Applicability of three fluorescent markers for growth estimations of the surf clam *Donax hanleyanus*

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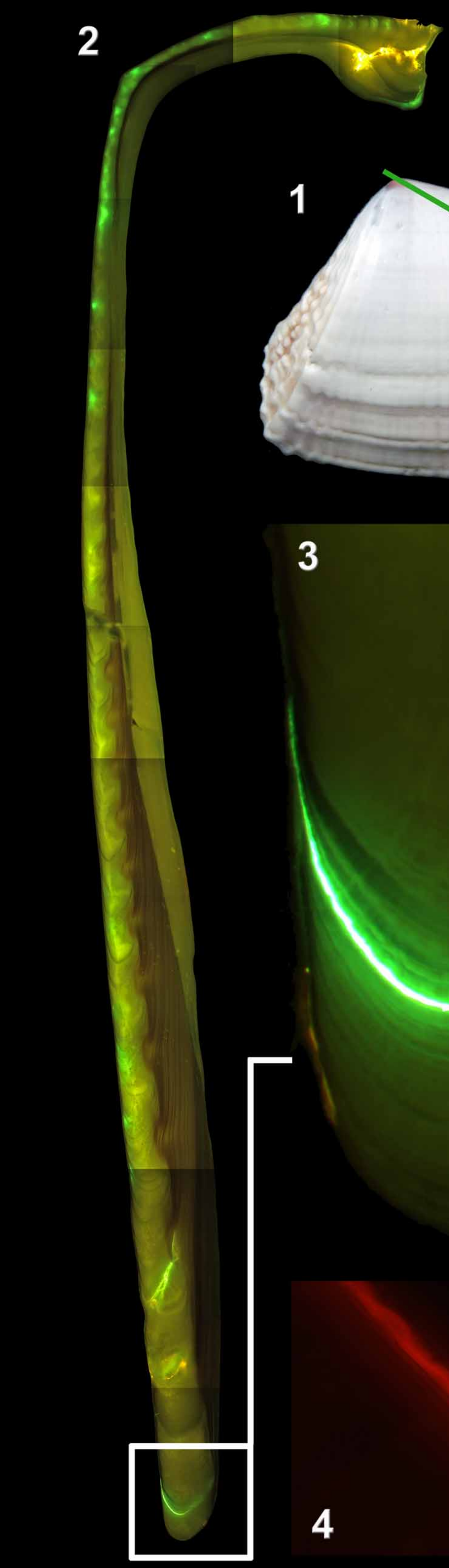

Introduction:
A non-lethal method providing growth marks is a precondition for many *in-vivo* experiments to investigate growth of marine invertebrates. This study determines the effectiveness of calcein, alizarin red and strontium chloride hexahydrate ($SrCl_2$)* in marking Argentinean surf clams (*D. hanleyanus*) under controlled conditions.

Material & Methods:
Wild specimens collected from Faro Querandí (Buenos Aires Province, Argentina) were tagged using different concentrations of each stain and several immersion periods (Table). After marking, clams were reared in a controlled aquaculture system to allow growth for 20 days. To detect the incorporated marks, shells were cuted (Fig. 1) and sections analyzed using fluorescence microscopy for Calcein and Alizarin Red (Fig. 2-4) and scanning electron microscopy for $SrCl_2$ (*).

Solution	Concentration (mg/l)	Staining time (hours)	Number of animals	Appearance of mark (%)	TOTAL (%)
Alizarine	50	3	15	3	13
Alizarine	50	6	15	8	
Alizarine	10	3	15	--	
Alizarine	10	6	15	2	
Calcein	100	3	15	7	28
Calcein	100	6	15	7	
Calcein	50	3	15	7	
Calcein	50	6	15	7	
$SrCl_2$	225	3	15		*
$SrCl_2$	225	6	15		
$SrCl_2$	900	3	15		
$SrCl_2$	900	6	15		
$SrCl_2$	2880	24	15		
Control	0	0	15	0	0
TOTAL			210		41

Results:
41% of all analysed samples were all able to produce visible growth marks in shells of *D. hanleyanus* (Table, Fig. 2-4). Alizarin Red (13% of analysed samples) showed not so clear flourescenc marks like Calcein (28% of analysed samples) (Fig. 3 and 4 respectively). Concentration and staining time had no significant influence. *Shells stained with $SrCl_2$. couldn´t analysed yet because of a defect of the microscope.

Conclusions:
Results demonstrate that Calcein was the best growth marker as it provides a long lasting, readily detected fluorescent mark that can be used to measure shell growth accurately, even at low concentrations and short immersion periods. In addition, marking with Calcein did not affect survivorship or growth and thus is considered to be the better stain for *D. hanleyanus*.

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