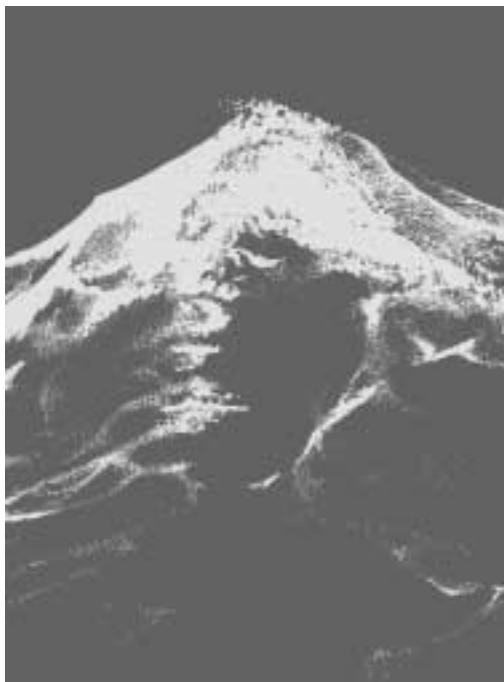


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Fate of juvenile school prawns, *Metapenaeus macleayi*, after simulated capture and escape from trawls

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Abstract. Two laboratory experiments were done to assess the fate of juvenile school prawns, *Metapenaeus macleayi*, after simulated multiple capture and escape from trawls. In the first experiment, prawns that were trawled and escaped one, five or 10 times, sustained some physical damage (mostly limited to the loss of antennae), but this was not significantly different from that sustained by control prawns that had not been trawled. Similarly, there were no significant differences between the different treatments and control prawns in their stress levels (as measured by changes in concentrations of L-lactate). Levels of L-lactate were greatest in all prawns immediately after the experiment started and then significantly reduced after 24 and 48 h. In the second experiment, treated prawns were trawled and escaped 10 times and then monitored for mortalities over 2 weeks. Compared with control prawns (that were not trawled), significantly more treated prawns died at the end of the 2 weeks, but the overall post-trawl survival rate was >89%. It is concluded that the multiple contact and escape of juvenile school prawns from trawls had minimal effect on their overall condition.

Extra keywords: by-catch, mesh size, mortality, penaeid, stress.

Introduction

Prawn trawling occurs in four estuaries in New South Wales, Australia, and is valued at approximately AU\$7 million per annum. Operators working in these fisheries mainly target school prawns, *Metapenaeus macleayi*, and, like the majority of prawn-trawl fisheries throughout the world, they also catch non-target organisms (termed by-catch; for reviews see Saila 1983; Andrew and Pepperell 1992; Alverson *et al.* 1994), comprising various small crustaceans, cephalopods and fish, which are usually discarded (Liggins and Kennelly 1996; Liggins *et al.* 1996). Over the past decade, various modifications to trawls (termed by-catch reduction devices) have been developed and legislated to improve selectivity and reduce by-catches of fish (for a review see Broadhurst 2000). An important issue that remains, however, involves the capture, discarding and subsequent mortality of small unwanted school prawns.

There is no minimum legal size for school prawns in New South Wales, although operators in most fisheries conform to industry-recommended 'counts', which can vary up to

approximately 150 to 180 prawns/500 g (i.e. mean weight of 3.3 to 2.7 g or mean carapace length (CL) of approx. 17 to 15 mm respectively). No formal studies have been done to quantify the selectivities of the minimum legal mesh sizes used in commercial trawls in New South Wales (45 mm in the trawl body and 40 mm in the codend), but it is apparent that large numbers of prawns smaller than the optimal size (i.e. <15 mm CL) are caught (Broadhurst and Kennelly 1996; Broadhurst *et al.* 1996) and then discarded. This is considered to be a major waste of prawn stocks, particularly since these small prawns could be expected to reach commercial size in a relatively short period of time (Glaister 1978).

