From this information, effective stress indicators for future *S. verreauxi* studies and practical strategies to mitigate physical damage and other negative impacts were identified.

Please cite Chapter 4 as:

CHAPTER FOUR

Eastern rock lobster
Chapter 4: Damage and physiological stress to juvenile eastern rock lobster (*Sagmariasus verreauxi*) discarded after trapping and hand collection

**J.C. Leland**¹,², P.A. Butcher¹, M.K. Broadhurst¹, B.D. Paterson³, D.G. Mayer⁴

¹**NSW Department of Primary Industries, Fisheries Conservation Technology Unit, National Marine Science Centre, PO Box 4321, Coffs Harbour, New South Wales 2450, Australia**
²**Marine Ecology Research Centre and National Marine Science Centre, School of Environment, Science and Engineering, Southern Cross University, PO Box 157, Lismore, New South Wales 2480, Australia**
³**Department of Agriculture, Fisheries and Forestry, Bribie Island Aquaculture Research Centre, PO Box 2066, Woorim, Queensland 4507, Australia**
⁴**Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, GPO Box 267, Brisbane, Queensland 4001, Australia**

**ABSTRACT**

Large numbers of *Sagmariasus verreauxi* are trapped and hand collected in Australia, but discarded due to size and quota restrictions, and under the unevaluated assumption of few impacts. To test the validity of enforced discarding, trapped and hand-collected *S. verreauxi* (49–143 mm carapace length – CL) were examined for external damage, placed into cages, transferred to aquaria and monitored (with controls) over three months. Haemolymph was non-repetitively sampled immediately and at one, three, and seven days to quantify stress. Most trapped (64%) and hand-collected (79%) specimens were undersized (<104 mm CL), with the latter method yielding broader ranges of sizes and moult stages. Within-trap *Octopus tetricus* predation caused the only mortalities (3.3%). Hand collection resulted in much greater antennae and pereopod loss than trapping (53 vs. 4%) but, compared to controls, both methods evoked benign physiological responses that resolved within a week. While most wounded *S. verreauxi* regenerated all or some missing appendages post-moult, their mean CLs were less than those from intact conspecifics. Simple strategies, including larger mesh sizes, and/or installing modifications to reduce bycatch in traps, careful hand collection, and appropriate release techniques might minimise impacts (including predation) to unwanted *S. verreauxi*, and help to control stock exploitation.
4.1 Introduction

In Australia, the eastern rock lobster (*Sagmariasus verreauxi*; previously *Jasus verreauxi*) is distributed across coastal reefs (~200 m depth) south from Tweed Heads, New South Wales (NSW) to Port MacDonnell, South Australia (Montgomery, 1992; Kailola et al., 1993). The largest of all palinurids, *S. verreauxi* can attain substantial carapace lengths (>260 mm CL) and weights (>8 kg; Kailola et al., 1993), and is considered an iconic, highly prized species, particularly in NSW where it supports important commercial (~130 mt; valued at $AUD 7.8 million annually; G. Liggins pers. com.) and recreational fisheries (~26 mt; Andrew et al., 1997).

A substantial proportion of the NSW *S. verreauxi* catch is taken from <10 m (hereafter referred to as shallow water) where, owing to positive age segregation by depth, there are large abundances of sublegal (<104 mm CL) juveniles (Montgomery, 1992). Shallow-water *S. verreauxi* are targeted with rectangular and round traps (Figure 7), that are regulated by legal dimensions for both commercial and recreational fishers (≤ 1.2 m ø or 1.2 × 1.2 × 1.2 m), and numbers for the latter (one trap per fisher). Although not restricted, commercial fishers typically use 20–140 traps per licence. Mesh size is not regulated for recreational gears, but most rectangular traps are made from either 50 × 50 mm or 75 × 50 mm welded wire mesh, while round traps generally have ribs with 35–40 mm spaces (Figure 7a and b). The minimum mesh size for commercial traps is 50 mm and there are plans to mandate “escape vents” in all traps, like those used in other crustacean trap fisheries (Murphy and Kruse, 1995; Arana et al., 2011), pending formal data on size and species selectivities.

In addition to trapping, both sectors can hand collect *S. verreauxi* while free diving (breath hold only), although this is almost entirely limited to recreational fishers. There are no definitive data on the proportion of the NSW recreational *S. verreauxi* catch taken by hand, but Henry and Lyle (2003) estimated that this method accounted for 32% of the national palinurid catch.
Figure 7. Three dimensional views of (a) rectangular and (b) round traps used to catch *Sagmariasus verreauxi* in New South Wales, Australia.

Along with gear regulations and minimum and maximum legal sizes (104 and 180 mm CL), NSW *S. verreauxi* are managed by quotas (annual catch for the commercial sector, typically 100–135 mt, or two individuals per day per recreational fisher) and obligatory discarding of ovigerous females (rare in shallow water). Because none of the fishing methods are 100% size or quota selective, some shallow-water *S. verreauxi* are discarded. Few quantitative commercial data are available, but Henry and Lyle (2003) estimated that recreational fishers nationally discarded 49% of their palinurid catches. Assuming a comparable trend in NSW, this would equate to ~5000 recreationally discarded *S. verreauxi*; including a large proportion of shallow-water juveniles. Two inherent assumptions
associated with such mandatory discarding are low associated fishing mortality and few deleterious sub-lethal impacts to discards.

Despite the need to elucidate the fate of discarded juvenile *S. verreauxi*, no scientific data have been collected. In fact, few studies have assessed impacts to discarded, trapped and hand-collected palinurids anywhere (but see Powrie and Tempero, 2009; Frisch and Hobbs, 2011). However, like other crustaceans caught by various fishing methods, which have been extensively studied (see Murphy and Kruse, 1995 for a review), the potential exists for some unaccounted fishing mortality and/or sub-lethal stress to unwanted individuals, with subsequent effects on biological processes. More specifically, previous studies have shown that negative impacts to discarded crustaceans typically are correlated to important and often cumulative factors, including their size (Brouwer et al., 2006), appendage loss (Uhlmann et al., 2009) or the fishing method (Powrie and Tempero, 2009) and/or the trap design (Butcher et al., 2012).

A major concern for discarded crustaceans is appendage loss, which often has a demonstrable relationship with reduced immunocompetence (Fotedar et al., 2001) or mortality (Uhlmann et al., 2009). In particular, loosing sensory or walking appendages can affect defence (Parsons and Eggleston, 2005), mobility and/or competitive ability (Juanes and Smith, 1995). Also, because crustaceans grow discontinuously, cumulative sub-lethal factors that affect ecdysial processes can alter moult increments (Juanes and Smith, 1995; Frisch and Hobbs, 2011) and ultimately reduce fisheries productivity. Mitigating such damage is considered important to reduce population and economic impacts (Parsons and Eggleston, 2007).

In addition to obvious physical damage, discarded crustaceans often undergo physiological changes. Such alterations can be quantified by monitoring temporal changes in haemolymph constituents, including: protein (measured as refractive index – RI; Butcher et al., 2012); haemocyte counts (Jussila et al., 2001); clotting capacity (Fotedar et al., 2001); glucose (Paterson et al., 1997);
lactate (Ridgway et al., 2006); calcium; magnesium; and potassium (Uhlmann et al., 2009). The utility of each parameter can be species-specific and generally depends on stressors inherent to a given fishing method (Butcher et al., 2012). Particular attention often has focused on haemocyte counts (Fotedar et al., 2001; Uhlmann et al., 2009) and glucose and lactate (Bergmann et al., 2001), which can positively correlate to hypoxia, handling and limb loss.

Given the above, two key aims of this study were to quantify the (i) relative size-selectivity associated with conventional trapping and hand collection of shallow-water *S. verreauxi*, and (ii) mortality, stress, physical damage and growth of discarded lobsters. Using this information, additional aims were to identify (iii) effective physiological stress indicators for future *S. verreauxi* studies and, where required, (iv) practical strategies to mitigate unaccounted fishing mortality and other negative impacts.

### 4.2 Materials and methods

#### 4.2.1 Control collection

Four to six weeks before starting the experiment, 22 juvenile *S. verreauxi* (80–110 mm CL) were caught from depths <10 m off Coffs Harbour (30°16’S, 153°05’E), NSW, Australia using rectangular traps (800 × 600 × 360 mm frame of 4 mm diameter (ø) steel rod (50 × 50 mm wire mesh), with two 190 × 145 mm side entrances of 50 mm wire mesh – Figure 7a) baited with sea mullet (*Mugil cephalus*). Undamaged individuals were individually housed in 30 l polyvinyl chloride cages (0.3 m ø × 0.4 m depth; described by Butcher et al., 2012) filled with seawater and immediately transported to the National Marine Science Centre (NMSC). All controls were released into three 3000 l holding tanks with bricks and tiles for habitat and with 5 l min⁻¹ of aerated seawater, prior to being used in the experiment.
4.2.2 Capture, discarding and monitoring of trapped and hand-collected *S. verreauxi*

*Sagmariasus verreauxi* were trapped with round (base ø and height of 600 and 280 mm, 21 vertical and 16 horizontal ribs spaced 35–40 mm apart with one 200 × 200 mm top entrance – Figure 7b) and rectangular traps (as per controls – see section 4.2.1.) at six randomly selected kelp-covered rocky reefs near Coffs Harbour. On each day (*n* = 23), researchers deployed 7–10 replicates of each trap for 24 h from rock platforms and/or boats depending on sea conditions.

Up to six free divers also hand collected *S. verreauxi* from the same areas (on 10 different days) without altering their normal techniques. Divers categorised *S. verreauxi* as either visible (e.g. carapace was observed) or hidden (e.g. only the antennae were seen), before noting their position (carapace towards, perpendicular to, or away from, the diver) during each capture attempt, estimating handling time (i.e. from the initial attempt to actual capture) and noting the presence of predators (mostly Sydney octopus, *Octopus tetricus*).

Irrespective of the fishing method, all individuals were checked for legal CL and immediately examined for any damage to their exoskeleton or appendages, caused during capture (termed ‘new’) or previously (‘old’). New wounds were identified as sealed or unsealed (following Ulhmann et al., 2009) and the handling time was recorded for each individual. Subsets of intact and damaged *S. verreauxi* (excluding those with old damage) were non-repetitively and randomly sampled for haemolymph (<30 s duration) immediately after being brought to the surface (termed ‘day 0’ samples – see section 4.2.4). These and all other treatment *S. verreauxi* were then individually ‘discarded’ into 30 l cages and supplied with fresh seawater during transfer to the NMSC (30–90 min). Appropriate numbers of controls were concurrently removed from their tanks; some were sampled for haemolymph (also termed day 0 – see section 4.2.4) at the NMSC, before all were individually caged and left for similar periods as treatments.
At the NMSC, the treatment and appropriate control *S. verreauxi* were removed from their cages (for <30 s) while they were sexed and their initial wet weight (WW – to the nearest 0.1 g) determined with a top-loading pan balance. The distal tip of either first pleopod was excised (~5 mm) and frozen at –4°C for moult-stage assessment as described by Lyle and MacDonald (1983) where: AB is post-moult; C is inter-moult and D is pre-moult comprising six subdivisions (D₀, D₁⁻, D₁⁺, D₂, and D₃⁻₄).

