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A NOTE ON THE MORTALITY ASSOCIATED WITH FLUORESCENT MARKING IN A GASTROPOD

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ABSTRACT: *Three groups of a total 716 whelks* (Buccinum undatum) *were exposed to the 50 mg l $^{-1}$ concentrations of three fluorescent dyes (Alizarin, Calcein and Oxytetracycline) for 24 h. While Oxytetracycline did not cause any acute mortality during the treatment and during the 17 days holding period (prior to release to field for mark-recapture experiment), some deaths occurred in groups treated with Alizarin and Calcein during these periods. Over the one year sampling period, the only recaptures were the whelks treated with Oxytetracyline.*

Keywords: Fluorescent, gastropod, marking, mortality.

BİR GASTROPOD'DA FLORESENT MARKALAMA İLE İLİŞKİLİ ÖLÜM ÜZERİNE BİR NOT

ÖZET: *Üç gruba ayrılmış 716 deniz salyangozu* (Buccinum undatum), *24 saatlik bir süre için üç ayrı floresent boyanın (Alizarin, Calcein ve Oxytetracycline) 50 mg l $^{-1}$ lik konsantrasyonlarına maruz bırakıldı. Boyama ve 17 günlük bakım (markalama-tekrar yakalama deneyi için sahaya salıverilmeden önce) süresince Oxytetracycline herhangi bir ani ölüme neden olmamasına rağmen, Alizarin ve Calcein'e maruz bırakılmış gruplarda ölüm olayları gözlendi. Bir yıldan fazla örnekleme süresince sadece Oxytetracycline ile muamele edilmiş deniz salyongozları yakalandı.*

Anahtar Kelimeler: Floresent, gastropod, markalama, ölüm.

INTRODUCTION

Different levels of fluorescent dyes are deposited at sites where calcification takes place, depending on the rate of calcification as a result of seasonal change in the growth rate of the organisms. Thus, areas with heavy fluorescent dye deposits in the hard structures of the organism could be observed under ultraviolet light. These dyes have been extensively used in fishes (1,2,3), but rarely in bivalves (4) and gastropods (5,6). Sire (5) applied both tag-

ging and marking methods to the Polynesian gastropod- *Turbo setosus;* the shell was tagged after drilling, wiring and chemical marking using fluorescent dyes to study sheel and operculum growth.

In general, there are three ways of applying markers to these animals: 1) in the food, 2) immersion of the animal into a dye solution, 3) injection. Sire (5) reported that the immersion method appeared to be better than injection for the Polynesian gastropod, *Turbo setosus*.

The two main aims of the chemical marking experiment were (a) to make the whelk's opercular rings clearly visible with fluorescent marker, and (b) to determine whether these rings are laid annually. These were primarily to determine the growth rate of this species. This experiment was not planned to assess the effect of fluorescent marking on the growth and mortality of the common whelk, *B. undatum* L. However, there were some interesting, results on the effect of these markers on the mortality of whelks which are worth mentioning.

MATERIALS AND METHODS

Animal were collected by pots in April 1989. The sampling area was approximately 0.5 miles off the coast of Douglas, Isle of Man, in the Irish Sea. Detailed information of the pots and sampling is given in Kideys (7). Animals were transported to the laboratory in the pots. On arrival at the laboratory whelks were held in tanks with running seawater.

Two methods of marking were performed; a) fixing a tag onto the shell and, b) chemical marking using fluorescent dyes.

The animals were tagged soon after arriving in the laboratory. Only animals with a shell length greater than 25 mm tagged succesfully. The details of the tagging are given elsewhere (7).