It is well established that one of the simplest options for reducing unwanted by-catches of organisms that are conspecific to the targeted species in trawls, involves increasing openings in the codend, via alterations to the hanging ratio, size and/or shape of the meshes (e.g. MacLennan 1992; Reeves *et al.* 1992; Broadhurst *et al.* 1999). However, less information is available on the benefits

of these sorts of changes for prawn-trawl fisheries, since there has been very little quantification of the fate of those prawns that enter trawls and then escape. Such assessments should precede attempts to improve selectivity because, unless a large proportion of escapees survive, simple modifications to gears will have little benefit in preserving stocks. Further, in fisheries that have high densities of trawling effort across small spatial and temporal scales (as is the case in the New South Wales estuarine prawn-trawl fisheries; Liggins *et al.* 1996), small prawns are likely to repeatedly contact trawls and so these assessments should include an examination of the effects of multiple contact and escape.

Studies have shown that many variables affect the damage and mortality of organisms that contact and escape from trawls (Kaiser and Spencer 1995; Chopin *et al.* 1996). For many organisms, sublethal disruptions and physical trauma can be cumulative and may contribute to longer-term mortalities (e.g. via an increased susceptibility to pathogens). One method of quantifying the severity of stress incurred by escaping crustaceans is to determine lactic acid concentrations in their haemolymph. The typical escape response of crustaceans to trawls involves repeated abdominal contractions that propel the animal backwards (Newland *et al.* 1992). This activity uses reserves of arginine phosphate in the abdominal muscle (Onnen and Zebe 1983), which is then anaerobically replenished through glycolysis. This process leads to the accumulation of lactic acid, which must be cleared when activity returns to normal. Measuring the concentration of lactate and reductions over time can therefore provide some indication of the severity of stress.

Because of the need to determine the benefits that any changes in the meshes used in trawls may have on stocks of school prawns, our aim in this study was to provide a first examination of the effects of repeated capture and escape on their physical damage, stress and mortality.

Materials and methods

Equipment used

Two experiments were done at the Cronulla Fisheries Centre's aquarium facilities between September and November 2001 using two 4000-L fibreglass holding tanks and 25 smaller fibreglass tanks (200 L). All tanks were supplied with seawater (at ambient temperature, approx. 18°C) at a rate of 2 L min⁻¹, aerated using air-stone diffusers and equipped with outflow pipes (Fig. 1a), designed to maintain constant water levels. The smaller tanks contained 600 g of sand substratum (Fig. 1a) and were evenly distributed on opposite sides of an enclosed room with a regulated 12:12-h photoperiod.

Two identical aluminium frames were constructed so that they could be inserted over each of the outflow pipes in the smaller tanks and fit between the inside wall of the tank and the outside wall of the outflow pipe (Fig. 1b). The first frame was rigged with a loose panel of mesh (3-mm diameter braided polyethylene twine, 40-mm stretched mesh between the knots) and designed to represent the posterior section of a trawl codend. Links of 40-mm galvanized metal chain (the same size as that used in the footropes of commercial trawls) were attached around

the perimeter of this frame (Fig. 1b). The second frame was rigged with a tightly hung panel of the same-sized mesh as that described above attached anterior to a panel of fine-meshed polyethylene designed to prevent prawns from passing through (Fig. 1b). The two aluminium frames could be placed along side each other and rotated freely throughout the entire volume of the 200-L tanks (Fig. 1c).

Collection of prawns

Approximately 2500 juvenile school prawns (<20 mm CL) were captured in the Hawkesbury River (33°42'S, 151°15'E) using a prawn trawl rigged with a fine-meshed codend (knotless nylon, 10-mm mesh size) towed for less than 20 min in shallow water (depths of between 6 and 12 m). At the end of each tow, the codend was emptied onto a sorting container. Live juvenile school prawns were removed, placed in holding tanks on the vessel and supplied with oxygen. These prawns were transported to the aquarium facility at the Cronulla Fisheries Centre and transferred (using buckets) to the 4000-L holding tanks. Prawns were allowed to acclimatize in the large holding tanks for at least 8 days, during which they were fed a diet of commercial fish pellets (at a rate of approximately 5% of their biomass every second day). All dead prawns were immediately removed and recorded.

