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Use of implant microtags for studies on populations of small reef fish

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ABSTRACT: Ecological studies on small reef fish could often be improved by distinguishing among individuals or cohorts within populations, but this has not been possible with conventional tagging methods. Historically, tagging of recently settled juveniles has proven to be particularly necessary and difficult. What is needed is a method for individually recognising small individuals. Here, use of 2 types of implant microtag are verified for this purpose, visible implant fluorescent tags (VIF) and coded wire tags (CWT). Retention rates of these tags were determined when injected into 2 size classes of a coral-reef damselfish, *Pomacentrus moluccensis* (10 to 20 mm juveniles, 25 to 40 mm adults). The influence of tagging on growth and survivorship was also measured. Microtag retention rates were high: 100% for new settlers and between 80 and 100% for adults. In addition survivorship and growth of juveniles and adults were not significantly different for fish with or without microtags. These microtags should provide the means to understand the importance of processes occurring within a few weeks of settlement and throughout the reef-associated phase of small fishes.

KEY WORDS: Microtag · Retention · Mortality · Survivorship · Growth · Reef fish · Pomacentridae

INTRODUCTION

Population studies often require recognition of either individuals or cohorts. Conventional methods used for tagging marine fishes are not appropriate for use in the majority of coral reef species because the tags are too large in proportion to the fish. They are particularly inadequate for marking recently settled juveniles, and this may be why this is one of the least understood phases in the life-history of benthic fishes. Several studies have found that loss of fish is greatest during the first days after settlement (Doherty & Sale 1985, Aldenhoven 1986, Victor 1986, Eckert 1987, Shulman & Ogden 1987, Meekan 1988, Sale & Ferrell 1988, Warner & Hughes 1988, Hixon & Beets 1989) but have not had the means to investigate this further. Without some degree of recognition, for example, it is not possible to look at degree of site attachment, variation in growth, size specific mortality, and ontogenetic shifts in microhabitat preferences or to distinguish mortality from migration. Distinguishing among these factors is essential for understanding the population ecology of reef fishes. This can only be fully achieved with recognition of individual fish.

Various methods have been employed to allow recognition of individual fish: intrinsic variables such as size or natural markings (Sale 1974, Reese 1975, Aldenhoven 1986, Connell & Jones 1991), fin clipping (Sale 1971), heat branding (Jones 1987), external tags (Randall 1961, Emery 1973, Fricke 1973), subcutaneous dyes (Kelly 1967, Hart & Pitcher 1969, Phinney & Matthews 1973, Lotrich & Meredith 1974, Thresher & Gronell 1978, Mapstone 1988), implanted diazo film (Heugel et al. 1977), internal marks (Brothers 1985, Volk et al. 1990), parasites (reviewed by Buckley & Blankenship 1990), liquid latex (Riley 1966, Forrester 1990) and internal tags (Bergman et al. 1968, Buckley et al. 1994). However, not all of these techniques are suitable for long-term recognition of small fish. Problems include negative effects on the fish, an inability to identify small individuals, the restricted time frame of some techniques (Mapstone 1988) and mark loss

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(Buckley et al. 1994). Juvenile fish grow fairly rapidly and therefore the effects of techniques such as heat branding and dyeing will wear off relatively more quickly than for individuals that grow more slowly.

Two types of implant microtag have been developed for fisheries research which may overcome these problems. They have been shown to have a low impact on juvenile survival and to have retention rates of >93% for 4 temperate reef fish species (Buckley et al. 1994). Histological examination revealed negligible tissue reaction to these microtags (Hargreaves & Le Brasseuer 1986, Fletcher et al. 1987, Bergman et al. 1992). The Visible Implant Fluorescent filament tag (VIF; Northwest Marine Technology, Inc., Shaw Island, WA, USA) and the binary-Coded Wire Tag (CWT; Jefferts et al. 1963) are biocompatible internal microtags (Buckley et al. 1994). The VIF tags are cylindrical polyester tags, which, when injected into translucent tissue and fluoresced by ultraviolet-A (UV-A) light, can be seen in live fish in the field. This attribute means that the fate of each cohort may be followed through time. CWTs are made from stainless steel wire and have unique batch or sequential codes etched onto their surfaces. Thus these microtags allow individual or cohort identification, with codes being deciphered under a binocular microscope. CWTs are magnetised and this feature is used to relocate them. Thus the use of both types of tag would give the ability to identify individuals after a long period of time.

