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## In situ giant clam growth rate behavior in relation to temperature: A one-year coupled study of high-frequency noninvasive valvometry and sclerochronology

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### Abstract

The life history of 15 giant clams, *Hippopus hippopus*, was studied in situ in the southern lagoon of New Caledonia; growth rate and animal behavior were studied both by sclerochronology and high-frequency noninvasive (HFNI) valvometry. Electrodes glued on each valve of each specimen recorded the shell-gaping behavior at 0.6-Hz frequency. A nonparametric regression model was used to model clam behavior. The daily increment thickness in the inner layer of five representative clams was measured. *H. hippopus* has its valves open during the day and partly closed during the night all year round, and shell growth is continuous. The cumulative growth using both techniques was similar, as was the mean daily thickness increment. The occurrence of one increment per day in *H. hippopus* shell was measured by valvometry. The five sclerochronological profiles were highly similar. Shell growth was significantly correlated to rising sea surface temperature (SST), up to 27°C. At the solar maximum, gaping behavior and increment thickness became erratic. SST- and solar irradiance-related stress could be related to physiological oxidative stress triggered by zooxanthellae symbionts. In the present context of globally increasing SST, our data indicate that the giant clams *H. hippopus* could live beyond their thermal comfort limits in summer in New Caledonia.

The coral reefs around the world represent a very diverse and vulnerable ecosystem, and many factors threaten their survival. Increased water temperature, solar irradiance, sedimentation, salinity changes, pollution, tourism, diseases, fishing practices, and high-energy storms are among them. Coral bleaching, one of the problems reported most often, occurs when the fragile balance between the animal and its symbiotic algae, the zooxanthellae, collapses; this has been quite often discussed in terms of maximum sea surface temperature (SST) and solar irradiance during the austral summer (Suggett and Smith 2011). Two major points of discussion are the adaptive capabilities of corals to rapid changes and the assumption that they are already living close to their thermal limits. There are predictions suggesting that increasing seawater temperature by only a very few degrees (Celsius) above summer values could have major effects (Brown 1997; Hoegh-Guldberg 2009).

Although it is often referred to as the whitening of corals, temperature-induced bleaching has been reported in all marine invertebrates that host zooxanthellae, including giant clams (Przeslawski et al. 2008). Their mantle bleaches (i.e., the zooxanthellae die) after exposure to high temperatures and high light intensities (Addressi, 2001; Buck et al. 2002). Together with corals, giant clams partly rely on zooxanthellae for the production of energy and metabolic processes. In coral, more than 50–90% of energy requirements are claimed to be provided through photo-

synthesis, although phyto- and zooplankton feeding are certainly also quite important (Muscatine 1990). In the reef, giant clams are living very close to corals, and we hypothesized that they could face a situation very close to that of corals, i.e., living close to their upper thermal limit.

The aim of the present work was to get greater insights into this possibility. We present an in situ study of growth-rate pattern and behavior in giant clams, *Hippopus hippopus*, living in a United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage Site unpolluted by human activity in the southern lagoon of New Caledonia (South Pacific) during a full growth season. This 1 yr of growth history was studied by combining, for the first time, two complementary techniques: high-frequency noninvasive (HFNI) valvometry (measuring shell-gaping activity) and sclerochronology. The HFNI valvometry is a new-generation, remote valvometry set-up enabling online study of the behavior of bivalve mollusks, freely living in their natural habitat. It allows autonomous long-term recordings (> 1 yr) of valve movements at high frequency without interfering with normal behavior. Here, we applied it for the very first time to the online recording of growth rate. Basically, the technique involves gluing one lightweight electrode on each valve and then measuring, at 0.6 Hz per individual, 24 h a day, 7 d a week, the distance between the valves (Tran et al. 2003; Chambon et al. 2007). Behavior patterns on daily, weekly, and monthly timescales are then mathematically constructed online (Sow et al. in press). To obtain the growth history on a daily scale, we took advantage of the fact that, in bivalve mollusks,

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calcification takes place in the mantle cavity, all over the shell's internal surface; if daily growth layers are produced, a consequence is that, when valves close every day, the minimal distance between electrodes also increases every day. To obtain a growth-rate index, we isolated these daily values and plotted them as a function of time. The same operation can be done with the maximum daily opening values, giving complementary behavioral information. Indeed, the maximal opening status is an index of a clam's welfare, as a decrease of valve opening is a major way to protect the soft body in endangered molluscan bivalves. Here we present the changes of minimum and maximum opening status in parallel to correlate growth rate and stress status with sclerochronology in the same clams.