All *S. verreauxi* were re-caged and randomly distributed among nine 3000 l holding tanks so that each held at least two controls and up to 18 treatments (maximum density of 20 caged individuals per tank). *Sagmariasus verreauxi* were alternately offered school prawns (*Metapenaeus macleayi*) or Australian sardine (*Sardinops sagax*) ad libitum (with uneaten food removed every two days) and monitored daily for 90 days. Temperature (°C) and salinity (PSU) were recorded (6 h intervals) with Greenspan data loggers in two of the holding tanks. At the end of the experiment, all *S. verreauxi* that did not moult were measured for CL (nearest 1 mm), carapace width (CW) and final WW.

### 4.2.3 Post-moult measures

Within 24 h after moultng, exuviae were measured with vernier callipers for initial CL and CW (at time of capture). Five days after moultng, each *S. verreauxi* was re-weighed, measured (for post-moult WW and CL) and any regeneration of damaged appendages recorded. Haemolymph samples were taken to quantify post-moult RI and clotting time (see section 4.2.4).

### 4.2.4 Haemolymph sampling

In addition to post-moults and some treatment and control *S. verreauxi* on day 0, other individuals were non-repetitively and randomly sampled for haemolymph on one, three and seven days after capture, before being returned to their respective cages and tanks. Each individual was held by the carapace (in ventral view) with the abdomen restrained while three samples were taken. The first sample (2 ml of haemolymph) was removed through the membrane at the basis of either fifth
pereopod using a 22-gauge needle. Approximately 0.05 ml was immediately placed onto a calibrated VetQuip™ VQ5600 refractometer and the RI recorded (scale: 1.340–1.360 nD units). The rest of the sample was stored in a 3.0 ml vial and immediately frozen in liquid nitrogen for subsequent analyses of glucose, lactate, calcium, magnesium and potassium concentrations (mmol l\(^{-1}\)) using an OLYMPUS™ AU400 automated clinical analyser.

The second sample (~0.20 ml of haemolymph) was extracted with a 25-gauge needle containing 0.20 ml of sodium-cacodylate anti-coagulant (see Jussila et al., 2001). Fixed samples (of known volumes) were refrigerated at 4°C for up to five days, before placement on a NEUBAUER™ haemocytometer, where the total numbers of relevant cells were counted under light microscopy (at 100×) to quantify total (THC) and differential haemocyte counts (DHC), which were volume adjusted. Obvious differences in size, morphology and granular content enabled hyalinocytes to be distinguished from two granular cell types, which were combined for DHC. This method was applied to giant mud crab (Scylla serrata) haemolymph by Butcher et al. (2012) and showed close agreement between repeated counts of stained and unstained cells from the same suspension.

To quantify clotting time, a third sample of haemolymph (0.02 ml) was extracted with a 22-gauge needle and syringe pre-loaded with 0.02 ml of filtered (60 µm) seawater at ambient temperature and expelled into a 1.5 ml vial. A pipette was used to immediately (<9 s from initial haemolymph extraction) transfer 25 µl of the mixture into a vertically positioned glass micro-pippetor tube (1.2 mm internal ø). Fluid haemolymph moved downward until it reached the bottom and then the tube was inverted 180°. This process was repeated until clotting occurred; defined by the cessation of downward movement. Clotting time for fluid haemolymph was capped at 120 s.

4.2.5 Data analyses

Appropriate generalised linear mixed (GLMM) or general linear (GLM) models (McCullagh and Nelder, 1989), using the GLMM procedure in GenStat (2010), were used to test the hypothesis of no
differences in (i) relative selectivity (e.g. catch composition), (ii) physical damage, (iii) haemolymph constituents, and (iv) moulting and growth due to the treatment (i.e. fishing and/or handling methods) of S. verreauxi and several other fixed factors (and interactions where appropriate – see section 4.3). For GLMMs in which sparse (or extreme) data precluded convergence, a simplified GLM (with spatial and temporal variances pooled) was used. Temporal and spatial random terms (capture date and location) were included in all relevant models.

Normal models (with transformation when necessary) were suitable for most parameters, but the Poisson model with a log-link was used for counts data (effects of the treatment of S. verreauxi, moult stage and size on the distribution of physical damage), while logistic models were used for binomial data. Where significant fixed factors exceeded two levels, differences among means were explored using post-hoc t-testing with the GenStat (2010) RPAIR procedure.

Pearson's Chi-square was used to analyse contingency tables of counts. Adjusted means (± SE for normal models only) are presented under equal weighting from all terms in the models. The significance of results was determined at $p < 0.05$.

4.3 Results

4.3.1 Relative species, size, sex and moult selectivity

In total, 158 S. verreauxi were caught (Figure 8), of which 25 (mean ± SD of 96.8 ± 15.6 mm CL) were from 157 deployments of round traps (relative catch-per-unit-of-effort (CPUE) of 0.16 per trap per 24 h), 36 (99.5 ± 9.2 mm CL) were from 119 deployments of rectangular traps (CPUE of 0.30 per trap per 24 h) in 1–4 m depth, with the remaining 97 (94.3 ± 19.4 mm CL) hand collected from 1–6 m deep (CPUE of 0.96 individual per diver per day). Bycatch comprised six blindsharks (Brachaelurus waddi; 420–510 mm total length – TL), one rock cale (Aplodactylus lophodon; 250 mm TL) and six large O. tetricus. The latter were caught in one round and five rectangular traps, and were also frequently observed by divers at all sampling locations.
There was no within-treatment sex bias ($\chi^2 = 3.89, df = 2; p > 0.05$) and no significant difference between the proportions of undersized trapped (combined), hand-collected or control *S. verreauxi* ($\chi^2 = 5.11, df = 2; p > 0.05$). Controls excluded, the fishing method significantly affected *S. verreauxi* CL (GLMM, $p < 0.01$) and moult stage ($\chi^2 = 39.74, df = 14; p < 0.001$). Specifically, compared to trapping, hand collection yielded greater proportions of undersized individuals (79 vs. 64%; Figure 8), and significantly more pre- and post-moult *S. verreauxi* (combined 76.3%), than inter-moults (24%; $\chi^2 = 32, df = 1; p < 0.001$; Table 9).

**Figure 8.** Size-frequency distributions (nearest 5 mm carapace length, CL) of control (black), trapped (combined; grey; $n = 59$) and hand-collected (white; $n = 97$) *Sagmariasus verreauxi*. Dashed line demarcates undersized (<104 mm CL) and legal individuals.
Table 9. Numbers of intact and damaged hand-collected and trapped (pooled) Sagmariasus verreauxi, with the numbers of post-, inter- and pre-moult (all six subdivisions combined) individuals expressed as percentages of the total catch. The respective numbers of individuals that moulted are given in parentheses.

| Moult stage | Hand-collected | | | Trapped | | |
|-------------|----------------|----------------|---|----------------|---|
|             | Intact | Damaged | % | Intact | Damaged | % |
| Post-moult  | 8 (0)  | 7 (0)   | 15.4 | 5 (0)  | 0 (0)   | 8.5 |
| Inter-moult | 13 (5) | 10 (7)  | 23.7 | 40 (2) | 2 (0)   | 71.2 |
| Pre-moult   | 25 (18)| 34 (20) | 60.9 | 12 (0) | 0 (0)   | 20.3 |

4.3.2 Mortality

Two undersized *S. verreauxi* (each in a round and rectangular trap) were being consumed by *O. tetricus* during retrieval. Both fatalities were excluded from subsequent analyses. All remaining *S. verreauxi* survived monitoring at the NMSC, across water temperatures of 17.4–21.9°C and salinities of 30–37 PSU. These results provided mortality estimates of 4.0, 2.7 and 0.0% for the round and rectangular traps and hand collection, respectively.

4.3.2 Physical damage

Although all discarded *S. verreauxi* survived, some were damaged. Three trapped and three hand-collected individuals (all undersized) had old damage comprising 1–3 missing pereopods. By comparison, only two trapped *S. verreauxi* (one from each trap) had a new single, sealed antenna wound (one half and three quarters missing, respectively), but 51 (53%) hand-collected individuals had new damage that included missing one or both antennae and/or their pereopods (typically 1–4, maximum of 7; Figure 9).

Antennae loss was not significantly influenced by any of the recorded fixed factors (GLMM, *p* > 0.05; Table 10). But the number of missing pereopods and total appendage loss were both significantly affected by fishing depth and diver, and were individually influenced by the visibility.
and handling time of specimens, respectively (GLMM, \(p < 0.05\); Table 10). Total damage was positively correlated with fishing depth and handling time. The effect of diver was due to one \(S. \text{verreauxi}\) missing four pereopods. Individuals that were visible during collection lost significantly more pereopods than those that were hidden (GLMM, \(p < 0.05\); both adjusted means approached zero, but that for visible \(S. \text{verreauxi}\) was greater).

Twenty-six hand-collected individuals were missing either part, or all, of one antenna, and 10 lost both (Figure 9). Only frontal attempts caused unsealed antennae wounds. Compared with pereopods, broken antennae more frequently resulted in unsealed rather than sealed wounds (6 vs. 24%; \(\chi^2 = 7.92, \ df = 1; \ p < 0.01\); Figure 9). There were no significant relationships between the proportions of unsealed wounds and sex, moult stage or CL (GLM, \(p > 0.05\)).

![Antennae and Pereopods](image.png)

**Figure 9.** Numbers of damaged hand-collected \(Sagmariasus \text{verreauxi}\) (\(n = 51\)) missing one or two antennae and one to seven pereopods, with the proportions of sealed (grey) and unsealed (white) wounds.
Table 10. Summary of fixed factors tested in generalised linear mixed models for significant effects on antennae, pereopod and total appendage loss among hand-collected *Sagmariasus verreauxi*. ns: not significant.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>Antennae</th>
<th>Pereopods</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Moult stage</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Initial CL</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Fishing depth</td>
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<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Handling time</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
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<tr>
<td>Visibility</td>
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<td>Attempt position</td>
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<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Diver</td>
<td>ns</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

* *p < 0.05, **p < 0.01, ***p < 0.001.

