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Growth estimations of the Argentinean wedge clam *Donax hanleyanus*: A comparison between length-frequency distribution and size-increment analysis

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ABSTRACT

Growth rates of the Argentinean wedge clam *Donax hanleyanus* were estimated comparing two different methods in the intertidal of the exposed sandy beach Mar de las Pampas: (i) results of a relatively shortly (49 days) tagging-recapture experiment using the *in situ* fluorescent marking (*IFM*) method and subsequent size-increment analyses were compared with results from (ii) length-frequency distributions (*LFD*) analysis originating from a time consuming 25 month quantitative sampling. Residuals, derived from *IFM* method and *LFD* analysis, were of similar magnitude and distribution, indicating that both methods are equally appropriate to estimate growth of *D. hanleyanus*. Comparing overall growth performance indices (*OGPs*) of several *Donax* species from different climate areas it resulted that growth of temperate bivalves can be estimated well by carrying out a relatively short-time tagging-recapture experiment using *IFM* but it is recommended to use both, the *IFM* as well as the *LFD* method to determine growth of tropical bivalves. Furthermore, an *in vitro* suitability test of the three stains strontium chloride hexahydrate, alizarin red and calcein resulted that the latter is useful as non-lethal growth marker for *D. hanleyanus*, emitting a bright green fluorescence band under blue light.

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1. Introduction

The growth rate of an organism provides basic ecological data and is one of the prime parameters to describe the respective population dynamics. In fisheries, growth rates linked with recruitment data are used to estimate the sustainable stock yield (Jennings et al., 2001; Hilborn and Walter, 2003; King, 2007). Growth rates of commercially and artisanally extracted bivalves have been well studied (e.g. McLachlan et al., 1996), via various methods such as (i) analysis of size-increments following mark-recapture experiments using tags, filed notches, painting labels and fluorescent stains, (ii) length-frequency distribution (*LFD*) analysis, (iii) shell growth ring analysis, (iv) analysis of stable isotopes (and (v) analysis of the autofluorescent age pigment lipofuscin (for references see Table 1). Estimations of growth and longevity resulting from differing methods are however, often contradictory (e.g. *Mesodesma mactroides*: Capezzani et al., 1971 calculated a life span of ~8 yrs for the Argentinean *Mesodesma* population; whereas Defeo et al., 1988 suggest for the Uruguayan *Mesodesma* population ~3.5 yrs). Current methods for growth and age determination of bivalves all have specific limitations. *LFD* analyses require well-defined age cohorts and normally large sample

sizes, invasive tagging-recapture methods promote physical disturbance and contingently uncharacteristic growth rates, whereas quantification of shell growth rings are affected by surface erosion and disturbance events (for revisions of growth methods see Griffiths and Griffiths, 1987; Richardson, 2001).

To overcome these limitations, a series of previous studies tested the suitability of various chemicals as shell growth markers in different marine invertebrates (Nakahara, 1961; Hidu and Hanks, 1968; Monaghan, 1993; Pricker and Schiel, 1993; Day et al., 1995; Peck et al., 1996). Within the diversity of markers, calcein has proven to be a suitable marker for several bivalves in order to investigate growth increments after marking (calcein: Kaehler and McQuaid, 1999; strontium: Fujikura et al., 2003; calcein: Heilmayer et al., 2005; calcein alizarin red and strontium chloride: Riascos et al., 2007; calcein: Riascos et al., 2008). The polyanionic calcein is a fluorescent compound that binds with calcium carbonate in biomineralised growing structures of organisms such as shells and which fluoresces lime-green when viewed under blue light (Wilson et al., 1987). However, no chemical markers were utilized by now to mark growth of donacid bivalves, for what reason it was implemented an *in vitro* suitability test of the stains alizarin red, calcein and strontium chloride.

Furthermore, to the best of our knowledge, comparisons of growth rate estimations of marine invertebrates, resulting from tagging-recapture experiments using the *in situ* fluorescent marking (*IFM*) method, and from the conventional *LFD* method, have not been made in the past. Previously, such comparisons between a direct and indirect method, respectively,

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Table 1

Growth rates of commercially and artisanally extracted bivalves have been well studied by a large number of authors, applying several methods: (i) analysis of size-increments following mark-recapture experiments, (ii) length-frequency distribution (LFD) analysis, (iii) shell growth ring analysis, (iv) analysis of stable isotopes and (v) analysis of the autofluorescent age pigment lipofuscin.

Analysis	Reference
i (tags)	Heald (1978), Riascos and Urban (2002)
i (filed notches)	Ropes and Merrill (1970), Richardson (1989), Richardson et al. (1990), McQuaid and Lindsay (2000), Laudien et al. (2003)
i (painting labels)	Seed (1969), Beal et al. (1999), Kesler et al. (2007)
i (fluorescent stains)	Hidu and Hanks (1968), Richardson et al. (1979), Parsons et al. (1993), Rowley and Mackinnon (1995), Kaehler and McQuaid (1999), Sato-Okoshi and Okoshi (2002), Heilmayer et al. (2005), Riascos et al. (2007), Miyaji et al. (2007)
ii	Nayar (1955), Alagarswami (1966), Talikhedkar et al. (1976), Arntz et al. (1987), Gaspar et al. (1999), Rocha-Barreira de Almeida et al. (2002)
iii	Capezzani et al. (1971), Ansell and Lagardère (1980), Guillou and Le Moal (1980), Sasaki (1981), Richardson (1989) Ramon et al. (1995), Fiori and Morsán (2004), Morsán and Orensanz (2004)
iv	Jones et al. (1983), Brey and Mackensen (1997), Heilmayer et al. (2003), Carré et al. (2005), Jones et al. (2005), Schöne et al. (2005)
v	Lomovasky et al. (2002)

were delicate inasmuch as investigations originated from different areas and analysed distinct species from disparate periods.