Experiment 1: analyses of physical damage and L-lactate after one, five and 10 trawls

Eight days after the prawns were captured (by which time all mortalities had stopped), the water level in one holding tank was lowered (to approx. 1000 L) and ice added to reduce the temperature to 12°C. This effectively anaesthetized the prawns. Two hundred and fifty prawns were selected at random, visually checked for signs of obvious physical damage (any damaged prawns were discarded) and placed in groups of 10 into each of the 25 smaller tanks (in the enclosed room). Because there was no evidence of any sexual dimorphism in the length/weight relationship of school prawns (see Results), individuals were randomly placed in tanks irrespective of their sex. These prawns were fed, monitored and left to acclimatize for a further 5 days. At the end of this period, 24 of the tanks were randomly assigned into four groups (three treatment groups and one control group) each with six replicate tanks. To maintain stocking densities throughout the experiments, prawns in the remaining tank (stock tank) were used to replace any mortalities in the treatment and control tanks. These prawns were marked for identification by cutting one uropod and excluded from all analyses.

On the first day of this experiment, the two aluminium frames were placed in the six tanks in treatment Group 1 and the frame with the 40-mm mesh and chain was rotated around each tank (Fig. 1c), so that all 10 prawns in each tank entered this panel and then passed through the meshes. To simulate repeated episodes of trawling and escape, this methodology was applied to the tanks in the remaining treatment groups and then repeated (with 3 min between successive 'trawls') five times in the six tanks holding treatment Group 2, and 10 times in the six tanks holding treatment Group 3. All of the control tanks simply had the two aluminium frames inserted and then removed without contacting any of the prawns.

At periods of 2 min, 24 and 48 h after the various procedures were done in the tanks, all prawns were removed from two randomly selected tanks in each of the three treatment groups and the control group (i.e. 10 prawns from two replicate tanks from each group after each period) using a scoop net. To restrict the activity of the prawns, they were removed along with a sufficient quantity of the sandy substratum in which they were buried.

Four randomly selected prawns from each tank were immediately secured in labelled aluminium satchels and kept frozen in liquid nitrogen for lactate analyses. Because of the significant costs involved, analyses of L-lactate were only done for three of these frozen prawns. Each of these prawns was ground to a homogenous powder in a mortar