Tagging studies generally assume that the tags have no influence on the variables to be measured, such as growth and survivorship. There is an increasing awareness of the need for verification of these assumptions of tagging (Emery & Wydoski 1987, Buckley & Blankenship 1990, McFarlane et al. 1990, Bergman et al. 1992), but to date very few coral reef fish studies have included the empirical evidence required. What is needed is a species-specific assessment of the effects of tagging This study examines the application of microtags to newly settled and adult damselfish of the species *Pomacentrus moluccensis*. It tests the reliability of microtag retention when injected singly and in combination, and the effects of tagging on fish growth and survivorship.

METHODS

This experiment included 2 phases, a laboratory phase (the first 45 d) and a field phase (the second 45 d). During the laboratory phase, fish were kept in aquaria because identifying individuals with lost tags was more precise in a confined environment. The field phase used isolated patch reefs to assess retention rates and impact of the microtags on fish on the reef. Censuses were performed after 9, 45 and 90 d to measure microtag retention rates and fish standard length. During the laboratory phase, survivorship was checked daily but during the field phase only once, after 90 d.

Laboratory phase. As stated in the 'Introduction', the ideal scenario for subsequent studies was to be able to use both types of microtag, the VIF and the CWT, in combination. However, it was possible that injection of 2 microtags into an individual may cause more problems than injection of 1 and each tag type may have unique effects. Hence, the experimental design included 3 treatments to allow assessment of each type of tag separately and in combination. The treatments were as follows: 1 CWT, 1 VIF and both microtags. The tagging process involves 2 steps, the first to anaesthetise the fish, the second to inject the microtag(s). Fish were immersed until unconscious, approximately 10 s, in a solution of quinaldine. The quinaldine solution which was found to be most effective was 1:15:4500, quinaldine:ethanol:sea water. The microtags were then injected with the aid of a binocular microscope, under the scales and into the dorsal muscle. All tags were implanted at least 1 tag length away from the insertion point to allow a healing area behind the tag (Buckley et al. 1994). When 2 microtags were to be injected, 1 was placed on each side of the fish. To minimise the damage incurred by the fish, particularly to the scales, each was placed in a click-seal polyethylene bag for the injection process. Sterile technique was employed, as far as possible, by washing the injector and microtags in an antiseptic solution prior to injection. Injection of a single microtag using this method took no more than 2 min, and no longer than 3 min for 2 microtags.

Two controls were used in the lab phase to assess the impact of anaesthetising and tagging and to assess the impact of the anaesthetising alone. Control 1 fish were placed in aquaria and were untouched other than for censusing to test the impact of anaesthetising and tagging. Control 2 fish were anaesthetised, placed in a polyethylene bag for 3 min and then released into the aquaria to test the impact of the anaesthetising alone. The time period chosen was the maximum time that any fish would be kept in a polyethylene bag.

All fish were kept in aquaria for 45 d. A total of 10 individuals, 2 from each tagging treatment and 2 from each control, were placed in each aquarium. Treatments and controls were allocated randomly among individual fish. Adults and new recruits were kept separately in 5 new recruit aquaria and 6 adult aquaria. For the purpose of this study new recruit refers to an individual of 10 to 20 mm standard length at the start of the experiment and adult refers to fish between 25 to 40 mm standard length at the start.

