African Journal of Marine Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tams20

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Published online: 08 Jan 2010.

To cite this article: SE Kerwath , A Götz , C Wilke , CG Attwood & WHH Sauer (2006) A comparative evaluation of three methods used to tag South African linefish, African Journal of Marine Science, 28:3-4, 637-643, DOI: 10.2989/18142320609504213

To link to this article: http://dx.doi.org/10.2989/18142320609504213

African Journal of Marine Science 2006, 28(3&4): 637–643
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AFRICAN JOURNAL OF MARINE SCIENCE
EISSN 1814–2338

A comparative evaluation of three methods used to tag South African linefish

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Tagging effects and loss rates of 60 Roman *Chrysoblephus laticeps* tagged with dart tags with barbs (D-tags), T-bar filaments (T-tags) and visible implant fluorescent elastomer (VIFE) tags were investigated. The fish were tagged and monitored in a controlled tank experiment over a period of 198 days. Application technique and underwater visibility of VIFE tags were assessed in a preliminary experiment on Roman and on fransmadam *Boobsoidia inornata*. The use of 25-gauge needles improved VIFE tag application. Whereas VIFE tagging caused fin rot in fransmadam, it had no negative effect on Roman. VIFE tag codes could be identified underwater from a distance of 3m under ambient light. There was no significant difference in growth rates among groups of Roman with different tags and controls, but rates of tag loss were high for D-tags (53%) and T-tags (73%). Although some of the VIFE marks were incomplete, all VIFE-tagged fish were individually recognised at the end of the study. The results highlight the need to take cognisance of the high tag loss rate of conventional tags during the design of mark and recapture studies.

Keywords: *Chrysoblephus laticeps*, dart tag, South Africa, tag loss, tag retention, T-bar anchor tag, VIFE tag

Introduction

Mark-and-recapture studies are commonly used to determine aspects of the biology, migration patterns and stock parameters of marine fish (Emery and Wydoski 1987 listed 1 400 studies). For the majority of these applications it is necessary to recognise individual fish that have been at liberty over long periods. Although increasingly replaced by more sophisticated systems such as passive integrated transponder (PIT) tags (Prentice *et al*. 1990a, 1990b), visible implant fluorescent elastomer (VIFE) tags (Beukers *et al.* 1995, Bailey *et al.* 1998, Willis and Babcock 1998) and coded wire tags (Haw *et al*. 1990, Bergman *et al.* 1992), the different types of dart tags with barbs (D-tags) and T-bar anchors (T-tags) are still commonly used worldwide (Carstens *et al.* 2003, Ortiz *et al.* 2003, Laurenson *et al.* 2005).

In South Africa, D- and T-tags have been used in large-scale tagging studies on commercially important linefish species (Mann 1999, Griffiths and Wilke 2002). Analysis and interpretation of data generated from these studies have a strong influence on fisheries management decisions. However, the validity of the conclusions relies on two assumptions: (1) tagging does not affect the normal biological functions of the fish, i.e. movement behaviour, growth, reproduction, mortality and predation; (2) the tags remain on the animals for the duration of the study, or their loss rate are known (Buckley and Blankenship 1990).

In the case of D- and T-tags, evidence for a breach of these assumptions is mounting. Attwood and Swart (2000) reported slower growth rates for two tagged sparids, galjoen *Dichistius capensis* and white steenbras *Lithognathus lithognathus*. Similar results were found for the sparid carpenter *Argyrozona argyrozona* (Brouwer and Griffiths 2004). Biological fouling on the tag causes drag (Hedgepeth *et al.* 1978) and may affect swimming performance (Serafy *et al.* 1995). The lesion created by the internal barb or anchors makes the fish vulnerable to infections (Roberts *et al.* 1973a, 1973b). Further, tag shedding rates have been found to be highly variable between tag types and species (Baglin *et al.* 1980a, 1980b, Davis *et al.* 1982, McFarlane *et al*. 1986, McGlennon and Partington 1997, Xiao *et al.* 1999).

Few studies adequately validate the use of the tag of choice in relation to the above-mentioned underlying assumptions. Buckley and Blankenship (1990) state that in many cases it appears that the choice or acceptability of tags is related more to historic use than to proven reliability. Furthermore, Bergman *et al.* (1992), Haw *et al.* (1990) and McFarlane and Beamish (1990) point out that the credibility

of tagging studies rests on demonstrating that assumptions about tag effects are correct.