4.3.3 Physiological effects

An initial GLMM revealed no significant effect of trap type on any haemolymph variable (\( p > 0.05 \)), and so data were pooled at this level for all subsequent analyses. Total haemocyte counts, DHC, initial RI, lactate and magnesium were all significantly affected by moult stage, while potassium was the only parameter affected by initial CL (GLMM, \( p < 0.01 \); Table 11). Parameters influenced by technical factors (i.e. a main or interactive effect of the treatment of *S. verreauxi*) were THC, DHC, lactate, calcium and potassium; variability among these was mostly explained by an interaction between the sampling day and treatment of *S. verreauxi* (\( p < 0.01 \); Table 11). Specifically, mean THC and DHC among trapped *S. verreauxi* were greater than those from control and hand-collected individuals on day 0, and then were mostly relatively lower across the remaining days (Figure 10a and b). Mean lactate and potassium concentrations were greatest among day 0 samples for hand-collected *S. verreauxi*, but were similar to those from the control and trapped individuals on all other days (Figure 11a and b). Mean calcium concentrations were lowest for trapped and hand-collected individuals on days 1, 3 and 7 (Figure 11c).
Figure 10. Adjusted mean (GLMM) (a) total and (b) differential haemocyte counts ($\times 10^5$) for control (black; $n = 22$), trapped (grey; $n = 54$) and hand-collected (white; $n = 92$) *Sagmariasus verreauxi*. 
Table 11. Summary of fixed factors tested in generalised linear mixed models for significant effects on haemolymph parameters among all monitored *Sagmariasus verreauxi* and those that were damaged (total haemocyte counts – THC; differential haemocyte counts – DHC; refractive index – RI). ns: not significant.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>THC</th>
<th>DHC</th>
<th>Initial RI</th>
<th>Glucose</th>
<th>Lactate</th>
<th>Calcium</th>
<th>Magnesium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>All <em>S. verreauxi</em></td>
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<tr>
<td>Treatment of <em>S. verreauxi</em> (T)</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
<td>ns</td>
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<td>Sex</td>
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<tr>
<td>Moult stage</td>
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<td>Initial CL</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>Handling time</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<td>Day sampled (D)</td>
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<td>Damaged <em>S. verreauxi</em></td>
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<td>Total appendage loss</td>
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<td>Wound type (W)</td>
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<td>ns</td>
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<tr>
<td>D × W</td>
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</table>

* p < 0.05, ** p < 0.01, *** p < 0.001.
Figure 11. Adjusted mean (GLMM) concentrations (mmol l\(^{-1}\)) of (a) lactate, (b) potassium and (c) calcium for control (black; \(n = 21\)), trapped (grey; \(n = 52\)) and hand-collected (white; \(n = 79\)) *Sagmariasus verreauxi.*
There was no interaction between the treatment of *S. verreauxi* and sample day for initial RI, glucose or magnesium concentrations (GLMM, \( p > 0.05 \); Table 11). Similarly, there were no significant differences in haemolymph clotting between groups (GLM, \( p > 0.05 \)), but of those *S. verreauxi* sampled on days 0, 1, 3 and 7 (\( n = 168 \)) only 22% clotted. The 59 individuals that moulted had greater clotting capacity; almost half (48%) clotted five days after moultning, but on the final sampling day only 22% of *S. verreauxi* (all combined) haemolymph clotted.

A reduced GLMM assessing only damaged *S. verreauxi* revealed a significant interaction between the sample day and wound-type for THC (\( p < 0.05 \); Table 11). For the sampled days, individuals with unsealed wounds had THC comparable to those that were not wounded, but lower than those with sealed wounds (Figure 12). A significant effect of sample day was found for DHC and potassium concentrations, while DHC and magnesium were mainly affected by wound type (GLMM, \( p < 0.05 \); Table 11). Specifically, mean DHC on day 1 (3.8 \( \times 10^5 \)) was greater than all other days (3.4 vs. 2.9 vs. 2.2 \( \times 10^5 \)) and *S. verreauxi* with sealed wounds had more granular cells (4.2 \( \times 10^5 \)) than intact individuals (2.4 \( \times 10^5 \)) and those with unsealed wounds (2.6 \( \times 10^5 \)). Individuals with unsealed wounds had lower mean magnesium concentrations (7.9 mmol l\(^{-1}\)) than those with sealed (9.6 mmol l\(^{-1}\)), or no wounds (8.5 mmol l\(^{-1}\)). Mean potassium concentrations were significantly greater on day 0 (10.0 mmol l\(^{-1}\)), but were then comparable for all other days (8.7 vs. 8.3 vs. 8.2 mmol l\(^{-1}\)). No other factors were significant (GLMM, \( p > 0.05 \); Table 11).

4.3.4 Moultng and growth

Appropriate GLMMs were applied to first test the hypothesis of no significant differences among the WWs of intact trapped, hand-collected and control *S. verreauxi* (none of which moulted between their initial capture and the start of the experiment). Then, we assessed factors explaining variability among the propensity of *S. verreauxi* to moult (which never occurred more than once for any individual), the time when this occurred and subsequent growth (CL in mm). For the first and last GLMMs, the initial CL was included as a covariate.
Figure 12. Adjusted mean (GLMM) total haemocyte count (×10⁵) for hand-collected *Sagmariasus verreauxi* with no wounds (black; *n* = 42), or wounds that were sealed (grey; *n* = 46) or unsealed (white; *n* = 11) over the four sampling days. No samples were taken for unsealed wounds on day 0.

A reduced GLMM of only intact individuals indicated that the predicted mean initial WW of hand-collected *S. verreauxi* (327 g) was significantly less than those of the trapped (393 g) and control individuals (396 g; *p* < 0.05). Moultng rates were significantly affected by (*p* < 0.05; Table 12) and dependent on (*χ² = 35.9, df = 6; *p* < 0.01) both treatment and moult stage. In total, moulting occurred among 22.7 and 46.3% of control and hand-collected *S. verreauxi* respectively, and significantly more frequently than among trapped individuals (2.2%; GLMM, *p* < 0.001). Further, late pre-moults (moult stage D₂ and D₃₄) had a significantly greater propensity to moult (100%), and post-moults a significantly lower propensity (0.0%), than both the inter-moults (C; 14%) and early pre-moults (D₀–D₁; 45%).
Table 12. Summary of fixed factors tested in generalised linear mixed models for significant effects on the moulting and growth of control, trapped (pooled) and hand-collected *Sagmariaus verreauxi* (carapace length – CL). ns: not significant.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>Propensity to moult</th>
<th>Days to moult</th>
<th>Post-moult CL</th>
<th>Regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of <em>S. verreauxi</em></td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Sex</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Moult stage</td>
<td>*</td>
<td>***</td>
<td>**</td>
<td>ns</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01, *** p < 0.001.

Days to moulting was significantly affected by moult stage (GLMM, p < 0.001), with *S. verreauxi* in stages C, D₀–D₁ and D₂–D₃–₄ averaging 57.6, 33.5 and 5.8 days respectively. Variability among subsequent growth (post-moult CL) was significantly affected by sex and initial moult stage (p < 0.05; Table 12). Females had significantly lower mean post-moult CLs than males (95.5 vs. 97.2 mm). Individuals in moult stages D₂ and D₃–₄ tended to have larger mean CL increases (99.9 and 97.7 mm) than all other moult stages (93.3–96.6 mm).

Of the 27 damaged hand-collected *S. verreauxi* that moulted, 11 regenerated all of their missing appendages (although slightly smaller in size). Six of these individuals were moult stages D₀–D₃–₄, but regeneration was not significantly influenced by the treatment of *S. verreauxi*, sex or moult stage (Table 12). For damaged *S. verreauxi* (moult stages D₀–D₃–₄ combined), the average time until moulting among those that regenerated missing appendages was 60.2 ± 18.0 d compared with 17.8 ± 2.5 d for those that did not. Two individuals (moult stage C) did not regenerate appendages, but all others did (including the most damaged individual). An abridged GLMM assessing moulting and growth for damaged *S. verreauxi* only, showed that post-moult CL was significantly affected by total appendage loss (p < 0.05), which was negatively correlated with growth increments. All other fixed factors were independent of the damage parameters (p > 0.05).
4.4 Discussion

Quantifying unaccounted fishing mortality is difficult, but understanding the relative importance of the various subcomponents is essential to validate management controls enforcing size and species selectivity, and especially discarding (He, 2010). While the absence of any discard mortality in this experiment is positive, like in previous studies (e.g. Joll, 1977; Brock et al., 2003) there was within-trap predation which, along with the observed physical and, to a lesser extent, physiological impacts to some discarded *S. verreauxi* might be sufficient to warrant mitigation strategies. Before prioritising the latter, it is necessary to consider the level of sub-lethal impacts to *S. verreauxi*, and their possible consequences beyond the limitations of this study.

Compared to hand collection, both traps caused few physical impacts to *S. verreauxi*. This result might reflect the comparatively benign capture mechanism, whereby individuals passively entered, fed and were then retrieved to the surface; probably with minimal interactions among conspecifics (e.g. low catch densities) and/or the trap meshes. Conversely, hand-collected *S. verreauxi* were physically removed (often with considerable force) from within and between rock crevices.

Such method-specific differences were probably sufficient to explain much of the bias in physical damage among hand-collected discards, but non-technical factors, including the sizes and pre-capture condition of individuals might also be important. For example, *S. verreauxi* were hand collected across proportionally smaller sizes than those that were trapped. While CL was not a significant predictor of appendage loss among *S. verreauxi*, previous studies have identified positive correlations between the size of trapped palinurids and appendage loss (e.g. Brouwer et al., 2006). Perhaps more importantly, trap catches were biased towards inter-moults, which are the individuals most likely to actively feed (Miller, 1990), and therefore might be in better physical condition than their pre- and post-moult conspecifics. In contrast, hand-collected *S. verreauxi* encompassed a broader range of the population including pre-ecdysial individuals, which were possibly more vulnerable (Powrie and Tempero, 2009); a conclusion supported by the relatively lower initial WWs of this group.
Despite physical damage to more than half of the hand-collected *S. verreauxi*, none died. However, two important caveats in interpreting this result are that the discards were protected in aquaria cages, and regularly offered food without competition or energy expenditure. At least some deaths could occur among damaged individuals discarded in the wild, with missing appendages potentially impacting on key chemical (e.g. sensory perception) and/or mechanical processes (e.g. mobility; Juanes and Smith, 1995); particularly locating shelter after release (Brown and Caputi, 1983). As one example of such impacts, Parsons and Eggleston (2005) observed that Caribbean spiny lobster (*Panulirus argus*) missing one antenna and two pereopods had compromised predator avoidance and self/cooperative defences; all of which increased their vulnerability to predation until subsequent regeneration (Juanes and Smith, 1995). More than 27% of hand-collected *S. verreauxi* in this study lost a similar combination (or both antennae) of appendages. Perhaps more importantly, this was associated with poor haemolymph clotting, which could attract predators while repelling conspecifics (Parsons and Eggleston, 2005).