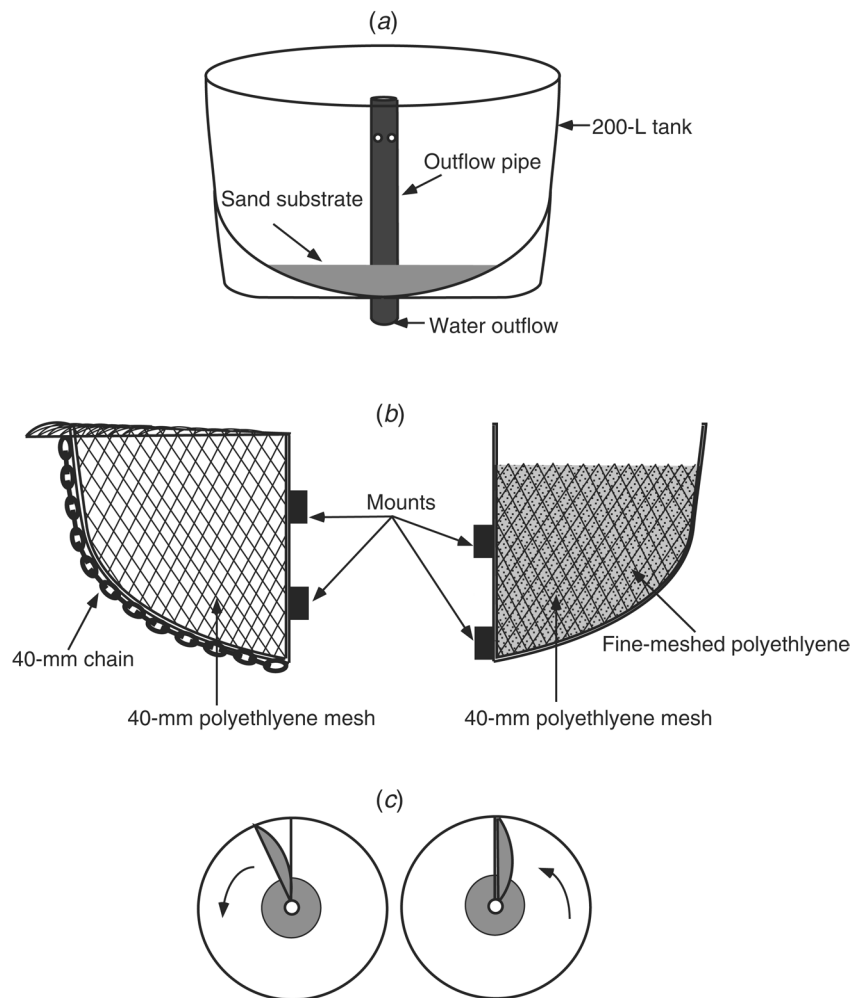


Fig. 1. Diagrammatic representation of: (a) the 200-L fibreglass tanks; (b) the aluminium frames and panels used in the experiments; and (c) the frames being rotated together in the treatment tanks.

that was pre-cooled with liquid nitrogen. Approximately 150 mg of the frozen, powdered tissue was then rapidly dispersed in 1.5 mL of 0.3 M perchloric acid in a 2-mL plastic centrifuge tube (by vortex mixing) and reweighed to determine the tissue mass. Each tissue-perchlorate suspension was centrifuged at 10000g for 10 min to compact the protein debris, and 1.5 mL of the supernatant was transferred to a new tube and kept frozen until neutralization. For neutralization, 1 mL of supernatant was added to 0.1 mL of 2 M KHCO_3 in a 2-mL plastic centrifuge tube, vortex-mixed and left on ice to allow the crystals to settle. The L-lactate concentration in each extract was determined spectrophotometrically using a Boehringer kit (Cat. no. 139 084) and a 200 mg L^{-1} L-lactate standard. The L-lactate concentration in each extracted prawn sample was calculated after correcting for dilution during neutralization and expressed in terms of $\mu\text{mol g}^{-1}$ of the homogenized prawn sample. The moisture content contributed by the sample to the extract volume after centrifugation was determined to be 71% of the sample weight.

Five of the remaining prawns from each tank were weighed, measured and visually assessed for physical trauma to their exoskeletons. Physical trauma was expressed in terms of percentage damage or loss (to the nearest 5%) for a total of 20 variables (Fig. 2).

With the exception of the rostrum and telson, all variables were examined on both sides of the prawns, and the means of these values were calculated to provide estimates of the damage for each variable. To provide an indication of total physical trauma for each prawn, we calculated the percentage of all 20 variables that showed evidence of any damage.

Experiment 2: analysis of mortality after 10 trawls

Experiment 2 was done immediately after Experiment 1 was completed. All prawns in the second 4000-L holding tank were anaesthetized as above. Two hundred were selected at random, individually checked for any signs of physical damage, removed and placed in groups of eight into each of the 25 smaller tanks. These prawns were fed and monitored as above and left to acclimatize for a further 5 days. At the end of this period, 24 tanks were labelled either as treatment or control tanks (i.e. 12 of each), and one was labelled as a stock tank.

The two aluminium frames were placed in all of the 12 treatment tanks and the frame with the 40-mm mesh panel and chain was rotated around each tank (as per the methodology described above). This was repeated 10 times (with 3 min between successive 'trawls'). All control