In the present study, we bridge this gap by assessing the suitability of both growth rate estimation methods *IFM* and *LDF* using data of the Argentinean wedge clam *Donax hanleyanus* Philippi, 1847 (Bivalvia: Donacidae), collected at the same time as well as at the same location.

2. Materials and methods

2.1. In vitro suitability test of three stains

2.1.1. Sampling and maintenance

In March 2005, 210 specimens of *D. hanleyanus*, covering the full range of anterior–posterior shell lengths (*apSL*: 21–32 mm) available during that month, were collected from the intertidal by excavating them with hands at the exposed sandy beach Mar de las Pampas (Province of Buenos Aires, Argentina: S37°19', W57°01') during spring tides. The *apSL* of all specimens was measured to the nearest 0.1 mm with a digital vernier calliper (Mitutoyo, model 500-161U). Specimens were maintained in the hatchery of the Instituto de Biología, Marina y Pesquera, 'Alte Storni' in three 350 l conical tanks equipped with a rounded lantern net (each with 70 specimens) containing filtered (using cartridge filters: [I] ECPP-010.7, 10 µm; [II] ECPP-005.7, 5 µm; [III] ECPP-001.7, 1 µm) and aerated circulating seawater under controlled conditions (salinity 34, water temperature 12–14 °C) at least for two weeks, before experiments were carried out. The analysis of the feeding behaviour of *D. hanleyanus* on the microalgae *Isochrysis galbana*, *Chaetoceros gracilis*, *Tetraselmis suecica* and a microalgae mix in proportion 1:1 (*I. galbana*, *C. gracilis*) had shown that wedge clams preferably feed on *I. galbana* (own unpublished data). Each tank of specimens were fed

daily with 38 l of *I. galbana* (600 cell l⁻¹, determined using a Neubauer counting chamber 0.1 mm deep and a surface of 0.0025 mm² area).

2.1.2. Staining experiment

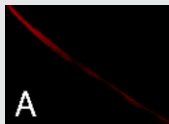
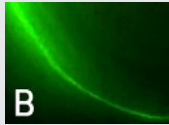
In order to test the suitability of three stains to mark shells of *D. hanleyanus*, the stains alizarin red (Sigma, CAS 130-22-3), calcein (Sigma, CAS 1461-15-0) and strontium chloride hexahydrate ([strontium chloride], Sigma, CAS 10025-70-4) were tested at different concentrations and immersion periods (Table 2), which for alizarin red and calcein were chosen based on previous studies (Day et al., 1995; Kaehler and McQuaid, 1999; Moran, 2000; Riascos et al., 2007). Following Fujikura et al. (2003) and Riascos et al. (2007) strontium chloride concentrations were used for the staining experiment 30, 120 and 360 times the strontium concentration of natural seawater of the South Atlantic Ocean (8.8 mg l⁻¹ on average: Mackenzie, 1964; Angino et al., 1966; de Villiers, 1999). For each treatment 15 randomly assigned wedge clams were available, which were pre-conditioned for two weeks, covering the full range of *apSL*. The staining process was standardised as follows: (i) specimens were placed in 2 l aquaria with aerated circulating seawater containing the respective stain; (ii) each aquarium was placed into the dark to prevent light degradations of the fluorescent chemicals during the immersion period; and (iii) after immersion, wedge clams were restored in the above mentioned 350 l conical tanks and reared in the hatchery for 20 days to allow growth. Dead animals were registered daily and extracted from the tanks.

2.1.3. Shell preparation and detection of growth marks

After the 20 day rearing period, test clams were sacrificed and the empty shells cleaned and dried at room temperature for 48 h. For the detection of incorporated marks, produced during the immersion in

Table 2

Photomicrographs of shell sections (A: alizarin red, 10 mg l⁻¹, 6 h, magnification 1000x; B: calcein, 50 mg l⁻¹, 3 h, magnification 400x) after staining with three different fluorescent stains, concentrations, immersion periods, results on quality of marks and mortality for treated Argentinean wedge clam *D. hanleyanus* under controlled conditions.

