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

Herrmann, M.[1,2]; Laudien, J.[1]; Arntz, W.E.[1]; Penchaszadeh, P.E.[2]

[1]Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, marko.herrmann@awi.de
[2]Lab. Invertebrados, Depto. Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

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


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'WO) 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 Al_2O_3 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:

$$\textit{absolute growth rate} = \frac{SL_2 - SL_1}{t_2 - t_1} = \frac{\Delta \mathrm{SL}}{\Delta \mathrm{t}}$$

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 mm – 18.98 mm).

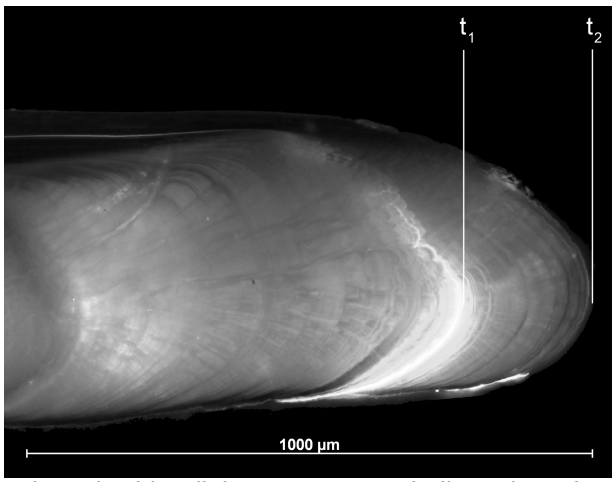

Fig. 2: Photomicrograph under blue light: transverse shell section of *Donax hanleyanus*, 14 days after (t_2) Calcein staining (t_1), the increment between t_1 and t_2 represents shell growth.

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 distict 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

BASHEY, F. (2004). A comparison of the suitability of Alizarin Red S and Calcein for inducing a nonlethally detectable mark in juvenile guppies. Trans. Am. Fish. Soc. 133: 1516-1523.

FUJIKURA, K., OKOSHI, K. & NAGANUMA, T. (2003). Strontium as a marker for estimation of microscopic growth rates in a bivalve. Mar. Ecol. Prog. Ser. 257: 295-301.
GOSSELIN, M.J., FERNANDEZ, E., GUZMÁN, N., LAZARETH, C.E. & ORTLIEB, L. (2006). Preliminary study of growth increments of the Peruvian mollusc shell Trachycardium procerum (Bivalvia) throughout the last half-million years. International Congress on Bivalvia, 22-27 July 2006, Universitat Autònoma de Barcelona, Spain.
GUZMÁN, N.G. (2004). Validation d'une approche scléroclimatologique sur la côte du Chili et du Pérou par l'analyse microstructurale et biogéochimique des coquilles du gastéropode Concholepas concholepas [Bruguière, 1789]. phd thesis 220 p.
HERRMANN, M., LAUDIEN, J., ARNTZ, W.E. & PENCHASZADEH, P.E. (2006a). Applicability of three fluorescent markers for growth estimations of the surf clam Donax hanleyanus. International Congress on Bivalvia, 22-27 July 2006, Universitat Autònoma de Barcelona, Spain.
KAEHLER, S. & MCQUAID, C.D. (1999). Use of the fluorochrome calcein as an in situ growth marker in the brown mussel Perna perna. Mar. Biol. 133: 455-460.
LAMARE, M.D. & MLADENOV, P.V. (2000). Modelling somatic growth in the sea urchin Evechinus chloroticus (Echinoidea: Echinometridae). J. Exp. Mar. Biol. Ecol. 243: 17-43.
LEIPS, J., BARIL, C.T., RODD, F.H., REZNICK, D.N., BASHEY, F., VISSER, G.J. & TRAVIS, J. (2001). The suitability of calcein to mark pocilid fish and a new method of detection. Am. Fish. Soc. 130 (3): 501-507.
MARSCHAL, C., GARRABOU, J., HARMELIN, J.G. & PICHON, M. (2004). A new method for measuring growth and age in the precious red coral Corallium rubrum (L.). Coral Reefs 23 (3): 423-432.
MEDEIROS-BERGEN, D.E. & EBERT, T.A. (1995). Growth, fecundity and mortality rates of two intertidal brittlestars (Echinodermata: Ophiuroidea) with contrasting modes of development. J. Exp. Mar. Biol. Ecol. 189 (1-2): 47-64.
MONAGHAN, J.P. (1993). Comparison of Calcein and Tetracycline as chemical markers in summer flounder. Trans. Am. Fish. Soc. 122: 298-301.
MORAN, A.L. (2000). Calcein as a marker in experimental studies newly-hatched gastropods. Mar. Biol. 137: 893-898.
PECK, L.S., BAKER, A.C. & CONWAY, L.Z. (1996). Strontium labelling of the shell of the Antarctic limpet Nacella concinna (Strebe 1908). J. Moll. Stud. 62: 315-325.
RIASCOS, J.M., GUZMAN, N., LAUDIEN, J., HEILMAYER, O. & OLIVA, M. (2006). Suitability of three stains to mark shells of Concholepas concholepas (Gastrpopda) and Mesodesma donacium (Bivalvia). J. Shellfish Res. 26 (1): 1-7.
ROUSSEAU, M., PLOUGUERNE, E., WAN, G., WAN, R., LOPEZ, E. & FOUCHEREAU-PERON, M. (2003). Biomineralisation markers during a phase of active growth in Pinctada margaritifera. Comp. Biochem. Physiol. A 135 (Part A): 271-278.
ROWLEY, R.J. & MACKINNON, D.I. (1995). Use of the fluorescent marker calcein in biomineralisation studies of brachiopods and other marine organisms. Bulletin de l'Institut Océanographique (Monaco) Spec. Issue 14 part 2: 111-120.
SCHÖNE, B.R., TANABE, K., DETTMAN, D.L. & SATO, S. (2003). Environmental controls on shell growth rates and 18O of the shallow-marine bivalve mollusk Phascosoma japonicum in Japan. Mar. Biol. 142 (3): 473-485.