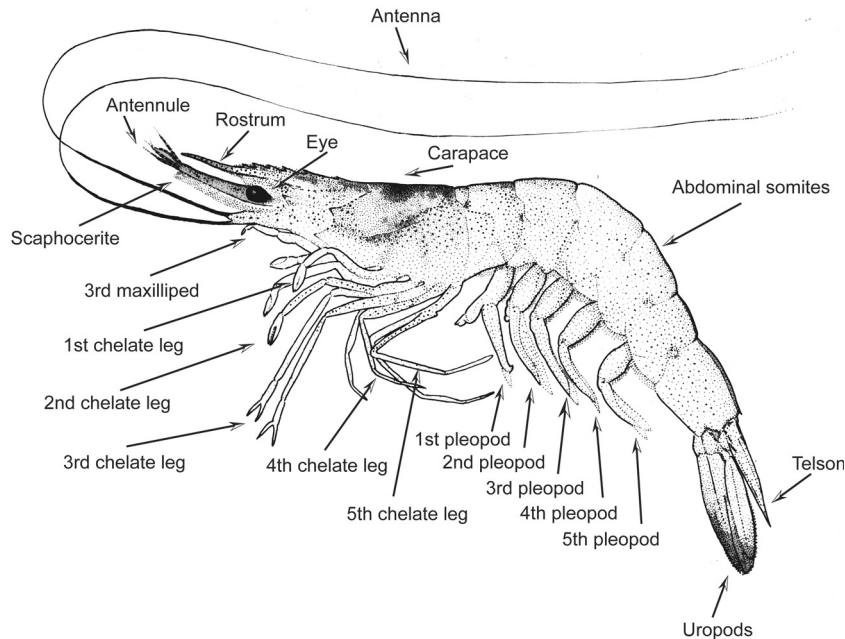


Fig. 2. Profile of a school prawn and the variables used to estimate physical damage.

tanks simply had the mesh panels inserted once and then removed without contacting any prawns. Over a period of 14 days, the prawns in all tanks were monitored twice daily for any mortalities. Where mortalities were detected, all dead individuals were removed from their tanks and replaced with the same number of live prawns (marked for identification by cutting one uropod) from the stock tank.

Statistical analyses

Using all available data, linear regressions of weight (g) and CL (mm) were calculated separately for males and females and then compared using the appropriate analysis of co-variance (this was done *a priori* to test the hypothesis of no sexual dimorphism). Two-sample Kolmogorov–Smirnov tests ($P = 0.05$) were used to compare the size-frequencies of prawns (pooled across sexes) between experiments.

In Experiment 1, where there were sufficient data for the various indicators of trauma (i.e. percentage physical damage and L-lactate concentrations), the appropriate ANOVA was used (Underwood 1981). In these analyses, the treatment (of prawns) and time (of sampling) were considered fixed factors and orthogonal to each other. Tanks were random (nested in treatment and time) and the data obtained from the randomly selected prawns per tank per sample time were the replicates. To increase the power of the test for the main effect of the treatment of prawns, where the F -ratio for tanks was non-significant at $P < 0.25$, the means squares for tanks and the residual were pooled to provide a new F -ratio denominator (Winer 1971). Significant differences detected in these analyses were investigated using Student–Newman–Keuls multiple comparisons.

The percentages of prawns surviving at the end of Experiment 2 (over 14 days) in each of the treatment and control tanks were calculated and compared using two-tailed t -tests. It was not possible to record mortalities over a shorter temporal scale (e.g. daily), because as individuals died, they were quickly consumed by the remaining individuals in the tanks (i.e. dead prawns disappeared before they could be counted). To examine the effects of repeated trawling on the growth of prawns, the CL and weight of a random sample of remaining prawns (six from each tank) at the end of Experiment 2 were analysed using the appropriate ANOVA. In these analyses, tanks were considered a random

factor nested in the treatment of prawns and the six prawns per tank were the replicates. In doing these analyses, we assumed that since there were no significant differences in the weight/length relationship between sexes (see Results) and all prawns were originally placed at random in each of the 200-L tanks (randomly assigned as either treatment or controls), the mean sizes of prawns in each of the tanks at the start of the experiment were the same.

Results

Prawns used in experiments

Approximately 900 of the 2500 prawns collected and placed in the 4000-L holding tanks died (97% of these mortalities occurred within 6 days of collection). Five prawns died (three as a result of jumping from tanks) during the acclimatizing period in the 200-L tanks for Experiment 1. There were no mortalities during the acclimatizing period for Experiment 2.

The sizes of prawns ranged from 8 to 19 mm CL and their distributions were not significantly different between experiments (Kolmogorov–Smirnov test). Analysis of co-variance failed to detect significant differences in regression coefficients ($F = 0.40$, $P > 0.05$) or elevations ($F = 0.05$, $P > 0.05$) between the regressions of logWt and logCL for males ($\log Wt = 2.8859 \log CL - 6.816$, $r^2 = 0.874$, $n = 331$) and females ($\log Wt = 2.9395 \log CL - 6.958$, $r^2 = 0.897$, $n = 140$). A common regression was therefore calculated as $\log Wt = 2.9045 \log CL - 6.8658$.

