Effects of subcutaneous fluorescent tags on the growth and survival of a newly settled coral reef fish, *Pomacentrus amboinensis* (Pomacentridae)

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Abstract Mortality rates are often confounded by unaccounted migration, and where tagging is used, tags themselves may bias demographic rates. We examined the retention rate of subcutaneous fluorescent elastomer tags on newly settled *Pomacentrus amboinensis* (Pomacentridae), and their influence on fish growth and survival. Fish were collected from light traps and marked with either a single tag, double tag or an uncured elastomer tag. There was 100% retention of all tags over the 14-day laboratory experiment. Survival was high for all treatments, ranging from 80% for double-tagged fish, 96% for the unmarked and single-tagged fish, to 100% for those with a single uncured tag. Fish marked with single tags, both cured and uncured, grew as fast as unmarked fish. In contrast, fish with double tags grew slower than the unmarked fish. These results indicate that marking newly settled coral reef fish with a single fluorescent elastomer is a useful means of short-term cohort, or batch, recognition. The high retention rate of the uncured elastomer tags increases the applicability of this technique for marking individuals underwater by alleviating the need to store it below 0^0C to extend its shelf life.

Keywords fluorescent elastomer, mortality, growth, tag retention, tropical reef fish, newly settled

Introduction

Settlement is a critical period in the life history of coral reef fishes (Searcy and Sponaugle 2001; Hoey and McCormick 2004). Recent studies have suggested that mortality may be disproportionately high in the days immediately following settlement (e.g. Doherty and Sale 1985; Sale and Ferrell 1988; Carr and Hixon 1995; Doherty et al. 2004; McCormick and Hoey 2004). However much of the evidence may be biased by the inability to distinguish unmarked individuals and cohorts, or by untested artefacts of the tagging method used. Obtaining accurate descriptions of the shape of mortality trajectories is important because the shape reveals where the processes that influence population abundances are most intense.

The ability to distinguish fish, either individuals or cohorts, is essential to provide unbiased estimates of the demographic rates of a population. The small size of newly settled coral reef fish, typically less than 20mm in length (Victor 1991), has precluded the use of conventional tagging methods (Buckley et al. 1994). Studies examining the demographics of recently settled coral reef fishes have relied largely on intrinsic methods, using variations in natural markings to identify individuals (e.g. Booth and Beretta 1994) or small size differences to distinguish successive recruitment cohorts (e.g. Robertson 1988; Sponaugle and Cowen 1997). Other studies have relied on the spatial isolation of suitable habitats (e.g. Meekan 1988; Forrester 1999) or the untested assumption that recently settled individuals are relatively site attached (e.g. Doherty and Sale 1985; Sale and Ferrell 1988). These intrinsic methods of identification have placed substantial restrictions on the spatial scale and the number of individual fishes examined. In addition, these studies have invariably biased their estimates of early post-settlement mortality through their inability to detect migration or subsequent natural settlement of individuals to the study site.

Several extrinsic identification methods have also been used to mark juvenile coral reef fishes. These include heat branding (Jones 1987), fin clipping (Booth 1991), and a variety of subcutaneous tags (e.g. acrylic paint: Malone et al. 1999; liquid latex, Forrester 1990; alphanumeric plastic film: Malone et al. 1999; coded wire: Beukers et al. 1995; fluorescent elastomer: Frederick 1997; Booth and Hixon 1999; Hoey and McCormick 2004; McCormick and Hoey 2004). However, poor *in situ* visibility, limited retention time, and reduced growth and survival of marked individuals has decreased the utility of many of these techniques, especially for very small individuals. To date no study has assessed the effects of marking newly settled coral reef fishes.

This study examines the application of a subcutaneous fluorescent elastomer (Northwest

Marine Technologies Inc.) to mark newly settled *Pomacentrus amboinensis* (Bleeker, 1868). This tag is a two-part biocompatible elastomer that is injected as a liquid and hardens to an inert flexible area that is visible under the scales and epidermis. Once the curing agent has been added to the elastomer it has a very limited shelf life, unless stored below 0^0C, thus limiting the feasibility of this technique for marking individuals in the field. The ability to use an uncured fluorescent elastomer injection to mark newly settled fish would greatly increase its application for tropical field based studies. The aim of this study is to assess the retention of cured and uncured fluorescent elastomer tags and the associated mortality and growth of newly settled *P. amboinensis*.