Stain	Concentration (mg l ⁻¹)	Immersion period (h)	Mortality (N)	Quality of mark	Incorporated mark
Alizarin red	10	3	0	No mark	
Alizarin red	10	6	1	Faint mark	
Alizarin red	50	3	2	Faint mark	
Alizarin red	50	6	1	Faint mark	
Calcein	50	3	1	Clear mark	
Calcein	50	6	2	Clear mark	
Calcein	100	3	1	Clear mark	
Calcein	100	6	2	Clear mark	
Strontium chloride ^I	264	3	2	No mark	No mark visible
Strontium chloride ^I	264	6	1	No mark	
Strontium chloride ^{II}	1056	3	1	No mark	
Strontium chloride ^{II}	1056	6	2	No mark	
Strontium chloride ^{III}	3168	24	3	No mark	
Control group	–	–	2	–	

30(I), 120 (II) and 360 (III) times, respectively, the concentration of strontium in Atlantic seawater (8.8 mg l⁻¹ on average: Mackenzie, 1964; Angino et al., 1966; de Villiers, 1999).

the respective stain solution, shells were embedded in Epoxicure resin (Distraltec LY 554 and HL 554) and transverse shell sections produced across the longest growth axis (Fig. 1). A Buehler diamond saw (model Isomet) was used for sectioning. Thereafter, cuts were successively polished on glass slides with different grades of Buehler silicon carbide powder (125–68–30–12–5 μm), and finally with 1 μm Buehler aluminium oxide suspension. Alizarin red and calcein marks were detected and photographed using the digital image processing software AxioVision release 4.6.3 (2008) with a fluorescence microscope (Zeiss Axio Imager Z1) under blue (450 to 490 nm) and red light (330 to 385 nm). In order to detect strontium chloride marks, shell sections were analysed under a Philips 515 scanning electron microscope (SEM) equipped with an EDAX 9100 X-ray microprobe system, whereby the electron beam was irradiated at an accelerating voltage of 15 kV and a lifetime of around 150 s.

2.2. Size-increment analysis

2.2.1. Growth marker

From the *in vitro* tests with alizarin red, calcein and strontium chloride it was evident, that marking with calcein does not affect survival or growth. Producing a clearly detectable fluorescent band, calcein was the most suitable stain, wherefore all clams used during the *in situ* experiment were exclusively stained with calcein. Results of the *in vitro* tests are detailed in the Results section.

2.2.2. Sampling, staining and *in situ* growth experiment

In order to study the growth of *D. hanleyanus* derived from the IFM, 240 specimens, covering the entire size range available (*apSL*: 5–32 mm), were collected at Mar de las Pampas in March 2006. Growth differences depending on size classes were analysed by dividing wedge clams into the three ontogenetic stages (determined previously based on histological analyses, Herrmann, 2009): (A) 90 recruits (<11 mm), (B) 20 juveniles (11–22 mm) and (C) 70 adults (>22 mm). The water temperature was set to resemble the ambient temperature of 20 °C. 180 specimens were stained with calcein (50 mg l⁻¹ for 3 h) as described above. Additionally, a non-treated control group of 60 specimens, randomly assigned, was maintained in a similar tank. After immersion, test and control clams were reared *in situ* in four replicated experimental cages in the exposed intertidal zone of Mar de las Pampas, whereby the three divided ontogenetic groups were stochastically independent distributed. Cubic cages consisted of round steel bars with a diameter of 1.5 cm and a side length of 40 cm, bonded with a 1 mm nylon mesh, to allow sediment (mean grain size = 0.37 mm: Marcomini et al., 2002; Herrmann et al., 2009) and microalgae (<50 μm : Coscarón, 1959) to pass through. The experimental set up was installed within the narrow *Donax*-belt (12 m: Herrmann et al., 2009) approximately 35 cm deep in the sediment and with minimal interspaces of 10 m, whereby the stocking rate of the experimental cages was not exceeded the natural

population abundance at Mar de las Pampas (max. abundance 531 ind. m⁻² Herrmann et al., 2009). Each cage was secured via an underground rope fixed to an anchor, buried in the sublittoral zone. To protect the experiment from curious tourists and bait searching anglers the setup was guarded 24 h over the entire experimental time. In order to guarantee measurement data from the highly dynamic exposed sandy beach Mar de las Pampas, every seventh day eight specimens were sampled from each experimental cage during a period of seven weeks by carefully sieving the sand through the cage mesh to avoid damage. Dead animals, noted as washed-out on the sediment surface, were registered daily and extracted from the experimental cages. Chi square analysis was performed to test the effects of the different experimental cages on mortality (Zar, 1999).

2.2.3. Shell preparation and detection of absolute growth rate

In order to calculate the absolute growth rate of *D. hanleyanus*, shells of sampled clams were prepared and analysed as described in Section 2.1.3, and longest growth axis were measured as shell length between umbo and shell margin (*umSL*) (mm) along time (*t*):

$$\text{absolute growth rate} = \frac{\text{umSL}_2 - \text{umSL}_1}{t_2 - t_1} = \frac{\Delta \text{umSL}}{\Delta t} \quad (1)$$

where *umSL*₁ is the initial shell length (mm) between umbo and shell margin before staining (*t*₁) and *umSL*₂ the final shell length (mm) between umbo and shell margin at the end of the experimental period (*t*₂) (Fig. 1).