Experiment 1: analyses of physical damage and L-lactate after one, five and 10 trawls

Four prawns died within 4 h of the various treatments being done. Two of these mortalities occurred in tanks from

Table 1. Experiment 1: summary of the physical damage to the four prawns that died within 4 h

Prawn no.	Treatment group	Carapace length (mm)	Weight (g)	Variable and percentage missing
1	Trawled once	16	2.8	Antenna (left): 80%; rostrum: 100% ; scaphocerite (left): fifth chelete leg (left): 10%
2	Trawled five times	15	2.9	Antenna (left): 80%; antenna (right): 20%
3	Trawled five times	14	2.7	Antenna (left): 20%; antenna (right): 20%
4	Trawled 10 times	16	2.7	Antenna (left): 100%; antenna (right): 100%; antennule (left): 20%; antennule (right): 20%; scaphocerite (left): 10%; scaphocerite (right): 10%; second chelete leg (left): 100%

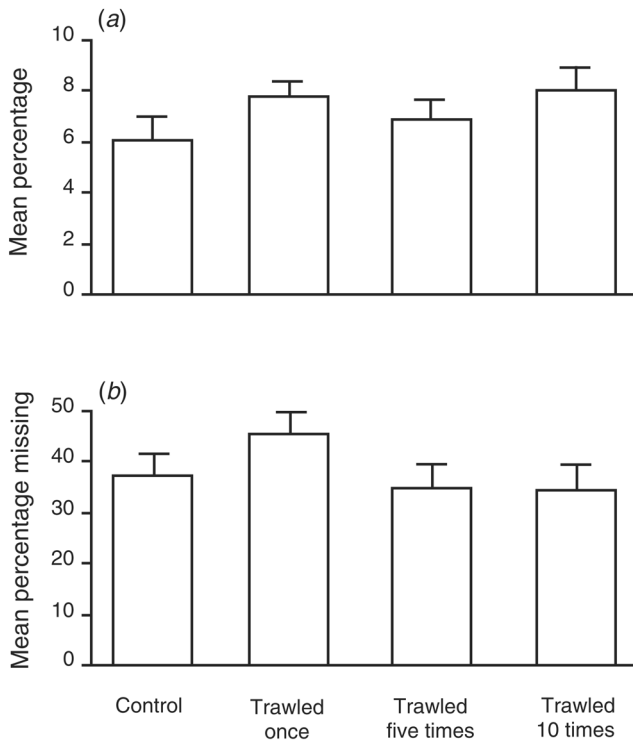


Fig. 3. Differences in mean percentage damage + 1 s.e. between the control and treatment prawns in Experiment 1 for: (a) total damage; and (b) antennae.

treatment Group 2 (trawled five times) and one each in tanks from treatment Groups 1 (trawled once) and 3 (trawled 10 times). Table 1 summarizes the physical damage sustained by these individuals. There were no mortalities in any of the control tanks.

Three samples of tissue (from prawns in different tanks) yielded results for L-lactate concentrations that were considered impossible (e.g. one negative and two outlying values) and attributed to errors incurred during processing. These values were substituted with the appropriate cell means and 3 df subtracted from the residual df. Subsequent ANOVA revealed that there were no significant differences for the main effect of treatment of prawns for levels of L-lactate or physical damage (Fig. 3; Table 2). A significant

effect of time after treatment was detected for L-lactate (Table 2) with Student–Newman–Keuls tests revealing that mean levels were elevated in all prawns at the 2-min sample time (mean ± s.e. = 7.78 ± 0.5 μmol g⁻¹) and then significantly lower at the 24-h (5.21 ± 0.36 μmol g⁻¹) and 48-h sample times (3.72 ± 0.32 μmol g⁻¹) (Fig. 4).

Experiment 2: analysis of mortality after being trawled 10 times

Significantly more prawns died in treatment tanks (12 individuals from seven tanks) than in control tanks (two individuals from two tanks) (*t*-value = -2.8, *P* < 0.05), providing an overall mortality rate of rate of 10.7% for prawns that were trawled and then escaped 10 times. The majority of dead individuals were at least partially consumed by the remaining prawns in the tanks, precluding any assessment of their physical damage. There were no significant differences detected between treatment and control prawns for their CL or weight at the end of this experiment (Table 3).