This study provides a comparative assessment of three tagging methods on Roman *Chrysoblephus laticeps*, a temperate sparid fish that is endemic to South Africa and represents an important component of the traditional handline fishery. Although the subject of numerous tagging studies with dart tags (e.g. Buxton and Allen 1989, Griffiths and Wilke 2002, Bullen and Mann 2004), the effects of the tags on this species have never been tested in a controlled experiment. The aims of this study were to validate the use of D-tags and T-tags and to test the feasibility of an alternative tag, the visible implant fluorescent elastomer (VIFE).

VIFE tagging has not been used previously on South African marine fish. The method was developed for batch tagging of juvenile bull trout *Salvelinus confluentus* and cutthroat trout *Oncorhynchus clarki* (Bonneau *et al.* 1995) and has been successfully applied to mark individual fish in various studies (Willis and Babcock 1998). VIFE tags consist of a viscous liquid elastomer that is injected into translucent tissue where it sets to form a permanent biocompatible mark that is fluorescent under UV-light. Potential advantages of the VIFE system include reduced effects on growth and mortality (Dewey and Zigler 1996) and possible underwater recognition of individual fish by SCUBA divers.

An experiment was conducted to investigate the application technique and underwater visibility of VIFE tags. A second species, fransmadam *Boobsoidia inornata*, was used to evaluate the visibility of tags on a species with a different colouration to that of *C. laticeps*. Tag retention and tagging effects of the two dart tags (D- and T-tag) and the VIFE tag on Roman were then investigated and compared to a control group.

Material and Methods

Preliminary experiment

Seven *C. laticeps* and nine *B. inornata* were caught by hook-and-line in False Bay, Western Cape province, South Africa, and transferred to two holding tanks (7 500l; diameter = 2m; height = 1.2m; open circulating seawater system; covered with shade cloth) at the Marine and Coastal Management Research Aquarium, Cape Town. After an acclimatisation period of five days, the fish were sequentially anaesthetised with a 2-phenoxy-ethanol solution (0.25ml l^{-1}; 80l container), then placed on a wet plastic-covered foam cushion and measured to the nearest millimetre fork length. Latex gloves were worn during handling to avoid epidermal damage and infections. The elastomer fluid (VIE Four Color Kit; Northwest Marine Technology, Inc., Shaw Island, Washington, USA) was then injected into the tissue between the fin rays. A maximum of five marks per fish was applied. All the available colours (green, orange, red and yellow) were used and marks were attempted on dorsal, anal and caudal fins. Two different instruments were used to apply the elastomer; the supplied tag applicator and a syringe with a 25-gauge needle. After the tagging was completed, the fish were carefully released back into the holding tanks. After a holding period of 17 days and fed on a diet of squid *Loligo vulgaris reynauldii*, white mussel *Donax serra* and red bait *Pyura stolonifera*, all fish were examined to assess their general health and the condition and the visibility of the tags. One fish of each species was released into a large observation tank (60 000l; diameter = 4m; height = 4.8m), in which tag recognition by a SCUBA diver was attempted under ambient light, torch light and UV-light.

Long-term experiment

A total of 100 Roman was caught and maintained in a similar manner as those in the preliminary experiment. After an acclimation period of five months, four groups of 15 healthy fish of similar size range were selected for the experiment. The fish were weighed to the nearest gram and measured to the nearest millimetre fork length. A digital photograph was taken of each fish for individual recognition, and fin or scale damage was noted.

The first group was tagged with barbed plastic D-tags (length 89mm, diameter 1.4mm; Hallprint, South Australia). The tag was inserted on the left side of the animal into the musculature below the posterior third of the dorsal fin, ensuring that the barb hooked in the pterygophores. The second group was tagged at the same position with T-bar anchor dart tags (Hallprint, South Australia). The tag was inserted in the musculature with a commercial tagging gun (Banok 203L series, Banok Company, Japan). The third group was marked with VIFE tags, using a 25-gauge needle. Four individual VIFE marks were placed into the caudal fin. The last group was not tagged and served as a control. All fish were released back into the holding tanks with five differently sized fish of each group in every tank to ensure standard conditions among groups, to minimise the impact of water quality, technical failures or disease, and to check which individuals experienced tag loss.