Although few trapped *S. verreauxi* were physically damaged, like their hand-collected conspecifics, they incurred quantifiable physiological impacts. However, irrespective of the capture method, the haemolymph chemistry changes were transitory, and probably would have few long-term effects on discards released into the wild. Nevertheless, the observed variations provide some insight into the reactions of *S. verreauxi* to capture-and-discarding mechanisms, and may help to prioritise appropriate stress indices for future studies.

In particular, changes to THC and DHC appeared to provide a coherent measure of explainable short-term impacts. Specifically, the observed initial elevation in THC among trapped individuals could have reflected their increased cardiovascular activity during retrieval (Jussila et al., 2001). Combined with stress from confinement under shallow sunlit water (in the presence of predators), this may also explain short-term elevations in their THC (see Hamann, 1975). Further, the short-term elevation in DHC among hand-collected individuals was likely in response to damage, which
mobilized granular cells. Stress, trauma or disease generally consume these cell types and reduce counts in crustaceans (Perazzolo et al., 2002; Wang and Chen, 2006), however a preceding increase is sometimes reported (e.g. Pascual et al., 2003).

The similarity in DHC among groups on day 7 indicated that the responses of hand-collected *S. verreauxi* were sufficient to prevent any infection or ongoing deterioration in cell counts. But like for physical damage, the question that remains is: what might occur outside the homogenous conditions of the aquaria (e.g. during low-salinity pulses)? Further research is required to elucidate the connection between the different cell types of *S. verreauxi* and their responses to changes in health and/or vigour under natural conditions.

Other measured haemolymph parameters were less definitive and/or sensitive to perturbations than THC and DHC. The elevated lactate among hand-collected individuals on day 0 may have been due to increased activity from handling resistance, yet the concentrations were low compared with the extremes possible (see Ocampo et al., 2003; Haupt et al., 2006). In any case, *S. verreauxi* certainly were not fatigued to the point of exhaustion (with the corresponding implications for predation or infection). It is also possible that the initial increased potassium concentrations among hand-collected individuals similarly reflected stress (e.g. Ulhmann et al., 2009) but, like for lactate, the short duration indicated that internal homeostasis was resumed quickly. By comparison, lower calcium concentrations in both trapped and hand-collected *S. verreauxi* (after day 0) than controls may have reflected differences in moult stage, including the relationship between bound calcium and circulating protein (Greenaway, 1985).

Beyond the immediate physical and short-term physiological impacts, there were more protracted consequences for the moulting and growth of some discarded *S. verreauxi*. Most of these differences were explained by the initial moult stage, and occurred irrespective of the fishing method. For example, discarded late pre-moults were clearly committed to moulting, and did so, but without
complete appendage regeneration (i.e. some were missing). Conversely, some of the discarded damaged inter- and early- pre-moults (predominantly hand collected) had comparatively delayed moulting, but did regenerate their appendages. However, such damage and the subsequent redirection of energy for limb regeneration usually results in decreased growth for palinurids (Brouwer et al., 2006). Similar impacts on growth have been reported for other crustaceans, but this may reflect injury rates (Juanes and Smith, 1995).

Despite the observed resilience of *S. verreauxi* to withstanding the capture-and-discarding mechanisms assessed in this study, the possibility for at least some discard predation discussed above, combined with the unnecessary capture and handling of sub-legal individuals warrants simple resolution strategies for both fishing methods. An obvious starting point for traps would be to regulate mesh size to more appropriately correspond to the morphology of a legal-size *S. verreauxi*. Alternatively, strategically positioned escape gaps (Treble et al., 1998; Arana et al., 2011) might be beneficial, especially for round traps which have asymmetrical mesh openings throughout, precluding the utility of a minimum mesh size. By allowing unwanted individuals to escape, such modifications would also reduce within-trap predation, which is a common problem among palinurid fisheries (Joll, 1977; Brock et al., 2003). Predation might also be mitigated through the use of so-called “two-chambered” traps that attract octopus away from confined lobsters (Brock et al., 2006).

Improving hand-collection size selection is more difficult. While it is possible to immediately measure and release undersized hand-collected *S. verreauxi* near their capture site (while underwater), because they are often partially hidden prior to capture, even very small specimens will still be handled and possibly damaged. Free divers should be encouraged to avoid contact with the antennae and, wherever possible, hold *S. verreauxi* by the carapace to minimise pereopod loss. Alternatively, the use of so-called “hand snares” (a pole and loop of twine designed to pass over the abdomen of lobsters) may warrant assessment, since Powrie and Tempero (2009) showed that, compared to gloved
hands, such gears significantly reduced appendage loss among southern rock lobster (*Jasus edwardsii*).

Other simple handling-and-release procedures might minimise the predation of discarded *S. verreauxi*. For example, O’Malley (2008) described a small release cage into which tagged Hawaiian lobster (*Panulirus marginatus*) were placed before it was lowered to the seabed and opened, thereby protecting discards from pelagic sharks and teleosts. The same concept might be an inexpensive solution for vessel-based trappers discarding *S. verreauxi*. Similarly, free divers could simply return illegal (or unwanted individuals) to the seabed and, if possible, back to their den.

The above changes to gear, fishing and operational techniques could minimise impacts to unwanted *S. verreauxi*, although more quantitative data are still required to assess the extent of potentially important subcomponents of unaccounted fishing mortality, including within trap predation, ghost fishing and also the fate of trapped discards for the commercial fishery in deeper waters. Obtaining such information, and developing coherent applied responses, will improve resource management. In the interim, the data presented here support size limits and quotas for helping to control the exploitation of *S. verreauxi* caught in shallow water off NSW.

Acknowledgements

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Chapter 5 – Prelude

Chapter 5 has been peer-reviewed and published in the international journal *Fisheries Research* (full citation details below). Chapter 5 is presented as it was published, except for where: i) small changes were required to maintain thesis coherence (e.g. in the Figure numbering) and ii) other minor textural changes were made to satisfy examiners comments.

The purpose of this Chapter was to determine the relative selectivities and efficiencies of popular recreational blue swimmer crab traps. A concurrent assessment of injury, physiological stress and short-term mortality among discarded *P. pelagicus*, and teleost bycatch, was used to quantify the immediate and short-term impacts to both groups. The objective was to identify potential mitigation practices and gear modifications that could reduce deleterious impacts, while assessing the validity of current management presumptions.

Please cite Chapter 5 as:

CHAPTER FIVE

Blue swimmer crab
Chapter 5: Relative trap efficiency for recreationally caught eastern Australian blue swimmer crab (*Portunus pelagicus*) and associated injury and mortality of discards

**J.C. Leland**1,2, P.A. Butcher1, M.K. Broadhurst1, B.D. Paterson3, D.G. Mayer4

1NSW Department of Primary Industries, Fisheries Conservation Technology Unit, National Marine Science Centre, PO Box 4321, Coffs Harbour, New South Wales 2450, Australia

2Marine Ecology Research Centre and National Marine Science Centre, School of Environment, Science and Engineering, Southern Cross University, PO Box 157, Lismore, New South Wales 2480, Australia

3Department of Agriculture, Fisheries and Forestry, Bribie Island Research Centre, PO Box 2066, Woorim, Queensland 4507, Australia

4Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, GPO Box 267, Brisbane, Queensland 4001, Australia

**ABSTRACT**

Australian recreational fishers targeting *Portunus pelagicus* are regulated by unverified gear restrictions which, combined with size, sex and bag limit regulations, result in >40% of their catches being discarded; all with unknown fate. To address these issues, we investigated the relative efficiency and temporal selectivity of “round”, “rectangular” and “wire” pots, and “hoop nets” set for 3, 6, and 24 h and the subsequent injury, physiological stress and mortality (in cages with controls over three days) of discarded *P. pelagicus* (37–85 mm carapace length – CL). Undersized (<60 mm CL) and ovigerous *P. pelagicus* comprised 22 and 4% of the total catch. Irrespective of soak time, round pots caught significantly more *P. pelagicus* and teleost bycatch. Five percent of individuals lost 1–3 appendages, usually during disentanglement, and only 1.1% of discarded *P. pelagicus* died (all within 24 h). Haemolymph parameters were mostly affected by biological, rather than technical factors. The results support the mandatory discarding of *P. pelagicus*, but pot selectivity might be improved via escape vents or larger mesh sizes.
5.1 Introduction

For many exploited aquatic species, unaccounted fishing mortality can represent a substantial component of their total fishing mortality (ICES, 2005) and accurate estimation is essential for effective stock management (King, 2007). More than six sub-components of unaccounted fishing mortality have been recognised. However, most attention has focused on the mortality caused during discarding (or “release”); not only because such estimates often are the easiest to acquire, but also because generally they are assumed to comprise the greatest proportion of unaccounted deaths (Broadhurst et al., 2006a).

Among recreational fisheries, most discard mortality studies have involved teleosts, reflecting not only concerns about effort and subsequent stock sustainability, but also the welfare of survivors (Bartholomew and Bohnsack, 2005; Arlinghaus et al., 2007). Until recently, much less attention had been directed towards assessing the fate of recreationally discarded crustaceans (Parsons and Eggleston, 2005; Butcher et al., 2012; Leland et al., 2013a, 2013b); perhaps owing to their relatively greater resilience to associated stressors (Wassenberg and Hill, 1993) and/or their perceived inability to feel pain (Elwood et al., 2009).

Like teleosts, crustacean discard mortality can be directly estimated over the short term (Wassenberg and Hill, 1993; Broadhurst et al., 2009), although finer-scale analyses of physiological impacts are also required to assess the potential for delayed mortality (Uhlmann et al., 2009; Leland et al., 2013a, b). Specifically, quantifying haemolymph constituents can identify changes in internal homeostasis (Poole et al., 2008; Uhlmann et al., 2009), which often correlate with discard-related stressors (e.g. air exposure and appendage loss) that can alter immunocompetence. Deviation from normal physiological function can be identified by quantifying the proportions of circulating haemocytes (Perazzolo et al., 2002), clotting time (Jussila et al., 2001), protein (by refractive index – RI) (Dall, 1975), glucose (Butcher et al., 2012) and lactate (Leland et al., 2013a). Such parameters can provide useful indices of possible longer-term impacts (e.g. infection), although the associated
cost-benefit relationships must be considered (Jussila et al., 2001; Uhlmann et al., 2009). Therefore, the consideration of previously applied measures is warranted; the utilities of which are often species- or procedure-specific (Uhlmann et al., 2009; Butcher et al., 2012).

