Effects of Staining and Streamer Tags on Survival and Growth of Juvenile School Prawns, *Metapenaeus macleayi*, Under Laboratory Conditions

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Abstract

Juvenile school prawns from two size classes (carapace length 11-20mm and 21-30mm) were marked with a stain (fast green FCF solution) or tagged with steamer tags under laboratory conditions to test the hypothesis that tagging or marking affects the survival and/or growth of prawns. The stain accumulated in all parts of the prawn, but after 14 days it was visible only in the gills. All surviving tagged prawns at the end of each experiment had their streamer tags intact with tag information still visible. Tagging and staining significantly reduced survival, but there was no significant difference in mortality (40-50%) between these two techniques. Mortality was at its highest for all treatments in the first week of the experiments, reflecting the stress associated with handling the prawn and applying the tag or stain. Mortality was not size- or sex-dependent and marking or tagging did not appear to affect growth. While the low survival rates are of concern, this laboratory study suggests that streamer tags are the most suitable method for use in mark-recapture population studies for juvenile school prawns.

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Introduction

The tagging or marking of prawns are widely used techniques for identifying animals for demographic studies and have been used for many years to examine dispersal, rates of growth and mortality, as well as numbers of individuals in wild populations (Penn 1976; Xiao and McShane 2000; Loneragan et al. 2002). Mark-recapture studies that identify individual animals offer the greatest potential for accurately estimating population parameters provided the individual marks or tags are retained and any detrimental effects of the marking process are quantified (Burnham et al. 1987). The effect that tagging has on an individual, either from tag-induced mortality (including tag loss) and/or differences in growth needs to be quantified when analysing tag-recapture data, otherwise estimates of mortality and even rates of recruitment to the fishable stock can be grossly underestimated. Likewise, tagging studies that are used to estimate growth parameters need to account for the effects of tagging on growth of the tagged individual.

There have been various types of marks used to study penaeid prawns (Farmer 1981; Penn 1976). As with all crustaceans, the marking of prawns is difficult due to the process of ecdysis that leads to the periodic shedding of the exoskeleton. Earlier tagging methods for prawns included intramuscular dyes or pigments (stains), internal tags, as well as the commonly known Petersen and Atkins type tags (Lucas et al. 1972; Glaister 1978). Although the method of staining prawns has its restrictions in tracking individual growth and movement, it has been useful as a marking technique in population studies. This procedure is considered less intrusive than physical tags making it more feasible for marking the smallest of juveniles (Costello 1959; Racek 1959; Kilma 1965). However past studies have shown that stain induced mortality and visibility of the stain vary greatly within and between prawn species (Dawson 1957; Costello 1964).

The various types of external tags used to study penaid prawns have been discussed by Kilma (1981) and Penn (1981). The streamer tag has been increasingly used since it was developed by Marullo et al. (1976) because it is easy to apply, does not appear to affect the normal behaviour of prawns and causes low mortality (Hill and Wassenberg 1985; Montgomery and Gray 1991). High tag-related mortalities in penaeids have been mainly due to the inexperience of the tagger, the poor condition of the prawn and/or the stage of the prawn's moult cycle when the tagging was

done, and the artificial environment in which the test animals were kept (Howe and Hoyt 1982; Benzie et al. 1995).

Recent studies on the effects of tagging penaeids have suggested that tag-induced mortality from streamer tags is size-dependent and as a consequence the range of prawn sizes that can be tagged may be limited (Penn 1976; Hill and Wassenberg 1985; Benzie et al. 1995). Likewise conclusions about the effect of tagging upon growth have varied between studies. Some laboratory and field studies have concluded that tagging does not affect growth in penaeids (Primavera and Caballero 1992; Montgomery et al. 1995). In contrast Menz and Blake (1980) stated that tagged *Penaeus vannamei* grew more slowly than unmarked individuals.