2.3. Length-frequency distribution analysis

2.3.1. Sampling and data collection

Quantitative samples of *D. hanleyanus* were collected from the same beach (Mar de las Pampas) from a series of stations (4 m intervals) at monthly intervals between December 2004 and December 2006. Sample stations were located along three transects separated by 20 m intervals and located perpendicular to the shoreline from the spring tide high water mark to the spring tide low water mark. At each station, three replicated sand samples (40 × 40 cm) were excavated to 35 cm depth using a 0.16 m² steel corer. Thereafter, samples were sieved individually over a 1 mm mesh and *apSL* of the retained wedge clams was determined as described above to obtain monthly LFD. Afterwards, following the methodology of Herrmann et al. (2009), a von Bertalanffy growth function was established from LFD using an asymptotic length (*L*_∞) of 44 mm and the growth constants (*K*) of 0.46 y⁻¹.

2.4. Comparison of methods

In order to compare growth estimates of both methods used in this study, LFD data were interpreted as size-at-age data (SAD). The IFM data set consisting of *umSL* values was converted to *apSL* data by the linear regression equation $\text{umSL} = 0.8381 \cdot \text{apSL} + 0.0037$ (*N* = 280, *r*² = 0.99). General von Bertalanffy growth functions (gVBGFs) were fitted to size-increment data (SID) resulting from IFM method and to SAD resulting from LFD analysis using the computation worksheet of Brey (2001), applying Microsoft Excel's SOLVER routine:

$$L_t = L_\infty [1 - e^{-K(t-t_0)}]^D \quad (2)$$

where *L*_{*t*} is the *apSL* (mm) at time *t*, *L*_∞ the mean asymptotic *apSL* (mm), *K* the growth constant (yr⁻¹), *D* determines the shape of the curve (inflection point if *D* > 1), and *t*₀ is the age when *apSL* equals zero. Both methods were compared by analysing the variance of the residuals of the gVBGFs.

Additionally, calculated overall growth performance (OGP) indices were useful to compare the VBGFs, since several authors (e.g. Pauly,

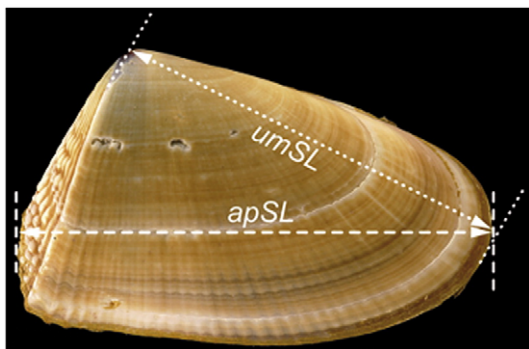


Fig. 1. Shell of *D. hanleyanus* indicating 'anterior-posterior shell length' (*apSL*) and 'shell length between umbo and shell margin' (*umSL*).

1979; Munro and Pauly, 1983; Moreau, et al. 1986; Laudien et al., 2003; Defeo and Cardoso, 2004) demonstrated the suitability of composite indices for *OGP* for inter- and intraspecific comparisons. *OGP* is proportional to the maximum rate of body mass increase during lifetime, i.e. the mass increase at the inflexion point of the *VBGF*, since few values of maximum body mass can be found in the literature and maximal mass is proportional to L_{∞} (Laudien et al., 2003; Herrmann et al., 2009). In this context, the *OGP* of *D. hanleyanus*, derived from both methods used in this study, was calculated as:

$$OGP = \log(K[L_{\infty}])^3 \quad (3)$$

and compared with results from several Donacidae at nearby sampling sites and those of different climate areas.

2.5. Statistical analysis

All statistical analyses were carried out using the statistical package *SPSS version 16.0.1* (2007). Differences were considered significant at a level of $\alpha = 5\%$ (Zar, 1999). Chi-square (χ^2) analyses were applied to determine if significant differences on mortality rates occurred by using stains to mark surf clams during *in vitro* suitability tests and *in situ* growth experiments. The relation between *umSL*₂ and daily growth rate was estimated by exponential regression analysis. Effects of *umSL*₂ and exposure time (7, 14, 21, 28, 35, 42 and 49 days) on growth rate were analysed by utilising a one-way ANCOVA (growth rate as dependent variable, days of exposure as fixed factors and initial length as covariate). Differences of growth rates within the three ontogenetic groups 'recruits', 'juveniles' and 'adults' were analysed by a one-way ANOVA with a Scheffé-procedure post hoc test. *LFD* analyses and tagging-recapture experiments using the *IFM* method and subsequent size-increment analyses, used to estimate growth of both surf clams, were compared by an ANOVA of the residuals of the *gVBGFs*.

3. Results

3.1. In vitro suitability test of three stains

Results of the *in vitro* suitability test of the three stains are summarised in Table 2. For *D. hanleyanus* alizarin red staining was less successful (Table 2A) than marking with calcein (Table 2B). The latter produced clearly visible fluorescent growth bands, easily distinguishable from the natural autofluorescence, at all concentrations and immersion periods (Table 2B). Strontium chloride was not detectable (Table 2). The numbers of dead wedge clams for each treatment are also listed in Table 2. After staining mortality was relatively low (9%) and did not statistically differ between treatments including the control group ($\chi^2 = 3.000$, $df = 2$, $p > 0.05$).