Australian recreational fishers target various marine crustaceans (Henry and Lyle, 2003). One species for which there are concerns about unaccounted fishing mortality is the blue swimmer crab (*Portunus pelagicus*). *Portunus pelagicus* are widely distributed throughout the Indo-West Pacific (Lai et al., 2010). Recently, using morphometric, morphological and genetic characteristics, Lai et al. (2010) revised *P. pelagicus* into four species, including *P. armatus*, *P. reticulatus* and *P. segnis*. However, for consistency, and because this revision did not assess reproductive compatibility and was not conclusive, in this study we report on *P. pelagicus*.

*Portunus pelagicus* are distributed throughout all Australian states (except Tasmania and Victoria), and are targeted by recreational fishers; predominantly using traps (which include both enclosed “pots” and open “hoop nets” – Figure 13). Recently, “round” and “rectangular” collapsible pots have been introduced and their popularity is increasing among both recreational and commercial fishers (Campbell and Sumpton, 2009; Butcher et al., 2012; Figure 13a and b), although the traditional rigid “wire” pots and hoop nets are still used (Figure 13c and d).

Owing to input control regulations (e.g. trap type, dimensions and mesh size, and minimum legal size, sex limits and quotas for *P. pelagicus*), >40% of the national recreational *P. pelagicus* catch (~3.9 million individuals p.a.) is discarded, representing the greatest proportion for any local crustacean (Henry and Lyle, 2003). Despite such discarding, few studies have assessed either the relative selectivity (i.e. for legal *P. pelagicus*) or efficiency (i.e. for total *P. pelagicus* catch) of existing recreational traps (to validate mesh sizes and/or configurations – but see Bellchambers and de Lestang, 2005; Boutson et al., 2009), or the associated damage and mortality of discards. Accurately
Figure 13. Diagrams and dimensions of (a) round, (b) rectangular and (c) wire pots and (d) hoop nets used to target *Portunus pelagicus* in Australia.
quantifying mortality among discarded undersized and ovigerous individuals is required for stock assessments (King, 2007).

It is well established that *P. pelagicus* caught and discarded from commercial trawls and gillnets can be injured and subsequently die, although the estimated impacts vary (Wassenberg and Hill, 1989, 1993; Broadhurst et al., 2009; Uhlmann et al., 2009). For example, Broadhurst et al. (2009) observed minimal injuries and low mortality (6%) among mature *P. pelagicus* discarded from gillnets after up to 2.5 h of air exposure. Uhlmann et al. (2009) reported that manually removing appendages from juvenile *P. pelagicus* (<25 mm CL) during disentanglement and discarding caused unsealed wounds, increased haemolymph loss and treatment-specific mortalities of up to 50%. Although similar wound data were not collected for trawled-and-discarded *P. pelagicus*, Wassenberg and Hill (1989, 1993) reported that up to 51% were injured (1–3 appendages missing) and mortalities approached 15%. These studies provide some evidence of a broad correlation between the extent of wounding and mortality.

Beyond the potential for some wounding among recreationally trapped-and-discarded *P. pelagicus*, there are other concerns. In particular, ovigerous *P. pelagicus* occur throughout the fishing season (in all relevant states) and their abundances generally peak during early summer (e.g. Potter et al., 2000; Johnson et al., 2010), before they leave the estuaries to spawn (Potter and de Lestang, 2000). Although size, quota and sex regulations vary, discarding is mandated in all states. Further, ovigerous female discards may have an increased propensity for fishing-related damage (see Bellchambers et al., 2005).

Given the above, the aims of this study were first to determine the relative selectivity and temporal efficiency of common recreational traps targeting *P. pelagicus*. Second, we sought to investigate the potential for physical injury, physiological effects and short-term mortality of discarded *P. pelagicus*
(with particular attention to undersized and ovigerous individuals) and also the immediate impacts to teleost bycatch.

5.2 Materials and methods

5.2.1 Study location and traps used

The study was done within a ~7 km² radius in Wallis Lake (32°19’18”S, 152°30’10”E); an estuary in New South Wales (NSW), Australia. Starting 16 November 2010, fifteen replicates of four common recreational *P. pelagicus* traps were used: (i) collapsible “round pots”; (ii) collapsible “rectangular pots”; (iii) rigid “wire pots”; and (iv) “hoop nets” (Figure 13; Table 13). All traps had their meshes measured (inside stretched mesh opening – SMO) to ensure within treatment uniformity, and were submerged in a similar estuary for >3 h before use to reduce the potential for confounding impacts on efficiency. On each fishing day (*n* = 14), the traps were baited with ~0.5 kg of sea mullet (*Mugil cephalus*) placed inside a wire-mesh bait bag (250 × 200 mm, with 10 × 10 mm mesh), before five replicates of each were set at random locations ~50 m apart to ensure independence (Williams and Hill, 1982) for 3, 6 and 24 h.

5.2.2 Experimental protocol

After each trap was retrieved, *P. pelagicus* and bycatch were concurrently removed, with the former being either emptied or untangled, and the time taken for these processes recorded. Non *P. pelagicus* bycatch (i.e. teleosts and other crustaceans) were identified, measured (for CL or total length – TL, nearest 1.0 mm), sexed if possible, classified as alive or dead (i.e. responsive or unresponsive to an external stimulus) and then released. Traps were assessed for damage (i.e. broken meshes) after each retrieval and any damaged traps were repaired or replaced.

All trapped *P. pelagicus* were measured with vernier callipers (nearest 1.0 mm) for CL (the distance between the frontal notch and the posterior carapace margin) and carapace width (CW – the distance between the tips of the ninth antero-lateral spines). Moult stage was assessed following Hay
Table 13. Technical specifications for round, rectangular and wire pots, and hoop nets used to target *Portunus pelagicus*. SMO – stretched mesh opening, Ø – diameter, \( n \) – number of entrances.

<table>
<thead>
<tr>
<th>Traps</th>
<th>Mesh Material</th>
<th>Shape</th>
<th>Material Ø (mm)</th>
<th>Nominal SMO (mm)</th>
<th>Entrance Type</th>
<th>( n )</th>
<th>Dimensions (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoop net</td>
<td>Polyamide monofilament</td>
<td>Diamond</td>
<td>0.5</td>
<td>150</td>
<td>Open mesh</td>
<td>1</td>
<td>750 Ø</td>
</tr>
<tr>
<td>Wire pot</td>
<td>Steel wire</td>
<td>Rectangular</td>
<td>2.0</td>
<td>75</td>
<td>Open funnel</td>
<td>2</td>
<td>30 × 15</td>
</tr>
<tr>
<td>Rectangular pot</td>
<td>Polyethylene multifilament</td>
<td>Square</td>
<td>1.0</td>
<td>90</td>
<td>Open ‘V’-shaped funnel</td>
<td>2</td>
<td>55 × 28</td>
</tr>
<tr>
<td>Round pot</td>
<td>Polyethylene multifilament</td>
<td>Diamond</td>
<td>1.0</td>
<td>50</td>
<td>Semi-closed funnel</td>
<td>4</td>
<td>30 × 20</td>
</tr>
</tbody>
</table>
et al. (2005): (i) early inter-moult – moderately flexible shell and some wear on chelae; (ii) late inter-moult – little or no flex in shell, and/or large, significant wear on chelae; or (iii) post-moult – clean and highly flexible shell, no wear on chelae. Sex was assessed, with the reproductive condition (i.e. ovigerous or non-ovigerous) of females noted. The pleonal flap of all *P. pelagicus*, and egg masses from visibly ovigerous females, were assessed for macroscopic damage. If present, the length (1.0 mm), or volume (mm$^3$) of the affected areas were recorded. Individuals were categorised as either “intact” (undamaged), “injured” (damaged appendages, carapace, pleonal flap or egg masses), or “previously damaged” (damage unrelated to our trapping). The position, type and number of any missing appendages (i.e. chelae, pereopods and swimmerets) were determined and these injuries were classed as “sealed” or “unsealed” following Uhlmann et al. (2009). Some intact *P. pelagicus* (37–72 mm CL) were immediately (within 20 s) sampled for haemolymph (see 2.3. below) following Butcher et al. (2012). Owing to insufficient numbers (for statistical analysis), injured individuals were not bled.

All *P. pelagicus* were then placed into individual 20 l polyvinyl chloride containers (PVC – 300 cm diameter (Ø) × 400 cm depth) with 18 mm Ø holes in the sides (n = 15) and lids (n = 5) to facilitate water exchange (termed “cages”; see Butcher et al., 2012) and their intervening handling time (“removal time”) recorded. The cages were immediately transported to a monitoring site (in the lake), where each was attached (~1 m apart) to a 300 m bottom-set line (in <2 m depth). During the experiment, other intact *P. pelagicus* (37–76 mm CL) were caught at night in <2 m depth using a spotlight and knotless PVC net, transported to the monitoring site and used as controls. Some undersized controls were also immediately sampled for haemolymph (see 2.3. below) prior to being caged.

All trapped-and-discarded and control *P. pelagicus* were monitored in the cages for three days. Any individuals that moulted or died during this period were noted, with the latter immediately removed. All survivors (i.e. trapped and control) were released with the distal tip of the left epipodite
(from the first maxilliped) removed to facilitate identifying recaptures during the study. This method was chosen instead of t-bar tagging because the latter often causes mortality (McPherson, 2002).

5.2.3 Haemolymph sampling

All haemolymph samples were taken through the ventral sinus of either fifth pereopod using a small-gauge syringe. Non-repetitive samples were taken from a subset of the trapped-and-discarded and control P. pelagicus; either immediately after trapping (termed “day 0”) or at the end of monitoring (termed “day 3”). Haemolymph samples were subsequently assessed for total (THC – \( \times 10^5 \)) and differential haemocyte counts (DHC – \( \times 10^5 \)) using a NEUBAUR™ haemocytometer under light microscopy (at 100\( \times \)) and the differentiation technique outlined by Butcher et al. (2012). Refractive index (Leavitt and Bayer, 1977) was determined using a VetQuip™ VQ5600 refractometer and converted to protein (mg ml\(^{-1}\)) following the procedures outlined by Butcher et al. (2012). Haemolymph samples were frozen in liquid nitrogen for subsequent analysis of glucose (mM) and lactate (mM) using an OLYMPUS™ AU400 automated clinical analyser and clotting time (s) was quantified with the modified capillary tube method of Jussila et al. (2001) (see also Leland et al., 2013b).