Material and Methods

Study species

Pomacentrus amboinensis is a ubiquitous pomacentrid that typically settles in high numbers to a variety of habitats on the northern Great Barrier Reef (Kerrigan 1994). *P. amboinensis* has a pelagic larval duration of 15 - 23 days and settles at 10.3 – 15.1mm standard length (Wellington and Victor 1989; Kerrigan 1996) with its juvenile body plan largely complete, and undergoes a rapid (less than 12h) change in body pigmentation (McCormick and Makey 1997; McCormick et al. 2002).

Tagging process

Late pelagic stage *P. amboinensis* were caught using light traps (for design see Stobutzki and Bellwood 1997). Traps were anchored approximately 50m from the nearest reef in 12 - 18m of water on the leeward side of Lizard Island on the northern Great Barrier Reef, Australia (14°41'S, 145°27'E). Fish were removed from the traps at 0730 hours, returned to the laboratory and maintained in 40 L aquaria with running seawater until they were tagged (~ 2 h). To minimise the damage incurred from handling during the injection process the fish were transferred to clear click-seal polyethylene bags containing a small amount of aerated seawater and tagged through the bag. The standard length (SL) of all fish was measured (± 0.1mm) prior to tagging. A subcutaneous fluorescent elastomer tag (Northwest Marine Technologies Inc.) was injected into the dorsal musculature with a 29-gauge hypodermic needle, the resultant tag being 1.5mm in length. Care was taken to ensure that the tag did not extend beyond the body to allow a healing area behind the tag (Buckley et al. 1994). Injection of a single tag using this technique took no longer than 60s, and no longer than 90s for two tags.

Experimental design

One hundred newly settled *P. amboinensis*, ranging in size from 11.7 to 13.4mm SL, from a single night's light trapping were randomly selected and allocated to one of four treatments: single tag, double tag, uncured tag and control (i.e. unmarked individuals). The fish were then batch tagged with a different coloured tag being used for each of the tagging treatments. Single tags, both cured and uncured, were injected into the mid-dorsal musculature and double tags were injected into the mid- and posterior-dorsal musculature. Five 10 L aquaria with fresh, flowing seawater and constant aeration were each stocked with 20 individuals; 5 from each treatment and the control. Fish were fed *Artemia* nauplii daily, and mortality assessed one hour after tagging and twice daily thereafter for 14 days. Upon detection, dead fish were removed, their tag/s identified and measured using calipers (SL , ± 0.1mm). After 14 days all fish were identified, measured (SL, ± 0.1mm), mean daily growth rates calculated and tag retention assessed visually. As fish were not individually identifiable, growth rates were estimated from the change in mean standard length for each treatment within each aquarium. All fish that died during the course of the experiment were excluded from the calculations.

Analyses

The survival rate of newly settled fish at the conclusion of the experiment was compared between the tagging treatments and the control using Friedman's analysis of variance by ranks. This non-parametric analysis of variance, which accounts for the non-independence of the treatments within each aquarium, was necessary due to the heteroscedasticity of the survival data. The mean daily growth rate was compared among the tagging treatments using a two factor Analysis of Variance (ANOVA) without replication. The statistical model consisted of two factors, tagging treatment and aquaria, thereby accounting for the non-independence of the treatments within each aquarium.

Results

Survival of newly settled *Pomacentrus amboinensis* was high for all treatments over the 14 day period, ranging from 80 ± 8.9%(SE) for double tagged fish, 96% ± 4%(SE) for the control and single tagged fish, to 100% for those with a single uncured tag (Fig. 1). Five of the recorded deaths occurred within one hour of the commencement of the experiment, with the further loss of 2 doubled tagged fish occurring after 36h (Fig. 1). Although the double tag treatment accounted for 5 of the 7 recorded deaths, statistically there was no difference between treatments in the number of fish remaining after 14 days (χ^2 = 6.33, df = 3, p = 0.10).

