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

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\begin{abstract}
Growth rates of the Argentinean wedge clam \textit{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 short-time (49 days) tagging-recapture experiment using the \textit{in situ} fluorescent marking (IFM) method and subsequent size-increment analyses were compared with results from (ii) \textit{length-frequency distributions} (LFD) analysis originating from a time consuming 25 month quantiative sampling. Residuals, derived from IFM method and \textit{LFD} analysis, were of similar magnitude and distribution, indicating that both methods are equally appropriate to estimate growth of \textit{D. hanleyanus}. Comparing overall growth performance indices (OGPs) of several \textit{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 \textit{LFD} method to determine growth of tropical bivalves. Furthermore, an \textit{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 \textit{D. hanleyanus}, emitting a bright green fluorescence band under blue light.

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\end{abstract}

\section{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, (ii) analysis of fluorescent marking, (iii) analysis of stable isotopes and (iv) 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 macrodyes: Capezzani et al., 1971 calculated a life span of \textasciitilde 8 yrs for the Argentinean \textit{Mesodesma} population; whereas Defeo et al., 1988 suggest for the Uruguayan \textit{Mesodesma} population \textasciitilde 3.5 yrs). Current methods for growth and age determination of bivalves all have specific limitations. \textit{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). The polyamionic 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 \textit{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 \textit{in situ} fluorescent marking (IFM) method, and from the conventional \textit{LFD} method, have not been made in the past. Previously, such comparisons between a direct and indirect method, respectively,
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 IRE and LDF using data of the Argentinian wedge clam Donax hanleyanus Philippi, 1847 (Bivalvia: Donaciidae), 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: 35°7′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 latern net (each with 70 specimens) containing 5 l filtered (using cartridge 1000x; B: calcein, 50 mg l⁻¹) and aerated circulating seawater containing the respective stain; (ii) each specimen 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 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.

2.1.2. 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), calcine (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 calcine were chosen based on previous studies (Day et al., 1995; Kaehler and McQuaid, 1999; Moran, 2000; Riascos et al., 2007). Following Fukijura 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>
<thead>
<tr>
<th>Stain</th>
<th>Concentration (mg l⁻¹)</th>
<th>Immersion period (h)</th>
<th>Mortality (N)</th>
<th>Quality of mark</th>
<th>Incorporated mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alizarin red</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>No mark</td>
<td>No mark visible</td>
</tr>
<tr>
<td>Alizarin red</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>Faint mark</td>
<td></td>
</tr>
<tr>
<td>Alizarin red</td>
<td>50</td>
<td>3</td>
<td>2</td>
<td>Faint mark</td>
<td></td>
</tr>
<tr>
<td>Alizarin red</td>
<td>50</td>
<td>6</td>
<td>1</td>
<td>Faint mark</td>
<td></td>
</tr>
<tr>
<td>Calcine</td>
<td>50</td>
<td>3</td>
<td>1</td>
<td>Clear mark</td>
<td></td>
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<tr>
<td>Calcine</td>
<td>50</td>
<td>6</td>
<td>2</td>
<td>Clear mark</td>
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<tr>
<td>Calcine</td>
<td>100</td>
<td>3</td>
<td>1</td>
<td>Clear mark</td>
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<tr>
<td>Calcine</td>
<td>100</td>
<td>6</td>
<td>2</td>
<td>Clear mark</td>
<td></td>
</tr>
<tr>
<td>Strontium chloride</td>
<td>264</td>
<td>3</td>
<td>2</td>
<td>No mark</td>
<td></td>
</tr>
<tr>
<td>Strontium chloride</td>
<td>264</td>
<td>6</td>
<td>1</td>
<td>No mark</td>
<td></td>
</tr>
<tr>
<td>Strontium chloride</td>
<td>1056</td>
<td>3</td>
<td>1</td>
<td>No mark</td>
<td></td>
</tr>
<tr>
<td>Strontium chloride</td>
<td>1056</td>
<td>6</td>
<td>2</td>
<td>No mark</td>
<td></td>
</tr>
<tr>
<td>Strontium chloride</td>
<td>3168</td>
<td>24</td>
<td>3</td>
<td>No mark</td>
<td></td>
</tr>
</tbody>
</table>