5.2.4 Data analyses

Generalised linear mixed models (GLMM) or general linear models (GLM) (McCullagh and Nelder, 1989) were appropriately applied, using the GLMM procedure from GenStat (2010). Where GLMMs were not converged because of sparse (or extreme) data, a simplified GLM was adopted. We tested the hypothesis of no differences in: (i) relative efficiency for P. pelagicus and teleosts among the four treatment traps; and (ii) physical damage; and (iii) haemolymph constituents among trapped and control P. pelagicus. Fixed factors included “treatment of P. pelagicus” (i.e. trapped-and-discarded or control crabs), “trap type”, “soak time” (i.e. deployment duration), “day sampled”, “CL”, “moult stage” and “sex” (and interactions where appropriate). To encompass the temporal variability in sampling, “capture date” was included as a random factor in all relevant models.
Parametric models with the normal distribution (and transformation when necessary) were suitable for most parameters, but the Poisson (with a log-link) and logistic models were used for counts and binomial data, respectively. Where significant fixed factors exceeded two levels, differences among means were explored using post-hoc t-testing with the GenStat (2010) RPAIR procedure.

The independence of the treatment (trapped vs. controls) of *P. pelagicus* and the total numbers surviving at the end of the experiment was investigated using the Fisher’s exact test. Pearson’s chi-square test was used to analyse contingency tables of counts. Adjusted means (± SE for normal models only) are presented under equal weighting from all terms in the models. Undersize *P. pelagicus* catches were analysed as both absolute (i.e. number of undersize) and proportional values (i.e. percentage undersize per trap type). The null hypotheses were rejected at *p* < 0.05.

### 5.3 Results

#### 5.3.1 Relative trap selectivity and temporal efficiency

In total, 277 *P. pelagicus* were caught (37–85 mm CL), of which 61 (22%) were undersized (37–59 mm CL) and 10 (4%) were ovigerous (62–72 mm CL). Irrespective of soak time, round pots were the most efficient (predicted mean of 0.89 individuals per trap), followed by rectangular pots, hoop nets and wire pots (0.20, 0.17 and 0.07 individuals per trap). No significant associations were found between trap type and sex ratio (χ² = 4.72, *df* = 3; *p* > 0.05), or moult stage (χ² = 3.73, *df* = 6; *p* > 0.05).

Catches of undersized (absolute and proportional), legal and total *P. pelagicus* varied significantly among trap types and within soak times (GLM, *p* < 0.05; Table 14; Figure 14). Specifically, compared to all other traps, round pots set for 6 and 24 h caught significantly more individuals in all categories (except for rectangular pots set for 6 and 24 h) (*t*-test, *p* < 0.05; Figure 14a, b and c). Within trap types, round and rectangular pots set for 24 h caught significantly more total *P. pelagicus* than those set for 3 and 6 h, while hoop nets reached their maximum efficiency after being set for 6 h.
(t-test, p < 0.05; Figure 14a). Total catches for wire pots were similar, irrespective of soak time (t-test, p > 0.05; Figure 14a). Absolute undersized catches from round and rectangular pots set for 6 h were similar (t-test, p > 0.05) and significantly greater than those from all wire-pots and hoop-nets (t-test, p < 0.05; Figure 14b). Compared with round and rectangular pots, hoop nets caught proportionally fewer undersized *P. pelagicus* (t-test, p < 0.05; Figure 15), while wire pots caught the same proportion as all other designs (t-test, p > 0.05; Figure 15).

The total non *P. pelagicus* bycatch comprised three giant mud crab (*Scylla serrata* – 84, 88 and 96 mm CL), and eight teleost species (n = 101; Table 15) and was significantly, and independently, affected by trap type and the soak time (GLMM, p < 0.001; Table 14). Irrespective of soak time, round pots caught more teleosts than the other traps (t-test, p < 0.05). For all traps combined, the amount of teleost bycatch was positively correlated with soak time (t-test, p < 0.05). Only one individual (tarwhine, *Rhabdosargus sarba*) died, and was observed being eaten by a *P. pelagicus* in a wire pot.

5.3.2 Trap clearance and injuries to discards

The time required to clear traps was significantly affected by *P. pelagicus* entanglement (mostly in hoop nets) (GLM, p < 0.001), but not soak time (GLMM, p > 0.05). Compared with all other traps, hoop nets were significantly more likely to have meshes broken (t-test, p < 0.05), with 1.3 ± 0.3 10.6 ± 0.3 and 5.7 ± 1.1 damaged meshes per trap set for 3, 6 and 24 h, respectively.

Only 15 (5%) *P. pelagicus* (52–79 mm CL) were injured (with 1–3 missing appendages, pleonal flap and/or ovarian damage) and of these, six had unsealed wounds (to their appendages or pleonal flaps). The wounded included one ovigerous and three non-ovigerous females and 10 males (two were undersized). *Portunus pelagicus* were never entangled (i.e. restricted by meshes) in wire pots, but this occurred in round (10%) and rectangular pots (41%), and hoop nets (94%), with respective mean disentanglement times of 23.0 ± 4.5, 58.4 ± 16.7 and 99.4 ± 12.5 s. Two round pot trapped individuals
Table 14. Summary of fixed factors analysed with generalised linear models to explain variability in absolute and proportion undersized (<60 mm CL), legal (≥ 60 mm CL) and total *Portunus pelagicus* catches, and teleost bycatch, from four trap types set for 3, 6 and 24 h soak times. ns – not significant.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>Absolute undersized</th>
<th>Proportion undersized</th>
<th>Legal</th>
<th>Total</th>
<th>Teleost bycatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trap type (T)</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Soak time (S)</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>T × S</td>
<td>***</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>ns</td>
</tr>
</tbody>
</table>

* p < 0.05, *** p < 0.001.
Figure 14. Mean (a) total, (b) absolute undersized and (c) legal catches of *Portunus pelagicus* by round, rectangular and wire pots, and hoop nets set for 3, 6 and 24 h (*n* = 5 replicate traps per soak time). Dissimilar letters indicate significant differences (*t*-test, *p* < 0.05).
had damaged pleonal flaps with 15 mm of the distal tip missing; injuries that occurred during the soak and removal, respectively. The only ovigerous female with a damaged egg mass (~10 mm³) was hoop netted.

There were no significant effects of trap type or soak time on chelae, pereopod and swimmeret injuries, nor total appendage loss (GLMM, \( p > 0.05 \)). There were no significant interactions between trap types and their soak time on injuries (GLMM, \( p > 0.05 \)). Twelve percent of the catch had previous damage (1–3 missing appendages). No discarded *P. pelagicus* were recaptured.

**Figure 15.** Percentage catches of undersized *Portunus pelagicus* from the four trap types. Dissimilar letters indicate significant differences (*t*-test, \( p < 0.05 \)).
Table 15. Total number (n) and mean total length (TL in mm ± SD, where relevant) of teleosts caught with each trap. -, none caught.

<table>
<thead>
<tr>
<th>Teleosts</th>
<th>Round pot</th>
<th>Rectangular pot</th>
<th>Wire pot</th>
<th>Hoop net</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>TL</td>
<td>n</td>
<td>TL</td>
</tr>
<tr>
<td>Toadfish (<em>Tetractenos</em> spp.)</td>
<td>34</td>
<td>156 ± 18</td>
<td>1</td>
<td>148</td>
</tr>
<tr>
<td>Yellowfin bream (<em>Acanthopagrus australis</em>)</td>
<td>20</td>
<td>137 ± 18</td>
<td>2</td>
<td>133 ± 17</td>
</tr>
<tr>
<td>Tarwhine (<em>Rhabdosargus sarba</em>)</td>
<td>20</td>
<td>129 ± 12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Silver biddy (<em>Gerres subfasciatus</em>)</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Striped catfish (<em>Plotosus lineatus</em>)</td>
<td>1</td>
<td>344</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leather jackets (<em>Monocanthus spp.</em>)</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>257 ± 46</td>
</tr>
<tr>
<td>Dusky flathead (<em>Platycephalus fuscus</em>)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Large tooth flounder (<em>Pseudorhombus arsius</em>)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
5.3.3 Mortality and monitoring

None of the control and only three trapped *P. pelagicus* died (all males and within 24 h of discarding), providing a non-significant mortality of 1.1% (Fisher’s exact test, \( p > 0.05 \)). Two fatalities were from hoop nets set for 6 and 24 h, and one was from a round pot set for 24 h. None of the three mortalities lost appendages, but one had an injured pleonal flap (with loss of anal tissue and an unsealed wound) and one sustained internal trauma (i.e. caused by concussion during measurement), while the other was uninjured. Removal times for these individuals were 2, 45 and 95 s. None of the monitored ovigerous *P. pelagicus* had changes in their ovarian condition, and all individuals vigorously swam away when released. Three non-ovigerous females (one injured) moulted during monitoring.

5.3.4 Haemolymph parameters

A preliminary GLMM showed no significant interaction between the treatment of *P. pelagicus* (control and trapped combined) and the day sampled for any haemolymph parameters (\( p > 0.05 \)). The treatment of *P. pelagicus* significantly affected their lactate and protein concentrations (GLM and GLMM, \( p < 0.05 \); Table 16). Specifically, mean lactate was significantly greater in *P. pelagicus* caught with wire pots (1.79 mM), than controls (0.60 mM) and those from round and rectangular pots (0.66 and 0.56 mM), and hoop nets (0.60 mM) (\( t \)-test, \( p < 0.05 \); Figure 16a). *Portunus pelagicus* caught with wire and rectangular pots had significantly greater protein concentrations (75.19 and 60.02 mg ml\(^{-1}\)) than those from round pots and hoop nets (46.57 and 37.67 mg ml\(^{-1}\)), and controls (42.12 mg ml\(^{-1}\)) (\( t \)-test, \( p < 0.05 \); Figure 16b).