Discussion

Overall, the results from this study showed that juvenile school prawns sustained minimal damage, stress and mortality after multiple contact and escape from simulated trawls. These results support the current use of minimum mesh sizes as a management tool to minimize the fishing mortality of small, unwanted school prawns and provide justification for future studies that seek to improve trawl selectivity via modifications to increase mesh openings.

In Experiment 1, the physical damage sustained by prawns was not significantly different between the control and the various treatments (total mean damage ranged from 6% to 8%; Fig. 3a) and was limited mostly to the loss of antennae (mean reductions between 34.5% and 45.4%; Fig. 3b). These results imply that the effects on prawns as a result of confinement within the tanks were no different to the effects of the simulated trawling, regardless of the number of ‘trawls’ (i.e. one, five or 10). This result may be explained by the behaviour of prawns during their contact and escape from the simulated trawl. Before starting Experiment 1, all prawns were buried in the sandy

Table 2. Experiment 1: summaries of *F*-ratios from ANOVA to determine effects on damage and stress of prawns as a result of different treatments (i.e. control prawns compared with prawns that were trawled one, five and 10 times), time (after treatment) and tanks, and of Student–Newman–Keuls tests for the significant *F*-ratio for L-lactate detected for the effect of time

P* < 0.05; *P* < 0.01. Residual df were 96 and 45 for physical variables and L-lactate respectively. Pld indicates that the main effect for tanks was non-significant at *P* < 0.25 and the sums of squares pooled with the residual. df for the *F*-test for the main effect of treatment of prawns when tanks were pooled = 3, 108. L-lactate data were ln(*x* + 1) transformed. 2-min sample time > 24-h sample time > 48-h sample time

Source of variation	df	Total physical damage	Antennae	L-lactate (μmol g ⁻¹)
Treatment of prawns (TP)	3	1.29	0.77	2.24
Time (T)	2	2.96	0.93	14.84**
T × TP	6	1.73	0.32	0.43
Tanks	12	0.99 ^{pld}	1.83	2.41*

Table 3. Experiment 2: summaries of *F*-ratios from ANOVA to determine effects on the growth of prawns after 14 days as a result of different treatments (i.e. control prawns compared with prawns that were trawled 10 times) and tanks Pld indicates that the main effect for tanks was non-significant at *P* < 0.25 and the sums of squares pooled with the residual; df for the *F*-test for the main effect of treatment of prawns when tanks were pooled = 1, 144

Source of variation	df	Carapace length (mm)	Weight (g)
Treatment of prawns	1	1.11	2.15
Tanks	22	0.72 ^{pld}	0.59 ^{pld}
Residual	120		

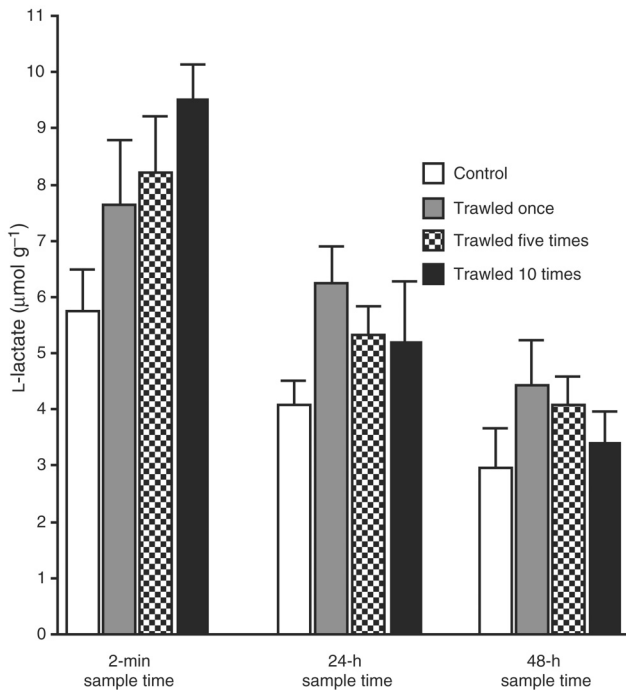


Fig. 4. Differences in mean L-lactate levels + 1 s.e. between the control and treatment prawns in Experiment 1 for each of the three sample times.