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).
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, 1950) to pass through. The experimental setup 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 caging 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):

\[
\text{absolute growth rate} = \frac{\text{umSL}_2-\text{umSL}_1}{t_2-t_1} = \frac{\Delta \text{umSL}}{\Delta t}
\]

where \(\text{umSL}_1\) is the initial shell length (mm) between umbo and shell margin before staining (\(t_1\)) and \(\text{umSL}_2\) 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 m\(^2\) 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 (\(L_\infty\)) 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 \(\text{apSL} = 0.8381 \times \text{umSL} + 0.0037 (N = 280, r^2 = 0.99)\). General von Bertalanffy growth functions (gVBGFs) were fitted to size-increment data (SAD) resulting from IFM method and to SAD resulting from LFD analysis using the computation worksheet of Brey (2001), applying Microsoft Excel’s SOLVER routine:

\[
L_t = L_\infty[1 - e^{-K(t-t_0)}]^{\Gamma}
\]

where \(L_t\) is the apSL (mm) at time \(t\), \(L_\infty\) the mean asymptotic apSL (mm), \(K\) the growth constant (yr\(^{-1}\)), \(\Gamma\) 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 (OCP) indices were useful to compare the VBGFs, since several authors (e.g. Pauly,
immersion periods (Table 2B). Strontium chloride was not detectable and did not statistically differ between treatments including the also listed in Table 2. After staining mortality was relatively low (9%) (Table 2). The numbers of dead wedge clams for each treatment are compared by an ANOVA of the residuals of the summarized in Table 2. For 3. Results with a Scheffé-procedure post hoc test.

analysed by utilising a one-way ANCOVA (growth rate as dependent exposure time (7, 14, 21, 28, 35, 42 and 49 days) on growth rate were was estimated by exponential regression analysis. Effects of 3.2. Size-increment analysis

of OGP = log(K/L∞)) 3 (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 α = 5% (Zar, 1999). Chi-square (χ²) 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. LID 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 (χ² = 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 (χ² = 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 µm d⁻¹ and 72 µm d⁻¹. The relationship between umSL 2 and daily growth rate was best described by an exponential function (Fig. 3). Both, umSL 2 (F 9,96 = 191.249, p < 0.001) and exposure time (F 9,96 = 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 (γ = 144.76 • e⁻⁰.⁰₃₀ₓ, r² = 0.91, N = 113); daily growth rates of recruits were significantly higher (Fig. 3, group A: 32.43 ± 11.21 µm d⁻¹ [mean ± SD]) compared to juveniles (Fig. 3, group B: 8.93 ± 5.24 µm d⁻¹) and adults (Fig. 3, group C: 0.41 ± 0.24 µm 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 y⁻¹ (r² = 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 yr⁻¹ (R² = 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 (r² = 0.99) (Fig. 4). The analysis of variance of the
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 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*: Helmlayer 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 µm d$^{-1}$ and 32.43 µm d$^{-1}$ whereby the daily growth rate was correlated to the umSL, 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 umSL (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 *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 intermitting 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 OGP 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, OGP 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. incarciatus* (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 (Sparr and Venema, 1998). On this account tagging-recapture experiments coupled with the IFM method are recommended to estimate adequate the growth of tropical
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. hanleyanus* (*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* (*Riscos 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* allows detection of seasonal growth (Appeldoorn, 1987) and specimens for clear cohort detection. On the other hand the measurements of (numbers in circles). Data sources: *D. trunculus* (1: Nayar, 1955; 2: Talikhedkar et al., 1976), *D. dentifer* (3; Vélez et al., 1985), *D. dentifer* (4; Riscos and Urban, 2002), *D. faba* (5; Alagarswami, 1966), *D. inornatus* (6, 7; Assell et al., 1972; 8: Nair et al., 1978; 9; Thippeswamy and Joseph, 1991), *D. stramineus* (10: Mcclachlan et al., 1996; 11: Rocha-Barreira de Almeida et al., 2002), *D. hanleyanus* (12: Penchasazadeh 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; Assell and Lagardère, 1980; 26: Guillou and Le Moal, 1980; 27: Bodoy, 1982; 38: Fernández et al., 1984; 29: Mâzé and Laborda, 1988; 30, 31: Ramon et al., 1995; 32: Gaspar et al., 1999; 33: Zechen et al., 2002), *D. vittatus* (34–37; Assell and Lagardère, 1980; 38: Guillou and Le Moal, 1980), *D. marinovichii* (39 before, 40 during and 41 after El Niño: *Arntz et al., 1987*), *D. serra* (42–45: de Villiers, 1975; 46–51: Lautien et al., 2003), *D. dentifer* (52; King, 1985; 53: Lautien et al., 2003).

### Table 3

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

<table>
<thead>
<tr>
<th>Category</th>
<th>IFM</th>
<th>LFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scientific aspects</td>
<td>Determination of daily growth</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>Direct growth estimation</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>Numbers of studied specimens necessary</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>Tourists effecting sampling</td>
<td>yes</td>
</tr>
<tr>
<td>Economic aspects</td>
<td>Costs</td>
<td>lower</td>
</tr>
<tr>
<td></td>
<td>Sampling time</td>
<td>6 h</td>
</tr>
<tr>
<td></td>
<td>Laboratory work</td>
<td>30 d</td>
</tr>
<tr>
<td></td>
<td>Expensive equipment necessary</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>Man power</td>
<td>more</td>
</tr>
<tr>
<td></td>
<td>Laboratory time</td>
<td>30 d</td>
</tr>
</tbody>
</table>

* After Herrmann et al. (2009).

\*Accepted during high season in the summer months January and February.

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.
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References