In addition to tagging, three groups of a total 716 individuals were also chemically marked with three fluorescent dyes. All there chemicals (Alizarin Sodium Sulfonate or Alizarin Red S No. A-3757 - reddish pink in colour; Calcein No. C-0875 -greenish yellow in colour; Oxytetracycline Hydrochloride No. O-5875- greenish yellow in colour) were supplied by Sigma Chem. Com. The experiments were conducted in a constant temperature room set at 13.5^{o}C. Fifty mg l^{-1} concentrations of these three chemicals were prepared in three 60 l tanks. In addition to aeration, recirculation of seawater was provided for each tank. The whelks were kept for 24 h under these conditions (9h dark-15 h light period) before being placed in holding tanks until release into the study area. The number of deaths which occurred during the chemical treatment and during the holding period (17 days) were recorded. On 12th May 1989, the live animals were released back to the same area where they were collected.

To recapture the marked whelks, pot sampling in the study area was carried out with monthly intervals until July 1990.

RESULTS

There were 6 and 10 deaths during treatment with Alizarin (223 individuals treated) and Calcein (260 individuals treated), respectively (Table 1). During the holding period (17 days

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prior to release, two of those treated with Alizarin and nine of those treated with Calcein died. No deaths occurred in the 233 oxytetracycline treated individuals during treatment or during holding (Table 1). Thirteen of the oxytetracycline treated whelks were recaptured whilst none from other treatments (Alizarin and Calcein) were found over one year sampling period.

Table 1. The Number of Dead Whelks During Treatment (24 Hours) and During the Holding Period (17 days) with Three Fluorescent Markers and Recaptures From each Group

Dye	Total treatment	Death during holding period	Death during	Recaptures
Alizarin	223	6	2	-
Calcein	260	10	9	-
Oxytetracycline	233	-	-	13

DISCUSSION

Firm conclusions about the effect of fluorescent markers used on the mortality of *B. undatum* can not be made from this study, since the main aim was to validate whether the opercular rings are annual or not. However, a short discussion on the results found here may be valuable for the future research on this subject.

Tagging itself has been shown to cause a significant mortality on welks under suboptimal conditions (i.e. space and seawater flow rate, 7). It might be suggested that the combined effects of tagging and fluorescent marking may even result in a greater mortality. In any case the findings is that, Alizarin and Calcein seem more acutely toxic in comparison to oxytetracycline (all 50 mg l^{-1} concentrations) since no deaths occurred during treatment (i.e. immersion) with the latter chemical, but did with the other two. The same suggestion may be valid in terms of long term mortality as 13 of the oxytetracycline treated animals were recaptured whilst none of those from Alizarin or Calcein treated groups was recaptured. Unfortunately, there is not much known on the effect of fluorescent dyes on the growth and mortality of gastropods since it is a comparatively recent marking method for this group (5, 6). Results from other studies confirm that, different dyes affect animals to different degrees, depending on the group , growth stage of the animal studied and on the dose of the dye. For example, while the exposure for 7 days to concentrations of Alizarin below 0.10 mg l^{-1} stopped growth and produced high mortality in larval *Mercenaria mercenaria* (4). Larvae subjected for 48 hours to the concentrations of Alizarin above 0.75 mg l^{-1} were unable to resume normal growth. These authors reported no deaths for the juveniles or adults of *M. mercenaria* when they were subjected to 0.25 to 20 mg l^{-1} of Alizarin for 7 days. Hettler (2) suggested that the survival of marina fish larvae of spot, *Leiostomus*

Doğa - Tr. J. of Zoology
17 (1993), 179-188

xanthurus and pinfish *Lagodon rhombides* were affected by trs buffered 100-500 mg l^{-1} solutions of tetracycline in a 1 % NaCl solution for a 1-2 hour immersion period. However, Weber & Ridgeway (1) report that diets containing tetracycline antibiotics (250 mg of tetracycline antibiotic per kilogram body weight per day for 4 consecutive days) did not affect growth or survival of pasific survival of Pasific salmon *(Oncorhyncus* spp.) even in the long term (2-year at sea).

The results of the present study indicates that some fluorescent markers are more toxic than the others. The toxic levels of different fluorescent markers are needed to be determined before being attempted to observe these markers in the hard structures of marine gastropods.

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