Previous studies (e.g. Buckley et al. 1994) have found that the ratio of muscle size to tag weight and size may be important in terms of the percentage of microtags retained. Therefore different sizes of microtag were used according to the size of *Pomacentrus moluccensis* tested. Standard-length microtags 1 mm long were injected into the adults and half-length microtags 0.5 mm long were injected into the new settlers.

Approximately 9 and 45 d after injection, tag status and fish standard length were determined. Tag rejection due to unacceptable location or tagging procedures is usually established by 30 d (Buckley & Blankenship 1990).

Field phase. After 45 d all tagged fish were placed onto patch reefs. Patch reefs were 2 m^2 in area, 20 m apart and consisted of a base of coral rubble above which were placed 4 hard live coral heads. Species used were *Pocillopora damicornis*, *Stylophora pistillata*, *Acropora nobilis* and *Acropora nasuta*. A total of 6 adults and 6 new settlers, 2 from each tagging treatment, were placed on each reef. A third control group was introduced at this stage to test for any effects of the entire laboratory phase. Approximately 100 m away from the first set of patch reefs, a second set of identical reefs was constructed for control 3 fish. These fish were captured from the nearby contiguous reef and placed on these patch reefs in the same densities as the treatments. The spatial isolation of the 2 sets of reefs was required to enable separation of individuals that had lost their tags from control individuals. This design was chosen to adequately test microtag retention rates, the issue which the authors consider to be the most important. A consequence of this is that whilst some aspects of mortality were adequately tested in the aquaria, any increased predation pressure that might be encountered when a fish is first released back onto the reef has not been tested here. All reefs were censused after 90 d. Recoveries of tagged fish were made by using UV-A light to fluoresce the VIF tags.

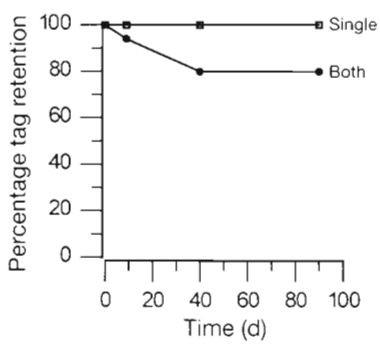

Fig. 1. *Pomacentrus moluccensis*. Retention rates for microtags injected singly and in combination into damselfish adults; n = 36

RESULTS

Retention

Percentage retention rates of microtags were 100% for all new recruits and adults when injected singly (Fig 1). They were lower when injected into adults in combination (80%; Fig. 1). The slightly lower figure for both microtags was due to losses with the larger size standard microtags. This indicates that use of standard microtags increases the likelihood of tag loss in this species and suggests that the half-length microtags would be more appropriate.

Survivorship

The percentage of fish remaining at 45 d was very high in new recruits (Fig. 2) and adults (Fig. 3) from the

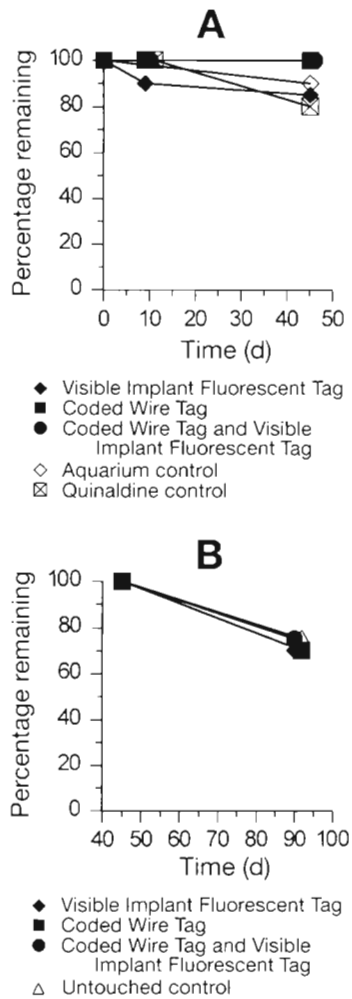

Fig. 2. *Pomacentrus moluccensis*. Comparison of percentage survivorship of new settlers in (A) the laboratory phase (B) the field phase of the experiment. For each line n = 10