The fish were fed to saturation two to three times a week with sardine *Sardinops sagax*, squid *Loligo vulgaris reynaudii* or white mussel. Tank temperature and water conditions were documented during feeding. Notes were made on abnormal behaviour, signs of infections and status of the tags. The fish were captured with a dip net after 40 days and 198 days and their condition was reassessed, which included wet mass, fork length, tag condition and fish condition. Digital photographs of each fish were taken to facilitate individual recognition of fish. Tag scars were photographed separately. VIFE tag condition was described using four categories:

(1) 'Complete' — tag was fully intact;
(2) 'Partially lost' — parts of the tag material were lost, but the tag was presumably still visible to a diver;
(3) 'Incomplete' — tag was barely detectable under normal light;
(4) 'Lost' — tag could not be detected, even after dissection of the fin.

Growth data analysis

To allow comparisons between growth rates of fish of different initial sizes, relative length increments (RLI) were calculated:

$$RLI = \frac{\Delta L}{L_{inf} - L_i} \quad (1)$$

where ΔL is the length increase over the observation period, L_{inf} the theoretical maximum length of the von Bertalanffy growth curve for Roman (Götz 2006) and L_i the initial length. Specific weight increments (SWI) were calculated after (Wootton 1999):

$$SWI = \log W_e - \log W_i \quad (2)$$

where W_i is the initial weight and W_e is the weight at the end of the observation period. After establishing the homogeneity of variance (F-test), differences between the groups were tested with ANOVA.

Results

Preliminary experiment

Tagging procedure

Although bigger fish took longer to be sedated, all the fish were motionless after five minutes in the anaesthetic bath. VIFE tags were initially applied to the dorsal, anal and caudal fins, but it soon became evident that the caudal fin was the most suitable fin for tag application, because it did not collapse. Furthermore, because the rays of the caudal fin are closely spaced, less material was needed to make suitably sized marks. However, the tag application proved to be difficult, especially on smaller individuals. The needle had to be inserted into the thin tissue between the fin rays without piercing through the tissue. Care had to be taken not to withdraw the needle too quickly; otherwise fluid oozed out of the entry wound and the mark was lost. Application times per mark varied between 20 seconds and 90 seconds. The applicator for the small needles that were provided with the tagging kit, presumably designed for batch tagging of juvenile fish, proved unsuitable because the needles quickly clogged and a lot of material was wasted. Also, tagging time was unnecessarily prolonged because of the slow flow of material through the narrow needles. The larger 25-gauge needles on 1cc syringes worked more efficiently on both small and large fish.

Survival and conditions during the observation period

All fish started swimming upright less than five minutes after release into the holding tanks, and no fish died during the tagging procedure. All Roman resumed feeding the following day, whereas fransmadam started feeding five days after the treatment. Tag loss, survival and loss of individual marks are summarised in Table 1.

After two days, five fransmadam showed signs of fin rot, and after 10 days all five had lost their caudal fin completely and died; only two of the remaining four appeared healthy. Two more fransmadam died with fin rot at Day 15 and only two appeared healthy after the 17-day experimental period.

All Roman survived the experimental period and there were no signs of fin rot or fungal infections. One fish showed a mild distension of the left eye, a condition referred to here as 'pop eye' disease. This condition is often caused by barotrauma after the rapid ascent of a fish from depth during capture. Gas permeates into the tissues in the tissues in the eye socked causing increased pressure and inflammation. Typically, the eye becomes distended and is eventually lost.

Tag loss

All tags inserted in fins other than the caudal fin were lost after 17 days. In all, five fransmadam lost their tags as a result of fin rot. All marks applied to the caudal fins of Roman were retained and remained visible, although some material was lost. All marks that were made with the larger 25-gauge needle were still complete after 17 days (Figure 1a).

Underwater detection

Although the water in the observation tank was turbid on the day of the assessment (visibility ~4m), marks were visible under natural light from a distance of 3m. The diver reported no difference in general detection of the marks between the two species. The ability to identify the different colours varied with the light conditions (Figure 1b). In natural light with low intensity, orange and green were easily confused with red and yellow respectively, especially on the larger Roman where a thick layer of tissue covered the tag. UV-light improved tag visibility and identification, but only when the diver was close to the fish (<1.5m). Direct artificial light (underwater camera strobe) made it more difficult to approach the fish and did not improve tag recognition.