3.2. Size-increment analysis

The described cages proved to be suitable for the *IFM* enclosure experiment in the exposed intertidal zone of Mar de las Pampas. All cages resisted the wave exposure during the entire experimental period. Visually it appeared that no difference was determined in the turbidity of water out- and inside the cages, no filter residue was recognisable on the mesh and no clogging of the mesh by sediment was registered, which indicates natural feeding conditions for the test specimens. Additionally, there was no distinguishable difference of grain size out- and inside of the cages (for details of grain size measurements see Herrmann et al., 2009), and no tidal current scouring was detectable indicating optimal near-natural conditions for the stained wedge clams and control specimens.

Calcein marks (Fig. 2) were conspicuous in 86% ($N = 155$) of the specimens from which growth increments (Fig. 2) were found and measured in 73% ($N = 113$). Over the 49 days of the experiment mortality was relatively low and ranged between 4% ($N = 9$) and 6%

($N = 14$) for the stained specimens and 5% ($N = 11$) for the control clams. Thus, calcein marking did not affect survivorship of *D. hanleyanus* ($\chi^2 = 0.384$, $df = 3$, $p = 0.943$) and therefore calcein is a useful non-lethal marker for field experiments.

As expected, maximum growth increments were found in juvenile *D. hanleyanus* (e.g. *umSL*₂ = 7.31 mm, growth increment of 1.86 mm after 45 days) (Fig. 2). Individual daily growth rate ranged between $8 \mu\text{m d}^{-1}$ and $72 \mu\text{m d}^{-1}$. The relationship between *umSL*₂ and daily growth rate was best described by an exponential function (Fig. 3). Both, *umSL*₂ ($F_{1,96} = 191.249$, $p < 0.001$) and exposure time ($F_{5,96} = 17.415$, $p < 0.001$) had significant effects on growth rate (one-way ANCOVA: growth rate as dependent variable, days of exposure as fixed factors and initial length as covariate). Growth decreased exponentially from recruits to adults ($y = 144.76 \cdot e^{-0.201x}$, $r^2 = 0.91$, $N = 113$): daily growth rates of recruits were significantly higher (Fig. 3, group A: $32.43 \pm 11.21 \mu\text{m d}^{-1}$ [mean \pm SD]) compared to juveniles (Fig. 3, group B: $8.93 \pm 5.24 \mu\text{m d}^{-1}$) and adults (Fig. 3, group C: $0.41 \pm 0.24 \mu\text{m d}^{-1}$) (one-way ANOVA with a Scheffé-procedure post hoc test, $F_{2,110} = 97.983$, $p < 0.001$).

A *gVBGF* was fitted to *SID*, originated from *IFM*, using the maximum length (*umSL* = 37 mm [analogical to *apSL* = 44 mm]) found at Mar de las Pampas as a fixed value of L_{∞} to calculate the growth constant $K = 0.41 \text{ yr}^{-1}$ ($r^2 = 0.69$).

3.3. Length-frequency distribution analysis

In order to analyse length-frequency distributions of *D. hanleyanus*, 2997 specimens were collected from Mar de las Pampas (first year $N = 1545$ ind., second year $N = 1452$ ind.) during a simultaneous study of 25 months (for details of data sets see Herrmann et al., 2008; Herrmann et al., 2009). The smallest live wedge clam recorded had an *apSL* of 4 mm and the largest measured 36 mm (*apSL*). The growth constant $K = 0.47 \text{ yr}^{-1}$ ($R_n = 0.202$) was computed by fitting a *gVBGF* to this data set, using the maximum length (*apSL* = 44 mm) found at Mar de las Pampas as a fixed value of L_{∞} .

3.4. Comparison of methods

The two methods used in this study were compared by residual analyses. Plotting residuals versus the estimated shell lengths showed a very good fit ($r^2 = 0.99$) (Fig. 4). The analysis of variance of the

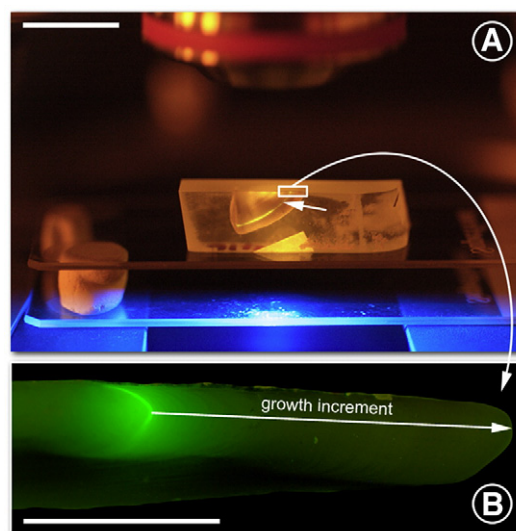


Fig. 2. Epoxycure resin block section of *D. hanleyanus* (*umSL*₂ = 7.31 mm) under a fluorescent microscope equipped with blue light, arrow indicates incorporated calcein mark after 45 days of experimental time, visible with naked eye (A). Resulting transverse shell thin section indicates a growth increment of 1.86 mm, arrow shows the direction of growth (B). Scale bars: (A) 10 mm, (B) 1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