substratum. During the first rotation of the frame containing the 40-mm mesh panel and chain, prawns propelled themselves away and towards the second frame (containing

the 40-mm and fine-meshed panels). Most individuals flicked back and forth at least two or three times, ‘escaped’ through the meshes in the first frame and then immediately burrowed into the sand. Similar behavioural responses to the frames were observed during the second successive rotation in the relevant treatments (i.e. five and 10 trawls), but by the third and fourth rotations, all prawns appeared to be exhausted and made no attempt to escape the path of the frame with the 40-mm mesh panel and chain or to bury themselves into the substratum (i.e. most individuals remained in the water column). It is apparent, therefore, that regardless of the level of repeated trawling, nearly all of the limited damage to prawns was done during the first rotation of the frame.

Analyses of the concentrations of L-lactate in the haemolymph of prawns in Experiment 1 support this conclusion, with ANOVA failing to detect any significant differences in mean levels for the main effect of the treatment of prawns (Table 2). A significant difference was detected among time for all prawns, with the highest mean levels (overall mean ± s.e. = 7.78 ± 0.5) recorded immediately after the aluminium frames were removed (i.e. at the 2-min sample time) (Fig. 4). These observed L-lactate concentrations are greater than those reported from the muscles of exercising yabbies, *Cherax destructor* (Phillips *et al.* 1977; Head and Baldwin 1986), but similar to those recorded from anoxic prawns and crabs (although the latter can reach up to 15 or 20 μmol g⁻¹; Taylor and Spicer 1987; Hill *et al.* 1991).

Concentrations of L-lactate in all prawns were significantly reduced at 24 h ($5.21 \pm 0.36 \mu\text{mol g}^{-1}$) and again at 48 h ($3.72 \pm 0.32 \mu\text{mol g}^{-1}$), indicating a protracted recovery from stress. Although comparable observations have been made for other crustaceans (e.g. *Homarus gammarus*; Bridges and Brand 1980), most studies showed that tissue and/or haemolymph lactate levels in crustaceans returned to normal levels within approximately 12 h following anaerobiosis (e.g. Taylor and Spicer 1987; Spicer *et al.* 1990; Hill *et al.* 1991). One explanation for the results observed in the present study is that the levels of L-lactate at 48 h represent minimum baseline rather than routine levels of the metabolite in active school prawns. In support of this, Taylor and Spicer (1987) recorded levels of around $4 \mu\text{mol g}^{-1}$ wet weight in resting *Palaemon elegans* that were similar in size to the individuals examined here (sample weights of 0.84–1.98 g), while Paterson (1993) measured comparable levels in the muscles of chilled, immobilized penaeid prawns, before packing for export.

Although significantly more prawns died in treatment tanks (12.5%) than in control tanks (2.1%) after 14 days in Experiment 2, the overall post-multiple-trawl survival rate was >89%. It was not possible to examine the damage sustained by individuals that died during this experiment, as all were either partially or totally consumed by remaining prawns in the tanks. However, two of the four prawns that died during Experiment 1 showed substantial damage and in particular, breakages of the scaphocerites and chelete legs that were mostly distal to the plane of autotomy (Table 1). This damage was probably caused during contact with the 40-mm chain attached around the perimeter of the aluminium frame, and may have contributed to the mortality of some of the treatment prawns in Experiment 2. There were no significant differences between treatment and control tanks for the sizes and weights of individuals, so none of the treatment prawns that survived to the end of Experiment 2 were adversely affected in terms of their growth (Table 3).

Assuming that the results and their interpretations described in this laboratory study reflect what occurs in the field, we conclude that there are minimal deleterious impacts on juvenile school prawns as a result of multiple contact and escape through the meshes of prawn trawls. Simple increases in the mesh openings (to improve selectivity) can therefore be considered an appropriate means for reducing the fishing mortality of juveniles. However, it is important to remember that the results from the present study were limited to prawns encountering and escaping from simulated trawls. Other papers have described that many factors (e.g. differences in trawls, methods of operation, time spent in the trawl, the type and quantity of by-catch in the codend) can negatively affect the damage, stress and mortality of organisms escaping from trawls (e.g. Kaiser and Spencer 1995; Chopin *et al.* 1996). Future studies on the fate of post-trawled school prawns would benefit from an assessment of these effects.

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