Long-term experiment

General conditions

Because the tanks were connected to an open seawater flow system, the temperature (12°–16°C, mean 14.3°C) and the water conditions were similar to those in the water adjacent to the aquarium. The turbidity of the water varied with sea conditions around the water intake to the aquarium. The initial size and weight of fish was not significantly different among the different treatment groups (ANOVA, $p = 0.89$ [length], $p = 0.96$ [weight]).

Observations after release and during feeding

All fish survived the tagging procedure. Irrespective of tagging method, all fish accepted food 1h after being returned to the tanks. There was no abnormal behaviour one day after the tagging. Some dart-tagged animals developed a bruise of 5–7mm diameter around the tag. During feeding, tagged fish showed no signs of restricted mobility and their behaviour was similar to that of untagged fish.

First assessment

The majority of the fish, independent of treatment or tank, showed no visible signs of distress or ill health after 40 days. Seven fish had minor abrasions of the upper caudal lobe and two fish had minor canine damage, presumably caused by bumping into the tank wall during capture attempts

Table 1: Summary of tag loss and fish conditions for VIFE-tagged *C. laticeps* (n = 7) and *B. inornata* (n = 9) after 17 days

Species	Condition			Retention of complete individual VIFE marks on surviving fish (%)
	Healthy	Unhealthy	Dead	
B. inornata	2	0	7	60
C. laticeps	6	1	0	73

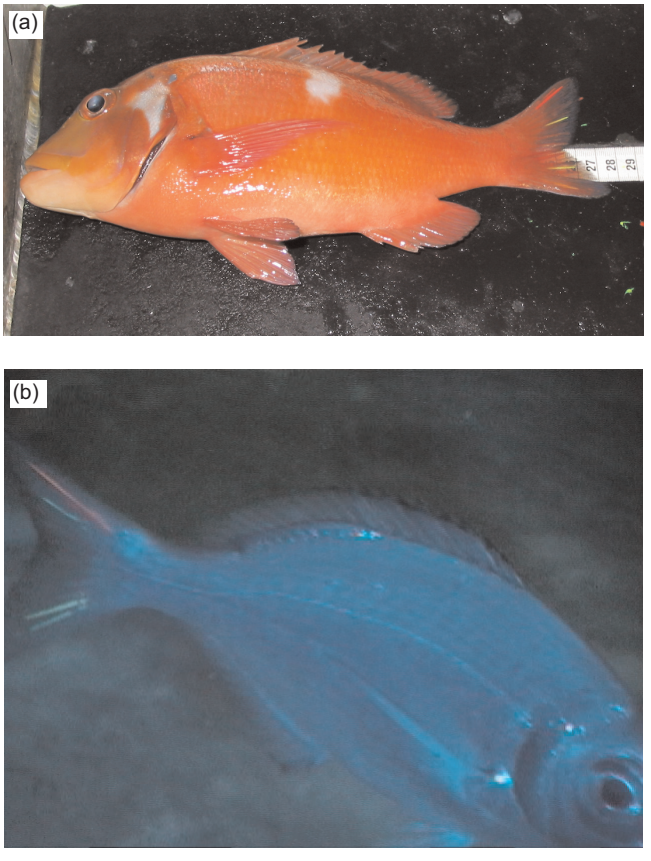

Figure 1: (a) *C. laticeps* with four complete VIFE marks (red-yellow-yellow-orange, dorsal to ventral); (b) *B. inornata* with four complete VIFE marks (orange-green-yellow-green, dorsal to ventral), photographed by a diver with an underwater camera under flashlight

or during flight reactions when disturbed by aquarium personnel. Two D-tags and one T-tag were shed during the first 40 days. The T-bar of the T-tag was broken off at one end. A total of 12 T-tags had exposed filaments and two D-tags had contact with the dorsal fin of the respective fish. This caused fin degradation at the contact point. In addition, one individual suffered from minor 'pop-eye' disease of the left eye. All VIFE-tagged fish could be individually identified, although a number of VIFE tags were partially lost or incomplete (Table 2).