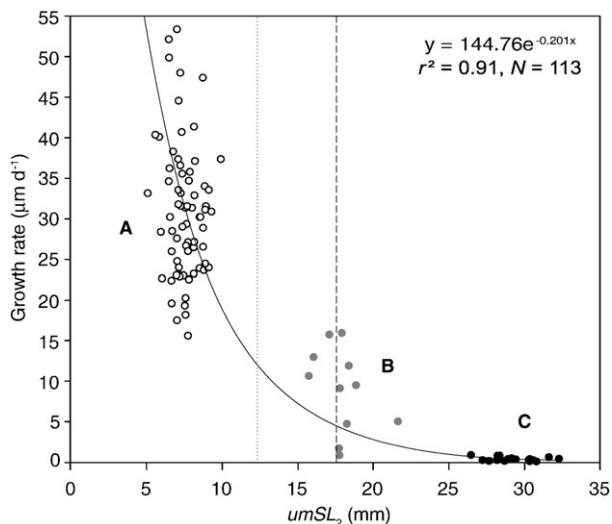


Fig. 3. *D. hanleyanus*. Relationship between final shell length between umbo and shell margin ($umSL_2$) at the end of the experimental period and growth rate ($\mu m d^{-1}$) within the three groups, defined following Herrmann (2009): \circ recruits (A: <11 mm $umSL_2$), \bullet juveniles (B: 11–22 mm $umSL_2$) and \bullet adults (C: >22 mm $umSL_2$). Dotted line indicates size at first maturity (13 mm) and dashed line indicate size of 50% population maturity (17 mm) (Herrmann, 2009).

residuals of the *gVBGFs* showed no significant difference between the two methods (ANOVA, $F_{1,64} = 2.153, p > 0.05$).

Computed *OGP* values of *D. hanleyanus*, resulting from *IFM* ($OGP = 4.45$) and *LFD* ($OGP = 4.60$), were plotted close to each other within the auximetric grid (Fig. 5, no. 16 and no. 17, respectively).

4. Discussion

Marks incorporated in *D. hanleyanus* shells demonstrated qualitative differences, depending on the stain type, concentration and immersion time. The fluorescence marker ‘calcein’ emitted a bright green fluorescence band under blue light, which was readily distinguished from naturally occurring autofluorescence, even in low concentrations and short immersion times. Alizarin red showed imprecise faint growth bands, however, only at higher concentrations and longer immersion periods. Strontium chloride did not produce any detectable growth mark, although high concentrations and long immersion periods were

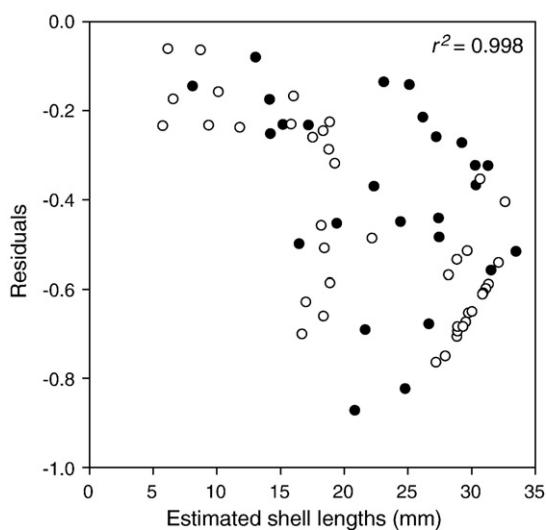


Fig. 4. *D. hanleyanus*. Residuals of estimated shell lengths of (\bullet) size-at-age data (SAD) converted from length-frequency distributions (LFD) analysis and of (\circ) size-increment data (SID) from *in situ* fluorescent marking (IFM) method, showing no significant difference (ANOVA, $F_{1,64} = 2.153, p > 0.05$).

used. The present results agree with previous observations that calcein produces clear marks in molluscs under controlled conditions, which enables short-term, high-resolution growth studies (e.g. *Haliotis rubra*: Day et al., 1995; *Perna perna*: Kaehler and McQuaid, 1999; *Adamussium colbecki*: Heilmayer et al., 2005; *Concholepas concholepas* and *Mesodesma donacium*: Riascos et al., 2007).

A variety of fluorochromes were tested and showed that calcein exhibits little toxicity (Wilson et al., 1987; Hales and Hurley, 1991; Monaghan, 1993; Day et al., 1995; Rowley and Mackinnon, 1995). In accordance with recent studies (Moran, 2000; Riascos et al., 2007) the present study revealed that calcein marking did not affect survivorship of *D. hanleyanus* during the *in vitro* and the *in situ* experiments, however performed well under relatively low concentrations and immersion periods. This shows that calcein can be recommended as a non-lethal marker for *D. hanleyanus*.

The distinct and narrow fluorescent band incorporated into the growing shell edge at the time of calcein exposure was successfully used as a datum point in growth measurements. Fluorescent marks were readily detected in stored samples at least two years after the experiment without visible degradation of the growth marks. The potential for using calcein as a growth-marker in long-term growth studies is therefore great (see also Rowley and Mackinnon, 1995; Kaehler and McQuaid, 1999; Moran, 2000; Riascos et al., 2007).