The school prawn *Metapenaeus macleayi* (Haswell) is one of a number of prawns caught along the east coast of Australia. It plays an extremely important role in marine and tidal ecosystems and forms the basis of an important commercial fishery in New South Wales, Australia (Glaister 1978). To date there has been limited information available on growth and mortality rates either of school prawns or of any metapenaeid species that lives in temperate waters. Ruello (1977) and Glaister (1978) determined tag-induced mortality in school prawns using Atkins type tags. However only small prawns (mean $\leq$ 20 mm CL) were examined over a short time period (five days) when moulting did not occur. Tagging trials using streamer tags have been done on other Australian penaeids (Montgomery and Gray 1991; Benzie et al. 1995; Wassenberg and Kerr 1990) but not on *M. macleayi*.

In the present paper we investigated the effect of tagging and staining on *M. macleayi* in the laboratory and whether tag or prawn size affected survival. This information was used to select a tag type for future studies on wild school prawns. The null hypothesis tested was that survival and/or growth of juvenile *M. macleayi* were unaffected by tagging (stain or streamer tag) or size of the tagged individual.

Methods

Equipment used

The hypothesis was tested on school prawns from the Hawkesbury River NSW, Australia. Two experiments were done at the aquarium facilities at the Cronulla Fisheries Research Centre of Excellence between No-

vember 2003 and April 2004 using three 4000L fibreglass holding tanks and 30 smaller fibreglass tanks (160L). All small tanks had a sand substratum (mean depth, 50-80mm), were supplied with flow through seawater (at ambient temperature, approx. 18-24°C), aerated using air-stone diffusers and equipped with outflow pipes designed to maintain 300 mm water levels. The tanks were evenly distributed on opposite sides of an enclosed room with a regulated 12:12 h photoperiod.

Collection and handling of prawns

Prawns were captured using a commercial prawn trawl vessel equipped with a standard prawn trawl net with a codend mesh size of 30 mm. Nets were towed for 10 minutes and then the captured prawns were placed into holding tanks supplied with oxygen. These holding tanks were transported to the aquarium facilities and prawns were placed into the large holding tanks. Prawns were allowed to acclimatise in these tanks for at least 7 days, during which they were fed a diet of commercial prawn pellets (Primo starter 3, 40% protein).

Treatments used

School prawns were grouped into two size classes (11-20 and 21-30 mm CL). Two sizes of streamer tags (small 12P, 43 mm long by 2 mm wide and large 4S, 63mm long by 3 mm wide both with a central notch) manufactured by Hallprint Pty Ltd were used. All tags were inserted through the articular membrane between the first and second abdominal segments of the prawns with needles of 0.7 mm diameter. Stained prawns were injected with a 0.2mls (0.5 % solution) of fast green FCF (Di Colours, Australia – benzyldiethyldiamino, hydroxytriphenyl – carbinol trisulphonic acid anyhydride: manufacturer HCA Australia), dissolved in distilled water (Ruello 1977). The solution was injected through the articular membrane between the fourth and fifth abdominal segment with a 0.5ml, 29-gauge needle. As the stain was not found to be visible for a sufficient time in the first experiment (see results) the treatment was not examined in the subsequent experiment.

Experimental procedure

School prawns were randomly assigned one of the treatments in each particular experiment (Table 1). Each individual prawn was measured (to the nearest 0.1 mm) between the base of the orbit of the eye and the centre of the posterior margin of the carapace (carapace length, CL). The gender of the prawn was determined by the presence of a thelycum (fe-

males) or petasma (males). The experiments ran for 70 days with each tank being monitored every 24 h for dead prawns, loose tags and exuviae. At the same time, water temperature and salinity were recorded from three randomly selected tanks. Dead prawns were replaced with prawns from reserve stocks in the larger stock tanks and were identified by removing part of their right uropod. These prawns remained identifiable throughout the experiment and maintained similar densities of prawns in each tank. Every two weeks two replicates of each treatment were randomly selected and the surviving prawns counted. The surviving prawns were then placed back into their respective treatment tank along with restocked prawns to maintain appropriate densities and were not processed again until the end of the experiment. Dead prawns and exuviae collected throughout the experiment and all prawns at the end of their selected time period were inspected for evidence of a tag wound and had their gender determined. Where applicable, the tag number of the prawn was also recorded.