Differences in the daily growth rate of newly settled *P. amboinensis* among tagging treatments was found to be marginally non-significant ($F_{3,12}$ = 3.08, p = 0.07). Whilst the mean daily growth rate of the control fish was similar to that of the fish with single tags, the growth of fish with double tags appeared lower than that of fish from the other treatments (Fig. 2). Surprisingly, newly settled *P. amboinensis* marked with the uncured tags exhibited a higher (but non significantly different) mean daily growth than fish that were either not marked or marked with a cured tag (Fig. 2). Retention rates of the fluorescent elastomer tag, both cured and uncured, were 100% for all surviving *P. amboinensis* over the 14-day experimental period. All tags were clearly visible to the naked eye at the conclusion of the experiment.

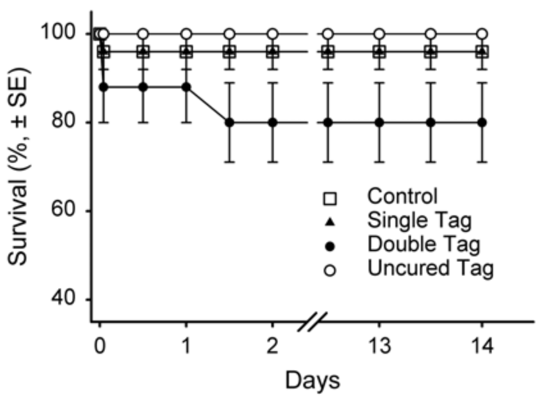

Fig. 1. Comparison of survivorship of newly settled *Pomacentrus amboinensis* marked with single cured tag, double cured tag, uncured tag or left unmarked (control) over a 14-day period.

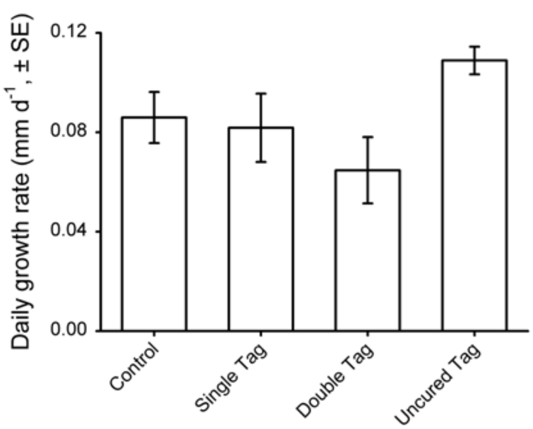

Fig. 2. Comparison of mean daily growth rate of newly settled *Pomacentrus amboinensis* marked with single cured tag, double cured tag, uncured tag or left unmarked (control) over a 14-day period.

Discussion

This study indicates that marking newly settled *Pomacentrus amboinensis* with a single fluorescent elastomer can provide a useful means of short-term cohort, or batch, recognition. Fish marked with single tags, both cured and not cured, had high rates of tag retention (100%) and showed no reduction in growth or survival. The complete retention of the elastomer tags that were not cured suggests that it may be more efficient and cost effective to use uncured than cured elastomer for tagging fish underwater, especially in warmer tropical waters. Surprisingly, fish marked with the uncured elastomer had higher growth rates than unmarked fish. Whilst this may be due to natural variation and the relatively small sample size, it may be related to the tag itself and warrants further investigation. Frederick (1997) reported a tag retention rate of 98% for a variety of juvenile coral reef fishes marked with the fluorescent elastomer over a 45-day period. However, the survival rates of tagged fishes were considerably lower than those of the present study, ranging from 60 – 87% in the laboratory and 44% in the field after 2 days for juvenile fishes (< 20mm SL), including two pomacentrids, *Chromis ovalis* (Steindachner, 1866) and *Dascyllus albisella* (Gill, 1862). In addition these fishes were collected from the reef, and having survived a period of time post-settlement may be more resilient to tagging stress than newly settled individuals.