There was no significant difference in specific weight increments among the different tag types and the control group after 40 days (Figure 2, ANOVA, p = 0.14). Only fish that retained their tags were included in the analysis. Length increment was not analysed after 40 days because of the high inherent error of length measurements in relation to the slow growth rate.

Table 2: Summary of VIFE tag condition for tagged *C. laticeps* after 40 days and 198 days

VIFE retention	Proportion (%)	
	40 days	198 days
Complete	42	25
Partially lost	33	37
Incomplete	25	38
Lost	0	0

Final assessment

After 198 days, one D-tagged fish and two control fish from different tanks had died. Two of them had developed 'pop eye' disease; one appeared to have an inflated intestine and was unable to control its buoyancy. Three of the remaining fish of different tanks (T-tag, D-tag and control) had developed mild 'pop-eye' disease on one side. The condition of the remaining 54 fish seemed unchanged since Day 40. All VIFE codes were identifiable, although several tags had been partially lost or were notably incomplete (Table 2). Because of the careful selection of clearly distinguishable fish within treatment groups and the photographic identification, all fish without tags were individually identified after 198 days. Dart tag losses could be clearly distinguished from untagged fish by the grey tag scars of 3–7mm diameter.

In all, 11 T-tags (73%) and eight D-tags (53%) were lost during the study period. Of these, six T-tags and one D-tag were lost without trace, presumably being flushed down the drainage system. All other tags were recovered on the day of tag loss. The filaments of six T-tags had split and the barbs of four D-tags were missing. A thin layer of algal growth covered tags shed after Day 100. No teeth marks were evident on any shed tag. The D-tags were shed at a constant rate, independent of time at liberty (Figure 3). The instantaneous tag loss rate was 0.0028 day^{-1} (linear regression, r = 0.85, p = 0.000).

Results from the 12 fish that retained either a T-tag or a D-tag at the end of the study period were pooled to achieve a meaningful sample size. There was no significant difference in growth among the VIFE tagged fish, the remaining dart-tagged fish and the control group at end of the experimental period (ANOVA, p = 0.43 for relative length increments [Figure 4] and p = 0.50 for specific weight increments [Figure 5]).

Discussion

To interpret the results of mark-and-recapture studies in a meaningful way, it is critical to test the effect of tags on growth and mortality of the study species and to assess the rate of tag loss (Buckley and Blankenship 1990). The fact that there was no difference in mortality or growth rate between tagged groups and control fish suggests that

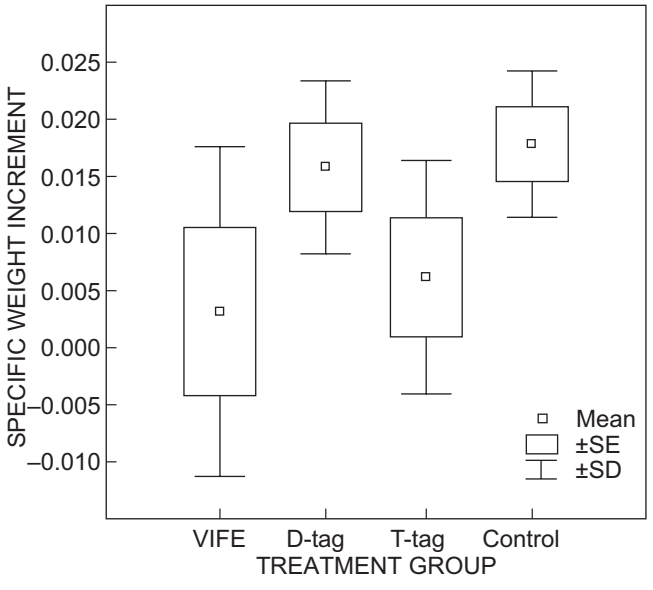

Figure 2: Comparison of specific weight increments between treatment groups after 40 days. Differences are not significant

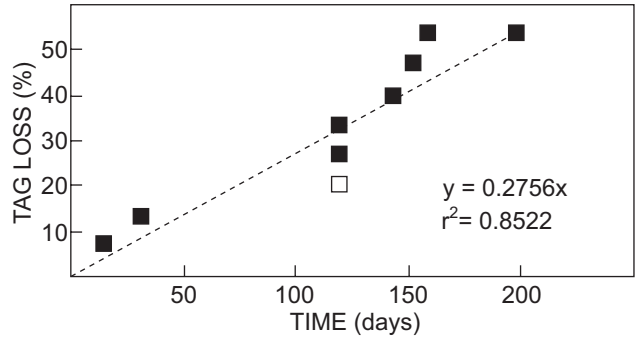

Figure 3: D-Tag loss over time during the 198-day experiment. The tag-loss date of one missing tag was plotted as if it occurred halfway between the two assessments, and is indicated by the white box