Table 1. Experimental design for estimating tagging mortality on Hawkesbury River school prawns

Experiment	Size of prawn	Treatment	Replicates	No. per tank
1	Small*	Small tag	10	20
		Stain	10	20
		Control	10	20
2	Large+	Small tag	10	15
		Large tag	10	15
		Control	10	15

* Prawns between 11 and 20mm carapace length
+ Prawns between 21 and 30mm carapace length

Analysis of data

Mortality was expressed as the proportion of treated prawns released that had died or shed their tag (stain) at the end of each time interval (14, 28, 42, 56 and 70 days). The null hypothesis was tested for data from each experiment using a two-factor analysis of variance (ANOVA). Student-Newman-Keuls (SNK) multiple comparisons were used to detect patterns in mean rates of mortality between treatments. To estimate rates of mortality a double exponential model of the form:

$$y = y_0 + a\,e^{bx} + c\,e^{dx}$$

was fitted to mean weekly mortality data of tagged and untagged prawns in each of the two experiments, with y being the cumulative proportion of

prawns which died in period x. The double exponential (or exponential mixture), which is the sum of a fast decaying term and a slower decaying term was fitted using a reparametrisation of the form:

$$y = y_0 + a\, b^x + c\, d^x$$

using the MLP software (Version 3.08, Numerical Algorithms Group, Oxford UK). This provided stable starting values for the five parameters in refitting the double exponential model using Sigmaplot (Version 8.02, SPSS Inc., Chicago, IL).

The distributions of lengths of prawns at the start and those of prawns that survived to the end of the experiment were compared using the Kolmogorov-Smirnov test to investigate the effects of tagging and staining on the growth of school prawns. To investigate any size-related survivability associated with the tagging of school prawns a Kolmogorov-Smirnov test was used to compare the initial carapace length of all prawns with the initial carapace length of prawns that survived to the end of the experiment. Chi-squared contingency tables were also done to compare sex ratios between school prawns recorded at the beginning of the experiment and those that survived to the end of the experiment. For all tests, analyses were done using both Statistica (Version 6, Tulsa, OK) and SPlus (Version 6.1, Insightful, Seattle, WA) with a P value of <0.01 considered significant (Zar 1974).

Results

Stain or tag loss and mortality

When the prawns in experiment 1 were injected with the fast green FCF solution, the stain immediately accumulated in all parts of the body including the head. By day 14 the stain was visible only in the gills and by day 70 all but one prawn was still showing evidence of stain. Streamer tags remained intact and legible throughout each experiment. There was no observed difference in burying or moulting behaviour between control, stained and tagged prawns. The only visible damage from the streamer tag was at the point of entry of the tag where a small black scar occurred, but this scar was lost at the first moult.

At the end of each experiment 98% and 97% of the treatment, control and restocked prawns were accounted for in experiments 1 and 2 re-

spectively. The most likely scenarios for the unaccountable were either that the prawns had escaped from the tanks, or that other prawns within the tanks had eaten them. Only one (small) and four (two small, two large) dislodged tags were found in experiments 1 and 2, respectively.

Deaths of untagged (control) prawns represent the mortality that can be attributed to factors other than tagging (e.g. handling and tank environment). Therefore, the differences in proportional mortality between untagged and the treated prawns provided an estimate of actual tag or stain induced mortality (Adjusted mortality, Table 2). Results from the two-factor ANOVA showed that the proportion of prawns that had died or shed their tags (stain) in each experiment differed significantly amongst treatments (Table 2). Mean mortality of untagged prawns (controls) was significantly less than stained or tagged prawns (ANOVA, $P < 0.01$) in both experiments. There were no significant differences in mean mortality between stained prawns or prawns with small tags (Experiment 1) nor between large and small tags (Experiment 2) (Table 2).