Other methods for marking cohorts of small coral reef fishes have generally been limited by their poor retention rates, high mortality, and often untested biocompatibility of the tagging material. Several studies have found that marking juvenile pomacentrids, *Dascyllus* spp. and *Stegastes* spp., (< 20mm SL) with tattooing dyes caused increased mortality, and the marks typically faded within two weeks of application (Forrester 1990; Wellington 1992; Booth 1995). Reduced growth has also been reported for the goby, *Coryphopterus glaucofraenum* (Gill, 1863), (< 25mm SL) marked with acrylic paint (Forrester 1995; Malone et al. 1999). In contrast, Beukers et al. (1995) successfully tagged juvenile *Pomacentrus moluccensis* (Bleeker, 1853) (11-20mm SL) using a fluorescent filament tag with one hundred percent tag retention and negligible impacts on survival and growth over a 90-day period. However, the injection procedure required aseptic conditions and a binocular microscope, placing considerable restrictions on its versatility, especially for marking fishes in the field.

In contrast, the use of multiple fluorescent elastomer tags to provide individual recognition of newly settled coral reef fish appears to be limited. The number of fish that can be uniquely marked with a single tag is simply the product of the number of elastomer colours available and the number of anatomical locations suitable for tagging. This could be greatly increased by the use of multiple tags, using a combination of colours and anatomical locations. However, the lower growth and survival of fish with double tags, albeit statistically non-significant, may bias estimates of demographic parameters. Frederick (1997) reported an inverse relationship between fish length and tagging mortality using the same fluorescent elastomer and suggested that the use of multiple tags was only feasible for larger fish (> 20mm SL). Coded wire tags have been used to provide individual recognition of juvenile *P. moluccensis* (11-20mm SL) without reducing growth or survivorship (Beukers et al. 1995). However, the injection procedure required aseptic conditions, and the coded wire tags are not visible *in situ* and must be removed for identification, placing considerable restrictions on their use. Malone et al. (1999) used alphanumeric plastic film to individually mark adult *C. glaucofraenum* (>25mm SL) with no effect on growth or survivorship over a 75-day period. The larger size of these tags (1 × 2.5mm) may preclude their use on smaller recently settled individuals.

The ability to successfully mark newly settled *P. amboinensis* with a single fluorescent elastomer removes many of the constraints of previous studies examining early post-settlement processes of coral reef fishes. However, prior to using this technique to directly estimate demographic rates of a population the field mortality of this technique should be assessed. For example, the presence of a tag may make a fish more visually conspicuous to predators, resulting in increased mortality or reduced growth as more energy is partitioned into predator avoidance.

The use of this marking technique may be enhanced by allowing a 1h recovery period after tagging as seventy-one percent of all tagging related mortality, which included the only reported loss of a single tagged fish, occurred within this period. Frederick (1997) similarly reported that fifty-three percent of all tagging related mortality occurred within two hours of marking. A recovery period would enable these losses to be detected and replaced prior to releasing them into an experimental framework. The high retention rate of the uncured elastomer tags in the present study increases the applicability of this technique for marking individuals in the field by alleviating the need to store the elastomer below 0^0C to extend its shelf life. Using this technique the fate of large numbers, or cohorts, of newly settled fish can be determined. This should enhance our understanding of the relative importance of early post-settlement processes in structuring reef fish populations by distinguishing mortality from migration, and the appearance of newly settled individuals from those previously censused.

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References

Beukers JS, Jones GP, Buckley RM (1995) Use of implant microtags for studies on populations of small reef fish. Mar Ecol Prog Ser 125: 61-66

Booth DJ (1991) The effects of sampling frequency on estimates of recruitment of the domino damselfish *Dascyllus aruanus* Gill. J Exp Mar Biol Ecol 145: 149-159

Booth DJ (1995) Juvenile groups in a coral-reef damselfish: density-dependent effects on individual fitness and population demography. Ecology 76: 91-106

Booth DJ, Beretta GA (1994) Seasonal recruitment, habitat associations and survival of a pomacentrid reef fish in the US Virgin Islands. Coral Reefs 13: 81-89

Booth DJ, Brosnan DM (1995) The role of recruitment dynamics in rocky shore and coral reef fish communities. Adv Ecol Res 26: 309-385

Booth DJ, Hixon MA (1999) Food ration and condition affect early survival of the coral reef damselfish, *Stegastes partitus*. Oecologia 121:364-368