The *in situ* experiment showed that specimens of *D. hanleyanus* grew between $0.41 \mu m d^{-1}$ and $32.43 \mu m d^{-1}$ whereby the daily growth rate was correlated to the $umSL_2$ as described by an exponential function, depending on individuals’ size classes Fig. 3A, B, C, respectively). Apparently, recruits of *D. hanleyanus* use like other molluscs (e.g. Spight et al., 1974; Calow, 1983; Hutchings and Haedrich, 1984; Adam, 1990; Sato, 1994; Campbell and Ming 2003) the major part of energy for growth until specimens reach the size at first maturity of approximately 11 mm *apSL* (Fig. 3, dotted line) (Herrmann, 2009).

The residuals derived from *IFM* and *LFD* were of similar magnitude and distribution, indicating that both methods are equally appropriate to estimate growth of *D. hanleyanus*. Growth of wedge clams calculated from the 49 days *in situ* experiment (*IFM*) in March–April conforms well to shell growth of the 25 months observation (*LFD*). Furthermore, *OGP* values of the Argentinean *D. hanleyanus* resulting from *IFM* (4.45, Fig. 5, no. 16) and *LFD* (4.60, Fig. 5, no. 17) of the present study shows small distances to values calculated from *LFD* data sets of other *D. hanleyanus* populations (for details of data sets see Herrmann et al., 2008, 2009) from Argentina (4.65, Fig. 5, no. 12), Uruguayan (4.46, Fig. 5, no. 13) and Brazil (4.17 and 4.32, Fig. 5, no. 14 and no. 15, respectively). Therefore it can be concluded that as an alternative to *LFD* analyses, tagging-recapture experiments using the *IFM* method and subsequent size-increment analysis are appropriate to estimate growth of the Argentinean wedge clam *D. hanleyanus*.

Moreover, the auximetric grid (Fig. 5) shows that the *OGP* is habitat-specific; species populating tropical–subtropical regions show lowest *OGP* (2.84–3.68, group A), temperate species have intermittent *OGP* (4.17–4.91, group B), while species of upwelling areas show the highest *OGP* (5.06–5.65, group C). The comparison of *OGPs* of several donacids indicates that tagging-recapture experiments using the *IFM* method and subsequent size-increment analyses are required to estimate growth of tropical *Donax* species. In this way, *OGPs* of *D. dentifer* (Fig. 5, no. 4) and *D. striatus* (Fig. 5, no. 11) demonstrate a relatively large distance to other tropical donacids such as *D. cuneatus* (Fig. 5, no. 1–2), *D. denticulatus* (Fig. 5, no. 3), *D. faba* (Fig. 5, no. 5), *D. incarnatus* (Fig. 5, no. 6–9) and *D. striatus* (Fig. 5, no. 10) and in contrast much lower distance to the temperate donacids *D. hanleyanus* (Fig. 5, no. 12–17), to *D. trunculus* (Fig. 5, no. 18–33) and to *D. vittatus* (Fig. 5, no. 34–38). Since tropical species exhibit continuous spawning events or recruit over a longer period, compared to temperate donacids, *LFD* analysis may not be useful for tropical species to estimate growth (Sparre and Venema, 1998). On this account tagging-recapture experiments coupled with the *IFM* method are recommended to estimate adequate the growth of tropical