Table 2. Analysis of variance of proportional mortality of school prawns (small and large) during the two experiments

Analyses of variance					
Exp.	Source	df	MS	F	p
1	Hawkesbury (small)				
	Treatment	2	7066	58.88	<0.01*
	Time	4	162	1.35	0.30
	Treatment x Time	8	196	1.63	0.20
2	Hawkesbury (large)				
	Treatment	2	0.43	8.76	<0.01*
	Time	4	0.06	1.22	0.34
	Treatment x Time	8	0.01	0.12	0.99
	Student-Newman-Keuls tests				
1	Small tag (0.68) **(0.53)** = Stain (0.64) **(0.49)** < Control (0.15)				
2	Small tag (0.75) **(0.40)** = Large tag (0.85) **(0.50)** < Control (0.35)				

Variances were homogeneous (Cochrans test, p>0.01). (=) in Student-Newman-Keuls tests denotes no significant differences between two means.
*denotes significant difference. Proportion in bold indicates adjusted mortality.

Trends in mortality amongst tagged and stained prawns were similar in the two experiments with rates of mortality being the highest during the first 2 weeks of the experiment for all treatments, particularly the first three to four days. Figures 1a and b show fitted lines to the mean weekly cumulative mortalities in both tagged (small tag) and untagged prawns for the entire duration of each experiment. Tagged animals in both experiments showed similar patterns of mortality over the 10 week study period,

with a rapid increase in mortality in the initial two weeks and a levelling off from week 4. Parameter estimates and fit statistics for tagged and untagged prawns derived from the double exponential model are shown in table 3 for each experiment.
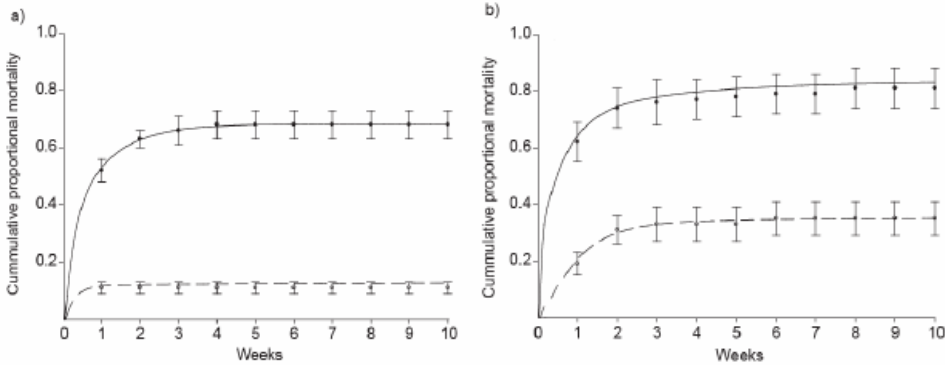

Fig. 1. Mean ± S.E. cumulative proportion of school prawns tagged with small streamer tags (●) or untagged (○) that died during experiments a) 1 and b) 2. The fit of the double exponential model is shown.

Table 3. Parameter estimates, standard errors and adjusted r squared values of mortality among prawns that were tagged (small tag) and untagged in Experiments 1 and 2

Exp		Tagged			Untagged	
		Coefficient	Std. Error		Coefficient	Std. Error
1	a	-37.37	3.58	a	-14.79	1.29
	b	-0.63	0.13	b	-0.53	0.06
	c	-39.75	4.21	c	-58.53	4832.12
	d	-0.14	0.01	d	0	0.02
	y0	68.2	0.14	y0	70.57	4832.38
		$R^2 = 0.99$			$R^2 = 0.91$	
2	a	-47.7	2.9	a	-42.59	2.55
	b	-0.23	0.02	b	-0.16	0.01
	c	-13.56	2.79	c	-57.75	35778.1
	d	-0.04	0.013	d	0	0.17
	y0	83.69	0.69	y0	91.66	35780.92
		$R^2 = 0.99$			$R^2 = 0.97$	

Moulting and growth

Daily patterns in the frequency of moulting were similar among all treatments within both experiments (Kolmogorov-Smirnov tests; p>0.1) although it was impossible to distinguish between exuviae from tagged and restocked prawns. The frequency of moulting of prawns initially peaked

within two weeks of the beginning of each experiment followed by several small irregular moulting events.

Mean lengths of prawns before and at the end of the each experiment did not differ significantly among treatments. Nor were there significant differences among treatments in the distributions of lengths of prawns at the beginning of each experiment or for those that survived to the end of each experiment (Table 4; Kolmogorov-Smirnov tests, $P > 0.1$).