Irrespective of the treatment of *P. pelagicus*, their glucose and lactate concentrations and DHC varied significantly between the two sampling days, and all by similar magnitudes (GLM and GLMM, \( p < 0.05 \); Table 16). Glucose was significantly greater in *P. pelagicus* sampled on day 3, than those sampled on day 0 (0.5 vs. 0.3 mM), while both lactate and DHC were significantly greater on day 0 than day 3 (1.1 vs. 0.6 mM and 12.2 vs. 6.8 \( \times 10^5 \)) (\( t \)-test, \( p < 0.05 \)).
Table 16. Summary of fixed factors tested in generalised linear (lactate and clotting time only) and mixed models for significant effects on haemolymph parameters among all monitored *Portunus pelagicus* and then only those that were trapped. THC – total haemocyte count, DHC – differential haemocyte count, CL – carapace length, ns – not significant.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>THC</th>
<th>DHC</th>
<th>Protein</th>
<th>Glucose</th>
<th>Lactate</th>
<th>Clotting ability</th>
<th>Clotting time</th>
</tr>
</thead>
<tbody>
<tr>
<td>All <em>P. pelagicus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment of <em>P. pelagicus</em></td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Day sampled</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Trapped <em>P. pelagicus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Moult stage</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Sex</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Day sampled</td>
<td>*</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001.*
Figure 16. Concentrations of haemolymph (a) lactate and (b) protein for control *Portunus pelagicus* and those caught by round, rectangular and wire pots, and hoop nets (across all soak times and sampling periods). Dissimilar letters indicate significant differences (*t*-test, \( p < 0.05 \)).

A reduced GLMM (i.e. omitting the controls) was used to assess the influence of biological factors and sampling day on the haemolymph parameters of only trapped *P. pelagicus*. Differential haemocyte counts were significantly and positively associated with CL (GLMM and *t*-test, \( p < 0.05 \); Table 16). Glucose and protein concentrations were significantly influenced by moult stage (GLMM, \( p < 0.01 \); Table 16), with the former significantly greater for post-moults (0.43 mM) and late inter-moults (0.50 mM), than their inter-moult conspecifics (0.35 mM – *t*-test, \( p < 0.05 \)). Protein was significantly greater in inter-moults and late inter-moults (53.7 and 58.2 mg ml\(^{-1}\)), than in post-moult individuals (40.0 mg ml\(^{-1}\) – *t*-test, \( p < 0.05 \)). Haemolymph clotting was significantly affected by sex
(GLM and GLMM, $p < 0.05$; Table 16) and was proportionally less common (58 vs. 92%), and took longer (23.9 vs. 52.9 s) for females. The day sampled significantly influenced glucose and lactate concentrations, THC and DHC – following the same trends as those in the complete models ($t$-test, $p < 0.05$; GLMM, $p < 0.001$; Table 16), except for THC, which was greater in day 0 samples.

5.4 Discussion

This study represents the first assessment of the relative selectivity and temporal efficiency of recreational traps for *P. pelagicus*, and the concomitant impacts to discards. While the low injury rates and mortality of discarded *P. pelagicus* validate existing management practices for controlling exploitation, simple changes to the design and/or operation of traps could minimise unwanted impacts. Relevant modifications can be identified by considering the potential mechanisms contributing towards the observed trap efficiency, and the subsequent effects on discards.

The efficiency of portunid traps is particularly sensitive to entrance design, but typically those with multiple, semi-closed openings are the most effective (Vazquez Archdale et al., 2006, 2007; Butcher et al., 2012). The same trend was observed here, with relatively greater catches in round pots; a characteristic that was also probably affected by their shape and/or small mesh size (Butcher et al., 2012). More specifically, because these traps were circular, all of the semi-closed entrances were close together (only ~200 mm apart), maximising bait access. Further, although the SMO of round pots measured ~50 mm (Table 13), their lateral openings were considerably smaller (only ~10–15 mm) and corresponded to the maximum width of a ~110 mm TL yellowfin bream, *Acanthopagrus australis* (Broadhurst et al., 2006b). Given the mean sizes of retained *A. australis* (~140 mm TL – Table 15), and other morphologically similar species (i.e. *R. sarba* and silver biddy, *Gerres subfasciatus*), and the fact that mesh openings were smaller than the narrowest observed *P. pelagicus* carapace depth, it is unlikely that many similar- or larger-sized teleosts, or *P. pelagicus* escaped from round pots.
Similarly, although rectangular pots were less efficient than round pots, which was probably due to fewer and more open entrances, their lateral mesh openings were also considerably less than their SMO (i.e. 50 vs. 90 mm). Such an effect probably explains the relatively larger proportions of undersized *P. pelagicus* and teleost bycatch retained by rectangular traps. Boutson et al. (2009) found that escape vents in collapsible rectangular pots (similar to those used here) reduced undersized *P. pelagicus* catches, and other bycatch (by 59 and 38%, respectively) without affecting legal-size catches. Similar modifications and/or increases in mesh size at strategic locations in the pots assessed here might improve their selectivity.

In addition to technical modifications, simply regulating the soak time of hoop nets could reduce their environmental impacts; both in terms of size selectivity and also the potential for lost netting. In this study, hoop nets were more selective than pots for legal-sized *P. pelagicus*, with maximum efficiency achieved at some point before 6 h. Such results may indicate density-dependant changes to entry or escape rates, probably because of the relatively small surface area (i.e. ~750 cm Ø × 300 cm) and the associated risk of antagonistic interactions among netted conspecifics (Smith and Hines, 1991). It is also possible that, owing to the large mesh size (i.e. ~150 mm), some small *P. pelagicus* were less securely entangled and were able to escape. Butcher et al. (2012) reported similar selectivity for hoop-netted *S. serrata*. Given the similarities in hoop-net selectivity among often sympatrically distributed NSW portunids, soak-time restrictions (e.g. <3 h) would reduce the potential for impacts from damaged hoop nets, while having minimal effects on catches.

Another consideration regarding hoop nets is the possibility of injuries, particularly among ovigerous females. In this study, 10 ovigerous females were caught and while only one was damaged, it was hoop netted. Injuries to *P. pelagicus* egg masses have been identified previously (see Bellchambers et al., 2005), but detailed studies are lacking. Given the substantial spatial and temporal variation reported for ovigerous *P. pelagicus* (Bellchambers and de Lestang, 2005; Johnson et al.,
2010) and the prevalence of mature females in trap catches (Smith et al., 2004), future research is warranted to determine if a stronger bias towards injuries exists among these individuals.

Hoop nets were not the only trap that injured *P. pelagicus*, and most appendage loss occurred during disentanglement. The tendency for crustaceans to autotomise appendages, as an escape strategy, is well known (e.g. Smith and Hines, 1991; Wasson et al., 2002) and although the subsequent changes to *P. pelagicus* growth rate are generally transitory, overall growth can be reduced (Paterson et al., 2007). Autotomy was observed in the current study (57% of injuries), but less frequently than for *S. serrata* discarded from the same traps by Butcher et al. (2012). *Portunus pelagicus* are comparatively less likely to autotomise appendages during handling, thus disentanglement can result in unsealed injuries (Uhlmann et al., 2009). Nevertheless, only 15% of all *P. pelagicus* were injured and very few died, a relationship that supports previous studies assessing the short-term fate of this species after discarding from other gears (Wassenberg and Hill, 1989, 1993; Broadhurst et al., 2009; Uhlmann et al., 2009).

The observed physiological responses of trapped *P. pelagicus* suggests few protracted impacts and/or mortalities beyond the monitoring period. For example, compared to previously reported ranges for *P. pelagicus* (Uhlmann et al., 2009) and *S. serrata* (Poole et al., 2008; Butcher et al., 2012), the observed lactate and protein concentrations in this study were low. Further, the effect of wire traps on lactate was caused by substantially elevated concentrations (~2 and 4 times greater) for two outliers. Individuals caught with rectangular and wire pots had relatively greater, and less competitive, bait access (without risk of entanglement), which may have lengthened the time for metabolic processes and the corresponding increase in haemolymph protein (Dall, 1975).

Nevertheless, post-trapping THC, and sex-specific clotting differences indicated that some stress occurred. Although stress generally reduces crustacean haemocyte counts (Uhlmann et al., 2009), initial THC elevations have also been reported (Pascual et al., 2003; Leland et al., 2013b). The
temporary increases in THC observed here were attributed to elevated within-trap cardiovascular activity (Jussila et al., 2001; Leland et al., 2013b), and may have been affected by intra-specific aggression. Aggressive behaviour may also partially explain the observed clotting impairments among female *P. pelagicus*, which have not been reported previously and are probably also indicative of stress (see Jussila et al., 2001; Uhlmann et al., 2009). Such changes may be important because they potentially could increase post-discard haemolymph loss and predation.

Most of the remaining haemolymph parameters were more affected by experimental and/or biological factors than trapping. For example, irrespective of treatment, DHC, glucose, and lactate varied between the two sampling times (and by similar magnitudes) — probably in response to caging and the subsequent reduced activity (for glucose and lactate) along with some confinement stress (Dall, 1975; Taylor et al., 2009). Given that only intact *P. pelagicus* were sampled, it is unlikely that observed alterations in DHC were indicative of an immunological response to trauma (or infection) (Perazzolo et al., 2002). Further, the observed relationship between CL and DHC, and lower protein and glucose concentrations among post- and inter-moult *P. pelagicus*, probably were related to cyclic changes associated with moulting (Dall, 1975; Chang, 1995); effects that have been reported for other crustaceans (Butcher et al., 2012; Leland et al., 2013b).

While the observed stress, injury and short-term mortality to discarded *P. pelagicus* in this study were all low, further research may be required to quantify other potential unwanted impacts of trapping. In particular, the greater relative efficiency and poor selectivity of round pots demonstrates a disparity between designs that affects discarding and effort regulation in the relevant states (i.e. NSW, Queensland and the Northern Territory). Further, owing to their performance, the popularity of round pots among both recreational and commercial fishers is exceeding that of other designs and assessing their ghost-fishing potential (i.e. before and after modification) is a priority. Such work should also encompass the other traps, particularly hoop nets. The indirect impacts of derelict traps may represent an important component of unaccounted fishing mortality for several key NSW species.
Acknowledgements

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CHAPTER SIX
General Discussion
Chapter 6: General Discussion

Substantial marine and freshwater crustacean fisheries exist around the world (FAO, 2010) and ~12% of the world’s population participates in recreational fishing (Cooke and Cowx, 2004). Discards in recreational fisheries are the functional equivalent of commercial fisheries bycatch discards and are increasingly being recognised as a conservation problem (Alverson et al., 1994; Granek, 2008). Quantification of this unaccounted fishing mortality subcomponent is needed (Cooke and Cowx, 2004). Accordingly, this study investigated stress assessment methods, trap selectivity and efficiency, injury, physiology and mortality of baited-trap discards. Here Chapters 2–5 are considered in terms of their commonalities, exceptions and limitations, along with the implications for recreational fisheries management and further research priorities.