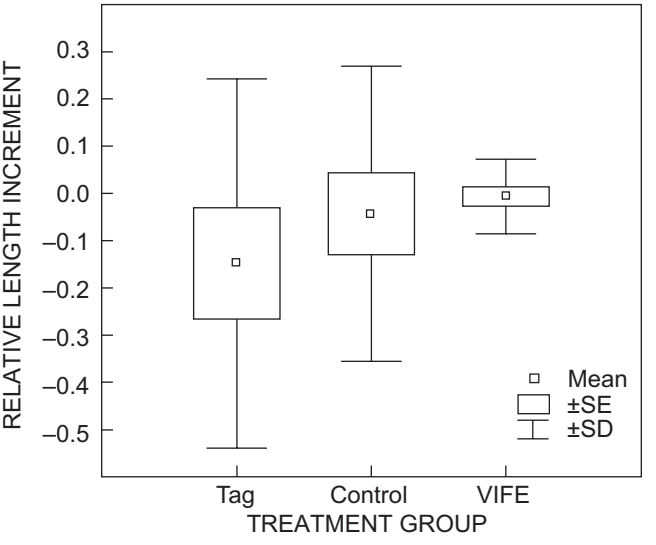

Figure 4: Comparison of relative length increments between treatment groups after 198 days. Data for D- and T-tags are pooled. Differences are not significant

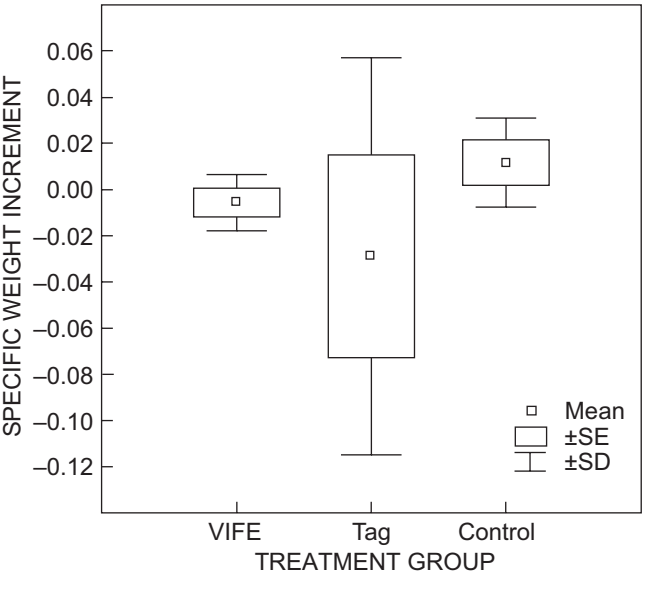

Figure 5: Comparison of specific weight increments between treatment groups after 198 days. Data for D- and T-tags are pooled. Differences are not significant

tagging experiments using tags investigated in this study are suitable to study Roman. This is supported by the low mortality rate (3%), and that the fish were feeding shortly after the tagging procedure and were generally in a healthy condition. The performance of the different tags therefore appears to be the major factor in the choice of tag for mark-and-recapture studies on this species.

VIFE tagging

Previous studies with VIFE tags indicate that they have a better retention rate and are less intrusive than dart tags (Willis and Babcock 1998). The current study clearly shows that VIFE tagging, if carried out correctly, is an effective method to individually mark Roman. However, the technique is more complicated than dart tagging and it requires more experience. The small needles provided with the tagging kit did not work well for fish of the size of Roman and should be replaced by 25-gauge needles, which facilitate speedy application. Also, individual VIFE tagging is limited to few positions for marking on the fish. In Roman, only the caudal fin proved to be suitable. With the four different fluorescent colours available and the two positions in the upper and lower lobe of the caudal fin, it is possible to mark 256 individual fish without duplication.

