Growth estimates of the Argentinean surf clam *Donax hanleyanus* (Bivalvia: Donacidae) derived from fluorescent marking

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**Introduction:**
Growth rate is a basic parameter to describe population dynamics and determines together with recruitment the sustainable yield. Due to the economical importance of bivalves growth rates are well studied using different methods (e.g. length-frequency analysis, tagging-recapture experiments, growth ring readings). Fluorescent marking is direct, efficient and allows micro-growth measurements. The aim of this study was to determine in situ growth rates of *D. hanleyanus* (Fig. 4) inhabiting exposed Argentinean sandy beaches.

**Material & Methods:**
- 260 *D. hanleyanus* (13.5 to 35.0 mm long) were collected at Mar Azul (Fig. 2) in March 2006.
- 195 specimens were stained (50 mg Calcein /l for 3 h, following Herrmann et al. 2006) in a dark aerated tank.
- A non-treated control group (65 *D. hanleyanus*) was maintained in resembling tanks without stain.
- Test clams were reared in situ using cages (Fig. 5) and sampled every week (5 weeks) (Fig. 8, 9).
- Empty shells of scarified clams were cleaned and dried at room temperature.
- For mark detection shells were cut transversely using a diamond saw before embedding in resin and successively polishing on glass slides (Silicon carbide powders; Aluminum oxide suspension).
- Fluorescence microscopical detection of marks and growth measurements using blue light.

**Results:**
Calcein provides a distinct green fluorescent mark conspicuous in 82 % of the shells. Growth increments were measured in ~50 % of the shells, in 21 % microgrowth rings were observed. Maximum growth was found in juveniles (e.g. SL = 16.41 ± 0.36 mm, 28 days). The relationship between SL and growth was best described by a potential function (Fig. 7).

**Conclusions:**
Calcein marking did not affect survivorship or growth of *D. hanleyanus* and thus is a useful non-lethal marker. Once incorporated Calcein emits green fluorescence under blue light, clearly distinguishable from autofluorescence (Fig. 1). The stain marks a distinct starting point, allowing individual growth measurements in contrast to collective growth estimates provided by other methods. For better insights the whole size range of the *D. hanleyanus* population should be covered and the experimental time prolonged, which however is difficult due to the wave exposure of the habitat.

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GROWTH ESTIMATES OF THE ARGENTINEAN SURF CLAM *Donax hanleyanus* (BIVALVIA: DONACIDAE) DERIVED FROM FLUORESCENT MARKING

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RESUMEN

Micro-growth increments of the Argentinean surf clam *Donax hanleyanus* were measured at the exposed sandy beach Mar Azul. After marking the shells with the fluorescence marker Calcein, animals were allowed to grow *in situ* for 36 days. The majority of the marked specimens showed a distinct fluorescent band reflecting the calcein staining time and allowing for growth estimates.

Palabras llaves: growth rate, fluorescent marking, Calcein, surf clam, *Donax hanleyanus*

INTRODUCCIÓN

Growth rate is one of the basic parameters to describe population dynamics; from a fisheries point of view growth, as well as recruitment determines the sustainable yield, which can be exploited from a stock. Growth rates of bivalves have been well studied, since many species are important for the fishing industry. A variety of methods, including length-frequency analysis, tagging-recapture experiments and readings of shell growth rings were used. Among these, marking methods are efficient in estimating bivalve growth rates because they are inexpensive and relatively easy to apply. However, it is difficult to detect micro-growth increments in shells on a scale of less than tens of micrometers, since such increases must be measured with a vernier caliper or by optical microscopy. The aim of the current study is to determine *in situ* growth rates of the bivalve *Donax hanleyanus* inhabiting an Argentinean exposed sandy beach.

MATERIAL Y MÉTODOS

![Experimental cage and installed cubes](image)

Fig. 1: Left: Experimental cage (40 cm × 40 cm × 40 cm) bonded with a 1-mm mesh, right: Installed cubes in the intertidal (*Donax*-belt) of the beach at Mar Azul (duration of experiment: 36 days).

260 specimens of the Argentinean surf clam *Donax hanleyanus* (anterior-posterior shell length [SL]: 13.47 to 34.95 mm) were collected alive at the exposed sandy beach Mar Azul, Province of Buenos Aires (37°20′S, 57°01′W0) in March 2006. To avoid high mortality rates during staining test bivalves were placed immediately in aerated tanks (circulating seawater). The water temperature was set to resemble the ambient temperature (17-20°C). 195 specimens were stained with Calcein (Sigma, CAS 1461-15-0; 50 mg/l for 3 h) in a dark tank to prevent light degradation of the fluorescent chemical during the immersion period. Additionally a non-treated control group of 65 specimens was maintained in resembling tanks. The concentration and immersion period were chosen in accordance to our
previous studies for marking *Donax hanleyanus* in controlled systems. After immersion test animals were reared *in situ* in three experimental cages (40 cm × 40 cm × 40 cm) bonded with a 1-mm mesh for 36 days (Fig. 1). Every seventh day samples were taken. Test bivalves were scarified, empty shells were cleaned and dried at room temperature. For the detection of incorporated growth marks produced during the immersion in the Calcein solution, a transverse shell section was cut across the longest growth axis with a Buehler diamond saw (model Isomet) before embedding in Epoxicure resin (Araldit GY 257) and Endurecedor (HY 5083). Thereafter the cuts were successively polished on glass slides with different grades of SiC powder (125-68-30-12), and finally with 1 µm Al₂O₃ suspension (Brot). Marks were detected by examination and photographing under a fluorescence microscope (Zeiss Axio Imager Z1) using blue light (460 to 490 nm, filter 9: 488009-0000-000). The growth of *Donax hanleyanus* was determined with a micrometer measuring the distance (µm) between the staining mark and the growth edge. Absolute growth rate was measured as shell growth along time:

\[
\text{absolute growth rate} = \frac{SL_2 - SL_1}{t_2 - t_1} = \frac{\Delta SL}{\Delta t}
\]

where, \(SL_1\) is the shell length before staining \((t_1)\) and \(SL_2\) the shell length at the end of the rearing period \((t_2)\).

RESULTADOS Y DISCUSIÓN

The distinct green fluorescence band of Calcein was visible in 81 % of the marked specimens. In 40 % of the spells growth increments were determined (Fig. 2). Maximal growth (362.86 µm) was found in juvenile shells \((SL = 15.89 \text{ mm} – 18.98 \text{ mm})\).

CONCLUSIONES

Calcein was found to be a useful growth marker for the surf clam *Donax hanleyanus*. Once incorporated into the shell, it emitted a bright green fluorescence under blue light, which was clearly distinguishable from natural autofluorescence. The distinct and narrow fluorescent band incorporated into the growing edge of the shells at the time of the Calcein staining could be successfully used as a distinct starting point for the time interval and thus for very exact growth measurements (µm scale) related to time. For better insights of growth from the whole *Donax hanleyanus* population at Mar Azul it would be necessary to run the experiment for a longer time, which however proved to be very difficult due to the conditions of the exposed habitat. In addition, Calcein marking did not affect survivorship or growth of *Donax hanleyanus* and thus is considered to be a useful non-lethal marker for *in situ* growth experiments with clams.

REFERENCIAS