6.1 Trap selectivity and efficiency

In their current form, the traps assessed here require simple modifications to improve their selectivity in terms of both target and non-target catches. The traps used to target S. serrata and P. pelagicus in this study were substantially less selective than those used for S. verreauxi and caught large amounts of teleosts. Recently, Rotherham et al. (2013) tested the utility of fitting escape vents to the round traps assessed here and reported significant reductions (i.e. 53–78%) in unwanted A. australis catches and identified the ideal gap size for minimising teleost bycatch. Grubert and Lee (2013) and Broadhurst et al. (2014) both demonstrated the effectiveness of installing escape vents into round collapsible pots to reduce undersized S. serrata catches (and bycatch), without negative effects on legal catches. Further, Broadhurst et al. (2014) reported a similar negative relationship between increased mesh size and undersized catches, although this approach also reduced legal crab catches. Similarly, escape vents are known to reduce undersized P. pelagicus catches (e.g. Boutson et al., 2009). Given the increasing popularity of round collapsible pots, their widespread deployment in sensitive juvenile fish habitats and the weight of evidence presented against them in their current form (Campbell and Sumpton, 2009; Butcher et al., 2012; Leland et al., 2013a; Rotherham et al., 2013)
regulatory changes in NSW requiring the fitting of escape vents, or increasing minimum mesh size, for both recreational and commercial traps are warranted. Similarly, catches of undersized lobster (and bycatch) in NSW could be mitigated through the enforcement of legislation mandating escape vents (e.g. Arana et al., 2011).

Unfortunately, hoop nets cannot be fit with escape vents to improve their selectivity. Fishers targeting *S. serrata* or *P. pelagicus* in NSW are allowed to possess a single pot, or up to five hoop nets (Tables 1 and 3). Although the disparity between these trap possession limits is justified in terms of catching efficiency, there might be other negative indirect consequences. For example, compared with collapsible pots, hoop nets are relatively cheap (e.g. ~AUD$27.00 vs. 6.00) and easily damaged and fishers might be more inclined to discard broken nets into the environment where they can adversely affect other estuarine species (Moore, 2008; Possatto et al., 2011). Further, traps are often lost and it is conceivable that a fisher using five hoop nets might be more likely to lose traps, than another fishing a single pot. Research is needed to investigate hoop net ghost fishing and the relationship between trap cost and pollution. Problems associated with hoop nets have been previously reported (Bartleet et al., 1993) and the relative simplicity with which other popular traps (e.g. round collapsible pots) can be modified indicate that the use of hoop nets should be banned in NSW.

### 6.2 Discard mortality

The absence of discard mortality for *S. serrata* and *S. verreauxi* and low mortality for *P. pelagicus* reported here is encouraging and supports enforced discarding to manage recreational crustacean-trap fisheries in shallow water. However, the isolated monitoring approach (i.e. of caged individuals) taken here is not absolutely representative of wild discarding. Decapods discarded into very shallow water (e.g. only 1–2 m depth) with generally homogenous bottom surfaces can quickly shelter under the substrate (Kirkwood and Brown, 1998). However, lobster discarded into the wild must descend through relatively deeper water (i.e. from 1–10 m), often in a fatigued state, before making an exposed transit to find cover (Brown and Caputi, 1983; Harris and Ulmestrand, 2004; Harris and Andrews,
2005). Such factors would have implications for lobster predation that were not investigated here (e.g. Brown and Caputi, 1983) and further research over different spatial and temporal scales is warranted.

One caveat for the mortality estimates reported here pertains to the consistently careful removal of crabs from traps; particularly hoop nets. Appendage loss, wound type and mortality can vary significantly depending on the removal method (e.g. slow and careful disentangling vs. quick and forceful removal) (Kennelly et al., 1990; Kirkwood and Brown, 1998; Uhlmann et al., 2009). For example, treatment-specific mortality of blue swimmer and spanner crabs removed and then discarded from commercial netted gears (i.e. gill and hoop nets) ranged upwards to 50 and 100%, respectively (Kennelly et al., 1990; Uhlmann et al., 2009). The potential for interaction between fisher behaviour and mortality was not thoroughly investigated in the present study, thus the mortality results probably underestimate actual mortality in the respective fisheries. Further studies should consider a broad range of fisher’s behaviours and how they might influence mortality among recreationally discarded crustacean species.

6.2.1 Physical injury and predation

Beyond the immediate need to seek shelter are other concerns, particularly for discarded decapods with missing appendages. Trapping caused comparatively few physical injuries among S. verreauxi and P. pelagicus (4 and 5% of their respective catches), but often caused appendage loss for S. serrata (18% of catch). Given the substantial anatomical and behavioural differences between lobsters and crabs and their interaction with different traps in the present study, such differential injury proportions might be expected (Kennelly et al., 1990; Powrie and Tempero, 2009; Uhlmann et al., 2009). However, the differences in physical injury among the relatively anatomically and behaviourally similar P. pelagicus and S. serrata (i.e. 5 vs. 18%), caught using the same traps and deployment durations, were probably influenced by differential tendencies for limb autotomy.
The regenerative capacity of crustaceans is well known, but multiple claw loss in natural populations (i.e. unfished) is generally rare (Smith and Hines, 1991; Juanes and Smith, 1995) and few studies have assessed the mortality of discarded crabs missing one or both claws (Barber and Cobb, 2007; Patterson et al., 2007). However, claw loss can cause competitive disadvantages among crabs (e.g. reduced defensive capacity) (Smith and Hines, 1991). Given the above, and the prevalence of antagonistic and cannibalistic behaviours among many crustaceans (Juanes and Smith, 1995; Møller et al., 2008; Wall et al., 2009), it is probable that some clawless *S. serrata* and *P. pelagicus* discarded into the wild would die. Similarly, lobsters missing one or both antennae are more susceptible to predation (Parsons, 2005; Parsons and Eggleston, 2005) and further research assessing such injuries is needed.

Generally, active collection (e.g. hand gathering) causes more injury than passive methods (e.g. trapping) (Cochrane, 2002; Ridgway et al., 2006; Powrie and Tempero, 2009). Previous studies have reported similar (or fewer) injuries among lobsters caught by SCUBA divers (Powrie and Tempero, 2009; Frisch and Hobbs, 2011) and physical injuries among deeper water hand-collected lobsters are unlikely to be an issue, because of regulatory (i.e. free diving depth restrictions) and natural limitations (i.e. breath-holding ability). However, such high injury rates clearly indicate the need for mitigation methods to reduce the impacts from hand collection, particularly when the increasing numbers of recreational fishers are considered. Practical investigations into innovative methods to avoid surface discarding (e.g. O’ Malley, 2008) for both trapped and hand-collected lobster are needed and might reduce the potential for immediate predation.

6.2.2 Physiological stress

The haemolymph analysis indices identified in Chapter 2 were used to definitively quantify sub-lethal stress in recreationally discarded *S. serrata*, *S. verreauxi* and *P. pelagicus*. The utilities of particular haemolymph parameters were species specific and strongly influenced by biological (e.g. moult stage and size) and environmental factors. Similar findings have been reported for commercial
discards (e.g. Poole et al., 2008; Uhlmann et al., 2009). Poor haemolymph clotting among recreational discards (i.e. irrespective of injury), has not been reported previously and is a good indicator of sub-lethal stress. In particular, the reduced clotting capacity of most *S. verreauxi* and female *P. pelagicus* raises other concerns for individuals discarded into the wild. For example, injured portunids and palinurids can attract predators (including conspecifics) (Parsons and Eggleston, 2005; Wall et al., 2009) and protracted clotting would increase haemolymph loss, while increasing the potential for attraction. Because *S. verreauxi* were discarded into very shallow depths (i.e. only up to 1 m) for captive monitoring, further research assessing the effect of depth on the haemolymph loss of injured individuals is required.

### 6.3 Growth

Although the results reported here for *S. verreauxi* showed no effect of injury on growth, further studies are warranted that assess the growth of injured-and-discarded lobster and crab in fished populations. Numerous studies have documented the negative impacts of injury on palinurid and portunid growth (e.g. Davis 1981; Brown and Caputi, 1983; Brouwer et al., 2006; Paterson et al., 2007). However, similar to the *S. verreauxi* growth results reported here, Frisch and Hobbs (2011) found no differences in growth between injured-and-discarded Australian painted spiny lobster (*Panulirus versicolour*) and their intact conspecifics, noting that the lack of negative impacts was probably due to non-repetitive injury (Frisch and Hobbs, 2011). Repetitive injury is positively correlated with fishing pressure and fished crab and lobster populations often show cumulative growth effects that differ from those that are unfished (e.g. Davis, 1981; Frisch and Hobbs, 2011). Further research is required to assess injury rates and their effect on growth among recreationally and commercially discarded crustaceans.

### 6.4 Ghost fishing

Another unaccounted fishing mortality subcomponent that requires consideration is ghost fishing. Although not specifically investigated here, the potential for any lost traps to “ghost fish” is probably
substantial. For example, Campbell and Sumpton (2009) reported that large numbers (~6 000) of similar, round collapsible pots (but with two open-funnel entrances) are lost annually in Queensland by professional fishers. Such derelict gears have considerable ghost-fishing potential (e.g. catching 223 $P. pelagicus$ per pot p.a.) and can persist for up to 5–10 years (Campbell and Sumpton, 2009). While perhaps less obvious, even small pieces of lost netting from hoop nets have negative environmental implications; either through entanglement, or ingestion by a range of other species (Moore, 2008; Possatto et al., 2011). While simple modifications to pots (i.e. increasing mesh sizes or installing escape vents), might reduce their ghost fishing (Campbell and Sumpton, 2009), such changes would be impractical for hoop nets. However, alternative trap components (e.g. corrodisable joints or biodegradable mesh) could be useful and warrant further research.

6.5 Summary conclusion

The low mortality reported here for $P. pelagicus$, and high survival for $S. serrata$ and $S. verreauxi$ justify the use of mandatory discarding to control the recreational exploitation of shallow-water crustacean stocks in NSW. Such enforced discarding is an effective management tool, but small changes to gear, fishing and operational techniques could minimise the negative impacts to recreationally discarded crabs and lobsters (and fish bycatch). Haemolymph clotting capacity can potentially be used to measure physiological changes caused by other factors including temperature, competitive interactions or habitat modification. Physiological stress and injury during capture-and-release may not cause substantial mortality among captive individuals, but could potentially compromise the survival of wild discards through increased predation and reduced competitiveness. Indirect impacts such as ghost fishing may represent an important component of unaccounted fishing mortality for several key NSW species and should be a priority for further investigative studies. The ultimate fate of crabs and lobsters discarded into the wild and in deeper water requires further investigation.
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