Table 4. Mean ($\pm$ S.D.) carapace length of treated prawns at the beginning of each experiment and those that survived to the end of each experiment. P value for all distribution comparisons among treatments

Exp.	Treatment	Start of experiment mean ($\pm$ S.E.)	KS p-value*	End of Experiment mean ($\pm$ S.D.)	KS p-value*
1	Control	16.88 ($\pm$0.10)		17.81 ($\pm$0.19)	
	Small tag	16.73 ($\pm$0.11)		17.84 ($\pm$0.20)	
	Stain	16.79 ($\pm$0.12)	p > 0.1	18.10 ($\pm$0.19)	p > 0.1
2	Control	24.28 ($\pm$0.17)		24.58 ($\pm$0.29)	
	Small tag	24.33 ($\pm$0.18)		24.53 ($\pm$0.29)	
	Large tag	24.50 ($\pm$0.17)	p > 0.1	24.93 ($\pm$0.25)	p > 0.1

Size and sex- related mortality

There were no significant differences in the mean lengths and distributions of initial tagged prawns and the mean lengths and distribution of lengths tagged prawns at release that survived to the end of each experiment (Fig. 2; Kolmogorov-Smirnov tests, $P > 0.05$). This indicated that rates of mortality of tag and stained prawns was not size selective. Also, there were no differences in mortality between genders for prawns with small tags (Chi-squared test, $P = 0.1$ and $P = 0.06$ for experiments 1 and 2 respectively) and large tags (Chi-squared test, $P = 0.1$, experiment 2).

Discussion

This study showed that tagging and staining significantly affected the survival (by up to 50%) of school prawns however there was no difference in mortality between sizes of tags used. It also showed that growth of

school prawns appeared to be unaffected by the tagging or staining process, and that mortality of tagged prawns was not size or gender dependent.
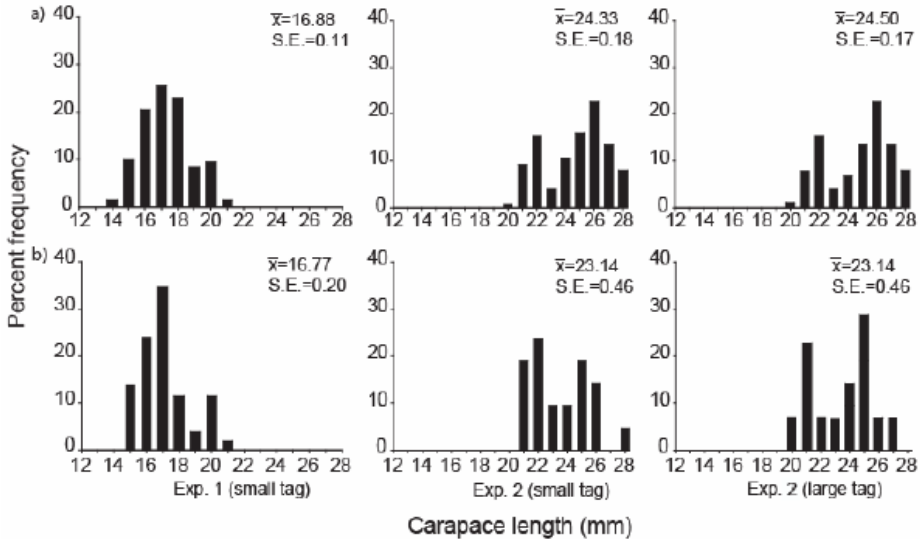

Fig. 2. Distributions of all small and large tagged school prawn carapace lengths at (a) the start of each experiment and (b) the distribution of initial school prawn carapace lengths of tagged small prawns that survived to the end of each experiment

Whilst there were no differences in rates of mortality between stained and tagged prawns, the stain was only evident in the head and gill area of the treated prawns for the first week of experiments and there was high variability in the visibility of the stain. Costello (1959) and Farmer (1981) found that there was minimal mortality in prawns that had been immersed in stain but the stain was only visible for a few days. Therefore, it appears that the initial trauma due to catching together with the stress associated with handling and the injection of the stain may play a significant role in the survival of stained prawns. The use of an injected stain as a marker in school prawns (11-20mm carapace length) may prove reliable for short-term fishery independent studies where individual information is not required. However in longer-term studies where the stain needs to be easily detected by untrained personnel such as commercial fishers and members of the general public, staining is unlikely to be a viable option.