The recognition of the individual VIFE marks during underwater visual assessments requires experience, especially because the combinations red-orange and yellow-green are easily confused. A powerful UV-torch would facilitate the SCUBA identification of individual marks and the detection

of red marks, which might be difficult to see at depth owing to the greater scatter of light with short wavelengths.

Tagging effects

Tagging may negatively affect growth, and increase mortality rate. Because VIFE tags are situated inside the fin tissue, potential problems associated with conventional tags (e.g. fouling and infections) are eliminated once the material is cured and the small puncture wound has healed. T- and D-tags can affect the growth rate of a fish in two ways: the fish has to expend more energy to overcome the additional drag of the tag (Serafy *et al.* 1995) and the fish uses more resources to fight infections caused by the tag (Roberts *et al.* 1973b). In tank experiments, food is readily available and the effects of additional drag on the energy expenditure of the fish might differ from *in situ* experiments. Roman is a benthic omnivore, feeding mainly on echinoderms and crustaceans (Buxton 1984), so it likely does not depend on speed or prolonged swimming; the effects of drag are therefore probably negligible.

Fin degradation and infections are mainly caused by tag contact with the fin during movement. This is generally the case when the tag becomes heavier with increasing biological fouling. Little biological fouling occurred during this study, which may be attributable to the filter system of the water supply. Tags on Roman *in vivo* accumulate more biological fouling than those on *in vitro* fish over the same period of time (SEK pers. obs.). Therefore, an increased infection rate *in vivo* may apply.

As a result of the anaesthetisation and the longer handling time, VIFE tagging could potentially cause higher mortality immediately after tagging. However this was not the case for Roman irrespective of the type of tag used. However, severe fin rot developed in fransmadam soon after VIFE tagging, causing mortality within five days. Willis and Babcock (1998) detected fin rot in 47% of VIFE-tagged *Pagrus auratus*, a temperate sparid fish from New Zealand, but did not attribute it directly to tagging. The present study serves to emphasise that tagging methods need to be tested across species and that results should not be generalised.

Tag loss

One of the main drawbacks in mark-and-recapture studies is the uncertainty in estimating tag loss. Evident in this study was that tag type and placement has a major effect on tag loss. Most notable was the high shedding rate of dart tags. Given that none of the tags had bite marks and picking on tags by other fish was never observed, effects of overcrowding can be excluded and it can be assumed that the tag loss rates are equally high for *in vivo* experiments. D-tags performed better than T-tags, probably because they are anchored between the pterygophores and their filaments are more rigid. Whether shedding is caused by a biological reaction (Bergman *et al.* 1992) remains to be established. Instantaneous tag loss rates for dart-tagged *P. auratus* were much lower (0.0006 day^{-1}; McGlennon and Partington 1997) than the tag loss in the present study (0.0028 day^{-1}), emphasising that tag loss rates can vary between closely related species.

The high short-term tag loss of VIFE tags in the preliminary experiment was likely attributable to tagging technique, because correct application is critical to retention rate (Willis and Babcock 1998). If properly inserted, VIFE tags had a higher retention rate than dart tags. All individual fish were recognisable after 198 days, although some of the implanted material was lost. In field studies, all 100 VIFE-tagged fish were individually identified after more than two years at liberty (SEK, unpublished data).

Conclusions

Dart tagging methods traditionally used in South African mark-and-recapture studies have a number of disadvantages, which have been highlighted in this study. The extent of negative effects on fish depends on the biology of the species under study and needs to be individually tested prior to field studies. For Roman, dart tags did not seem to have a negative effect on growth and survival, but the high tag loss rate makes long-term studies inefficient. The feasibility of tagging programmes needs to be revised through rigorous testing of the effects of tagging and tag-loss rates of all species. VIFE tagging is an effective alternative in scientific tagging programmes, but it should be used on a smaller scale that does not rely on recapture reporting from the general public, especially in ecological studies that examine juvenile dispersal (Buckley and Blankenship 1990) and assessment of site fidelity (Willis *et al.* 2001), in which underwater detection of individual fish is required.

Acknowledgements — We thank the research aquarium staff and the technicians from Marine and Coastal Management's inshore section for their logistic support. The 'Two Oceans' aquarium kindly provided the fish used in the preliminary experiment. The study was partly funded by the National Research Foundation.

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Manuscript received March 2006; accepted July 2006