Buckley RM, West JE, Doty DC (1994) Internal micro-tag systems for marking juvenile reef fishes. Bull Mar Sci 55: 848-857

Carr MH, Hixon MA (1995) Predation effects on early post-settlement survivorship of coral-reef fishes. Mar Ecol Prog Ser 124: 31-42

Doherty PJ, Dufour V, Galzin R, Hixon MA, Meekan MG, Planes S (2004) High mortality during settlement is a population bottleneck for a tropical surgeonfish. Ecology 85: 2422-2428

Doherty PJ, Sale PF (1985) Predation on juvenile coral reef fishes: an exclusion experiment. Coral Reefs 4: 225-234

Forrester GE (1990) Factors influencing the juvenile demography of a coral reef fish. Ecology 71: 1666-1681

Forrester GE (1995) Strong density-dependent survival and recruitment regulate the abundance of a coral reef fish. Oecologia 103: 275-282

Forrester GE (1999) The influence of adult density on larval settlement in a coral reef fish, *Coryphopterus glaucofraenum*. Coral Reefs 18: 85-89

Frederick JL (1997) Evaluation of fluorescent elastomer injection as a method for marking small fish. Bull Mar Sci 61: 399-408

Hoey AS, McCormick MI (2004) Selective predation for low body condition at the larval-juvenile transition of a coral reef fish. Oecologia 139: 23-29

Jones GP (1987) Competitive interactions among adults and juveniles in a coral reef fish. Ecology 68: 1534-1547

Kerrigan BA (1994) Post-settlement growth and body composition in relation to food availability in a juvenile tropical reef fish. Mar Ecol Prog Ser 111: 7-15

Kerrigan BA (1996) Temporal patterns in size and condition at settlement in two tropical reef fishes (Pomacentridae: *Pomacentrus amboinensis* and *P.*

nagasakiensis). Mar Ecol Prog Ser 135: 27-41
Malone JC, Forrester GE, Steele MA (1999) Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. J Exp Mar Biol Ecol 237: 243-253
McCormick MI (1993) Development and changes at settlement in the barbel structure of the reef fish, *Upeneus tragula* (Mullidae). Environ Biol Fish 37: 269-282
McCormick MI, Hoey AS (2004) Larval growth history determines juvenile growth and survival in a tropical marine fish. Oikos 106: 225-242
McCormick MI, Makey LJ (1997) Post-settlement transition in coral reef fishes: overlooked complexity in niche shifts. Mar Ecol Prog Ser 153: 247-257
McCormick MI, Makey L, Dufour V (2002) Comparative study of metamorphosis in tropical reef fishes. Mar Biol 141:841-853
Meekan MG (1988) Settlement and mortality patterns of juvenile reef fishes at Lizard Island northern Great Barrier Reef. Proc 6th Int Coral Reef Symp, Australia 2: 779-784
Robertson DR (1988) Abundances of surgeonfishes on patch reefs in Caribbean Panama: due to settlement, or post-settlement events? Mar Biol 97: 495-501
Sale PF, Ferrell DJ (1988) Early survivorship of juvenile coral reef fishes. Coral Reefs 7: 117-124
Searcy SP, Sponaugle S (2001) Selective mortality during the larval-juvenile transition in two coral reef fishes. Ecology 82: 2452-2470
Shand J (1997) Ontogenetic changes in retinal structure and visual acuity: a comparative study of coral-reef teleosts with differing post-settlement lifestyles. Environ Biol Fish 49: 307-322
Sponaugle S, Cowen R (1997) Early life history traits and recruitment patterns of Caribbean wrasses (Labridae). Ecol Monog 67:177-202
Stobutzki IC, Bellwood DR (1997) Sustained swimming abilities of the late pelagic stages of coral reef fishes. Mar Ecol Prog Ser 149:35-41
Victor BC (1991) Settlement strategies and biogeography of reef fishes. In: Sale PF (ed) The Ecology of Fishes on Coral Reefs. Academic Press, San Diego, pp 231-260
Wellington GM (1992) Habitat selection and juvenile persistence control the distribution of two closely related Caribbean damselfishes. Oecologia 90: 500-508
Wellington GM, Victor BC (1989) Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). Mar Biol 101:557-567