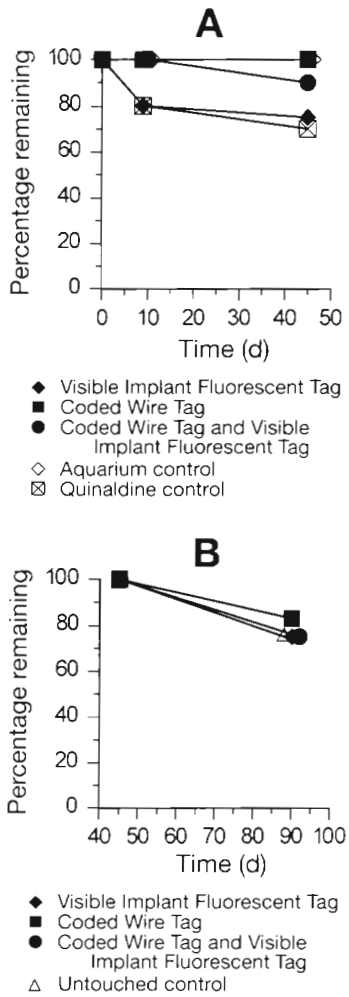

Fig. 3. *Pomacentrus moluccensis*. Comparison of percentage survivorship of adults in (A) the laboratory phase (B) the field phase of the experiment. For each line n = 12

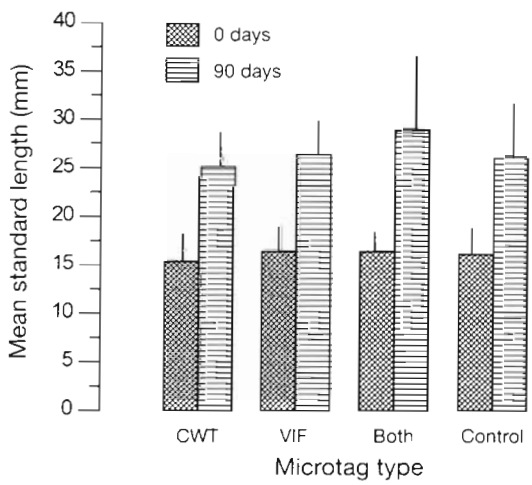

Fig. 4. *Pomacentrus moluccensis*. Standard lengths (mm, $\bar{x} \pm$ SD) of new settlers at time of tagging and 90 d later. n = 40

Table 1. *Pomacentrus moluccensis*. ANOVA tables comparing the final standard length mm^{-1} of VIF, CWT, Both and Control 3 individuals for new settlers and adults. Variances homogeneous in both new settlers (Cochran's $Q = 0.3623$) and adults (Cochran's $Q = 0.3566$)

Source	df	SS	MS	*F*	p
New settlers					
Between	3	65.4705	21.8235	1.22	0.3232
Within	26	466.099	17.9269		
Total	29	531.569			
Adults					
Between	3	237.278	79.0928	2.75	0.0581
Within	33	948.272	28.7355		
Total	36	1185.55			

aquarium control (90%, 100%), CWT (100%, 100%) and both microtag (100%, 90%) treatments. The lower survivorship shown by the VIF and quinaldine controls (new recruits: 85%, 80%; adults: 75%, 70%) was probably an aquarium-specific phenomenon due to conspecific aggression rather than any aspect of the tagging procedure. The results indicate that survivorship of individuals with both types of tag is good and it is therefore counterintuitive to consider that single VIF tags could increase mortality. In addition, several individuals that died had fin damage of some description with some severe cases having the entire caudal fin missing Losses in all groups were greater, but did not exceed 23%, during the field phase of the experiment and were comparable between treatment and control groups.