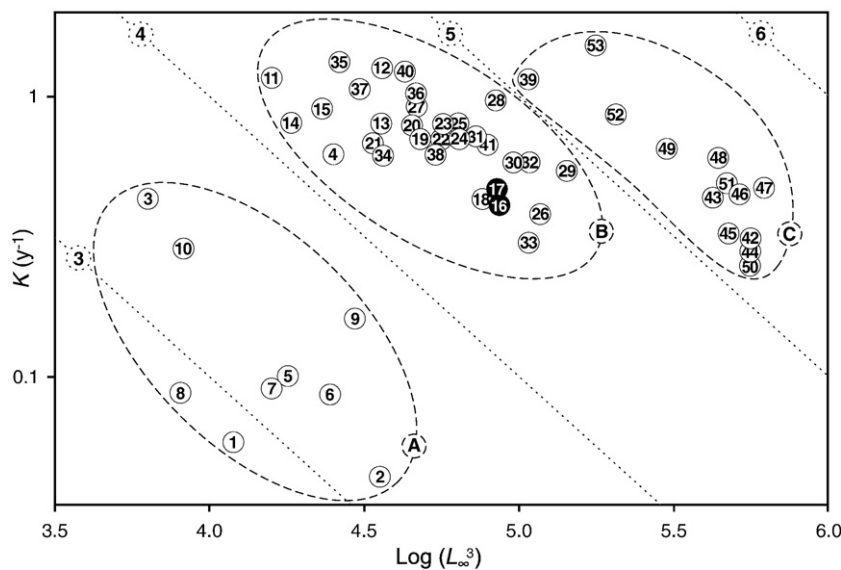


Fig. 5. The auximetric grid compares overall growth performance indices (OGPs) of the Argentinean *D. hanleyanus* from present study (●) with several *Donax* species from different areas (○). Donacids are grouped by the plot (dashed lines) in tropical–subtropical (A), temperate (B) and upwelling species (C). Diagonal dotted lines indicate equal values of OGP (numbers in circles). Data sources: *D. cuneatus* (1: Nayar, 1955; 2: Talikhedkar et al., 1976), *D. denticulatus* (3: Vélez et al., 1985), *D. dentifer* (4: Riascos and Urban, 2002), *D. faba* (5: Alagarswami, 1966), *D. incarnatus* (6, 7: Ansell et al., 1972; 8: Nair et al., 1978; 9: Thippeswamy and Joseph, 1991), *D. striatus* (10: McLachlan et al., 1996; 11: Rocha-Barreira de Almeida et al., 2002), *D. hanleyanus* (12: Penchaszadeh and Olivier, 1975; 13: Defeo, 1996; 14, 15: Cardoso and Veloso, 2003; 16: present study estimated from IFM; 17: present study estimated from LFD), *D. trunculus* (18–25: Ansell and Lagardère, 1980; 26: Guillou and Le Moal, 1980; 27: Bodoy, 1982; 28: Fernández et al., 1984; 29: Mazé and Laborda, 1988; 30, 31: Ramon et al., 1995; 32: Gaspar et al., 1999; 33: Zeichen et al., 2002), *D. vittatus* (34–37: Ansell and Lagardère, 1980; 38: Guillou and Le Moal, 1980), *D. marincovich* (39 before, 40 during and 41 after El Niño: Arntz et al., 1987), *D. serra* (42–45: de Villiers, 1975; 46–51: Laudien et al., 2003), *D. deltoides* (52: King, 1985; 53: Laudien et al., 2003).

species. Furthermore, even the effect of climate anomalies may be detected with the help of the auximetric grid, as indicated by the upwelling surf clam *D. marincovich* (Arntz et al., 1987), sampled in Peru throughout normal upwelling years (Fig. 5, no. 39) in comparison to the population sampled during (Fig. 5, no. 40) and shortly after an El Niño (EN) event 1982–83 (Fig. 5, no. 41). Also the OGP of the tropical *D. dentifer* (Riascos and Urban 2002), collected during the EN event 1997–98 (Fig. 5, no. 4), clustered with the temperate species (Fig. 5, group B), which indicates the abnormality during the climate anomaly.

However, both, the IFM and the LFD method, have advantages and disadvantages, which are summarised in Table 3. The decided advantage of the first mentioned one is accuracy, allowing daily growth rate measurements of *D. hanleyanus*; furthermore a relatively low number of specimens are needed in comparison to the LFD analyses. The IFM can thus also be used for scattered populations difficult to sample enough specimens for clear cohort detection. On the other hand the LFD analysis allows detection of seasonal growth (Appeldoorn, 1987) and specimens can live on after data collection. Also it can be assumed that *Donax* maybe growth more naturally using the LFD method with monthly disturbance, than applying the IFM method with weekly disturbance, induced by sampling respectively. Although the present study showed similar growth parameters calculated from LFD and IFM, the latter

method may not always detect growth of adults accurately, due to the slow growth rate and the short experimental time period. Therefore the IFM must be applied over an adequate period, dependent on the growth rate of the species. Whereas, long study periods, necessary for the LFD analysis, are vulnerable to bias caused by alongshore migration of cohorts, which may not be clearly followed. Although several surf clam species seem to favour river mouths for settlement and successively migrate alongshore (Tarifeño-Silva, 1980; Donn, 1987; Jaramillo et al., 1994; Lastra and McLachlan, 1996), such an ontogenetic alongshore migration was not recorded during the present study as there are no rivers or dry riverbeds located in the sampling site. Furthermore, the histograms did not display certain size classes unexpectedly (dis) appearing. Disadvantageous for the IFM is that carrying out only short-time experiments may lead to an underestimation of L_{∞} due to a possible lack of large adult specimens. Here LFD analyses are more precise due to the longer sampling period. Furthermore, strong wave exposure can destroy an entire *in situ* experiment, but collection for the LFD analysis may be postponed.

In addition to said population dynamic aspects future growth studies should balance also the feasibility of both methods. At the present study, the IFM method was more timesaving towards the LFD method. First mentioned method showed results after only one and a half months experimental time and another three months of laboratory time. The LFD method with 25 months sampling time however was much more time-consuming and also costs were twice that of the IFM method. However, for the IFM method more man power was used in the field compared to the second method where monthly sampling was comparatively easy.

5. Conclusions

Both methods, applied in the present study, are suitable to estimate growth of the Argentinean wedge clam *D. hanleyanus*. Consequently, it is recommended to estimate growth of temperate bivalves by carrying out a relatively short-time tagging-recapture experiment using IFM but to determine growth of tropical bivalves it is suggested to use both, the IFM as well as the LFD method.

Table 3
Attributes of *in situ* fluorescent marking (IFM) and length-frequency distributions (LFD) analysis.

	Category	IFM	LFD
Scientific aspects	Determination of daily growth	yes	no
	Direct growth estimation	yes	no
	Numbers of studied specimens necessary	240	2997 ^a
	Tourists effecting sampling	yes	no ^b
Economic aspects	Costs	lower	higher
	Sampling time	6 h	1 d
	Laboratory work	30 d	6 h
	Expensive equipment necessary	yes	no
	Man power	more	less

^a After Herrmann et al. (2009).

^b Accepted during high season in the summer month January and February.

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