Only seven dislodged streamer tags out of the 500 originally stocked tagged prawns were found throughout the three experiments. There was no evidence of treated prawns surviving to the end of the experiment without their tags intact. Therefore, either the prawns died with the tag

attached and were eaten leaving the tag behind, or they lost their tag and then were eaten. Tag shedding has often been associated with experiments to quantify the survival of tagged prawns (Benzie et al. 1995; Montgomery et al. 1995). Authors attributed the incidences of dislodged tags to the moulting behaviour of prawns, a problem also faced by other tagging experiments on crustaceans (Prentice and Rensel 1977). In our study prawns consistently moulted and streamer tag loss was considered negligible. The tags used have a central notch in which the body of the prawn sits, to reduce the risk of tag loss (Marullo et al. 1976).

In regards to behaviour, prawns with both small and large streamer tags were observed to swim, bury and moult normally. These treated prawns also appeared to have no trouble in shedding their moult with complete exuviae found across all tanks in each experiment. In similar studies on tiger prawns, Hill and Wassenberg (1985) found there to be no significant difference in the time and method it takes for streamer tagged and untagged prawns to complete ecdysis. In this study for both experiments the main frequency of moulting was within weeks one and two followed by a series of several smaller events. There were no significant differences in the frequency of moulting among treated and control (untagged) prawns for the two experiments.

Overall survival rates in school prawns as a direct result of tagging with streamer tags were lower compared to those recorded for other penaeid species (Primavera and Caballero 1992; Hill and Wassenberg 1985; Montgomery et al. 1995). Mortality rates for both tagged and stained prawns in both experiments were greatest over the first two weeks, suggesting that the high mortality in this smaller penaeid species maybe attributable to the initial effects of handling and more importantly the application of the actual tag or stain. This becomes evident as rates slowed after the first 2-4 weeks to a point where mortality was comparable to those of the untagged prawns (control) in each experiment. Estimates of rates of tag-induced mortality for other penaeids have been derived using an exponential decay model (Lucas et al. 1972; Montgomery et al. 1995). In this study a double exponential model accounted for data within the range of standard errors for replicate groups about each mean (Figs. 1a & b). The resulting high adjusted r squared values (>0.90) for tagged and untagged prawns in both experiments (Table 3) indicates that this exponential 'mixture' model is suitable for describing tag-induced mortality in school prawns.

The result in Experiment 1 that tagging or staining does not appear to affect growth is supported by other studies (Farmer 1981; Hill and Was-

senberg 1985; Montgomery et al. 1995). However the non-significant increase in growth for all treatments from Experiment 2 may have been the result of a higher stocking density (total weight of prawns per m^2) than Experiment 1. High stocking densities have been shown to increase mortality and inhibit growth in both untagged and tagged/marked individuals (Maguire and Leedow 1983; Jewett 1986) and therefore appear to have confounded any treatment effects on growth in this particular experiment. Within the size range of prawns studied our results demonstrated that the mortality associated with tagging was not size-dependent. This is in contrast to other studies that report that the survival rate of prawns tagged with streamer tags significantly decreased in prawns of a carapace length less than 20mm (Hill and Wassenberg 1985; Wassenberg and Kerr 1990). This study also showed that mortality associated with tagging and staining was not dependent upon the sex of the prawn.

We have shown that school prawns can be tagged with streamer tags but that rates of survival are low. The low rates of survival can be countered in experiments done in the wild if large numbers of prawns can be tagged. However any extrapolation of estimates of mortality from laboratory experiments should be verified with experiments in the field. Streamer tags are easy to apply, are readily identified in the catches of commercial and recreational fishers with the added benefit of an individual identity. In contrast the staining of prawns has no less impact upon mortality than streamer tags but the stain is difficult to detect in prawns after one week. We conclude that streamer tags are the most suitable method for use in mark-recapture population studies for juvenile school prawns.

Acknowledgements

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