Growth

There was no significant difference in standard lengths between treatments and controls after 90 d, for either juveniles (Fig. 4, Table 1) or adults (Fig. 5, Table 1). Juveniles grew approximately 10 mm in 90 d in all tagging treatments and controls (Fig. 3). Average growth in length was considerably less for adults, but there was no negative effect due to implanting single or double tags.

DISCUSSION

This study shows that the use of microtags in a small coral-reef damselfish, *Pomacentrus moluccensis*, can be a useful tool for providing long-term individual recognition. It shows that reliability of microtag retention is high for both new recruits and adults when tags are injected singly (100%, 100%) and in combination

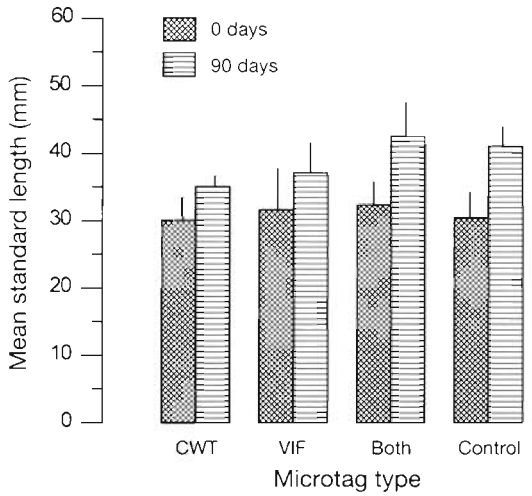

Fig. 5. *Pomacentrus moluccensis*. Standard lengths (mm, $\bar{x} \pm$ SD) of adults at time of tagging and after 90 d. n = 48

(100%, 80%) and also shows that there is negligible impact on growth and survivorship. Tagging is a good technique for recognition of individuals of 11 mm standard length or more, and it may be that fish smaller than this could be tagged with practice. There is an indication from this study that size of microtags used can have an important effect on tag retention, and for *P. moluccensis*, the half-length microtags are more reliable when 2 microtags are to be used in combination. This confirms findings elsewhere that tag size in relation to muscle size was an important factor in determining tag retention rates. It is probable that any microtag losses occurred within 45 d of injection, because all fish remaining on the patch reefs were tagged. If tag loss occurred in the field it was also accompanied by either mortality or dispersal. Previous work has indicated that these fish are extremely site attached, rarely moving more than 2 m (Mapstone 1988). Mapstone (1988) marked 99 *P. moluccensis* and found that only 1 moved further than 2 m over 3 yr. Fish losses in all groups were greater, but did not exceed 23%, during the field phase of the experiment and were comparable between treatment and control groups. Many predators colonised these patch reefs during the experiment and hence when individuals disappeared from the reefs it was more likely to be due to a fatality than to dispersal. Perhaps disorientation when first released onto the reefs increased fish vulnerability to predation.

Using this technique it should now be possible to answer major questions in reef fish ecology. For example, it should now be possible to measure the importance of immigration and emigration from populations or experimental units for individuals of 11 mm standard length or more. This will include new settlers for most reef fish species. Many scarids and acanthurids settle at <6 mm, but with practice these too may be possible to tag.

Some authors have suggested that there is no temporal or spatial variation in mortality rates (Victor 1986, Doherty & Fowler 1994). Without well-replicated tagging programmes it is impossible to fully ascertain the processes occurring, for example to separate mortality from migration. This inability to distinguish between processes has often been accepted as an assumption of settlement studies (Aldenhoven 1986, Eckert 1987, Meekan 1988, Sale & Ferrell 1988). It is mostly because of these problems that little is known about the mortality rates of new settlers and juvenile coral reef fish (Victor 1986). Therefore, these microtags should provide a means to understand the importance of processes occurring within a few weeks of settlement and in shaping reef fish populations.

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