As regards sclerochronology, the term was first applied to a radiographic study on coral skeletons by Buddemeier et al. (1974) and Hudson et al. (1976), and its meaning has been recently reviewed by Gröcke and Gillikin (2008). The technique can be applied to mollusk shells (postmortem examination) and allows the historical reconstruction of shell-layer thickness changes, i.e., growth rate, in the temporal context in which the shell layers formed. The focus of sclerochronology is to characterize growth patterns as a function of time and constraints by environmental cues, and/or "zeitgeber," and/or biological rhythms. It is routinely applied to deduce life history traits and is widely used in paleoclimatology, in which well-known examples are daily or annual growth in reef coral and mollusk shells.

In New Caledonia, water temperature closely follows air temperature changes, with a maximum in March,  $\sim 27\text{--}28^\circ\text{C}$ , and a minimum in July–August,  $\sim 21^\circ\text{C}$  (Le Borgne et al. 2010). We demonstrate here that by the end of the summer period, when SST reaches  $27\text{--}28^\circ\text{C}$  and solar irradiance is still reaching maximum annual values, the daily shell growth rate of *H. hippopus* is highly variable; its mean value is decreasing and is associated with strong behavioral signs of stress.

## Methods

**Samples**—Sixteen *H. hippopus* giant clams were placed in late August 2007 on the Ioro reef ( $166^\circ 57'\text{E}$ ,  $22^\circ 23'\text{S}$ ), located 1.7 km from the shoreline in the Havannah channel, southern New Caledonia (Fig. 1A–C). One animal died on 06 February 2008, a few days after Tropical Cyclone Gene approached New Caledonia, and it was excluded from all calculations. The Ioro reef, which *H. hippopus* naturally inhabits, is a permanently immersed reef, with a minimum depth of 0.5–1 m at low tide. The animals were collected by a local fisherman in early August 2007 from the Goro coral reef ( $\sim 10$  km from the experimental site) offshore from the Goro tribe area. The animals were then placed on a concrete platform ( $1 \times 1.1$  m) supporting 17 Netlon cages (one per animal and one for the submerged electronics), firmly fixed on a dead *Porites* coral. The depth of the full set-up varied from  $\sim 3$  m to 5 m according to the tide amplitudes, which vary from  $\sim 1$  m to 1.8 m. The full set-up was left in place from 24 August 2007 to 18 August 2008. During that time, in situ

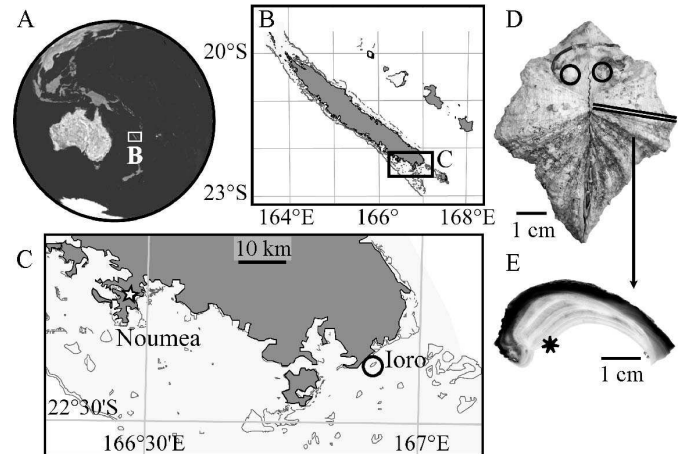


Fig. 1. (A–C) Location of the Ioro reef in the southern lagoon of New Caledonia. (D) The giant clam *Hippopus hippopus* a4 after 1 yr on site. The two electrodes are inside the open circles. The parallel bars represent the location of the shell slices cut off for the sclerochronology study. (E) Shell slice showing the growth line deposition pattern and the position of the last-deposited increment (asterisk). Maps: copyright IRD—Cartography department.

behavior and growth rate were studied by HFNI valvometry, by online analysis. After collection, a sclerochronological analysis was performed on the shells of five specimens. At the beginning of the recording period, the *H. hippopus* sizes were comparable (whole body mass,  $575 \pm 25$  g; maximum length,  $133 \pm 8$  mm) and the animals were around 2 yr old (according to postmortem analysis).

**HFNI valvometry**—HFNI valvometry allows the gathering of information on animal behavior in normal in situ environmental conditions, without formal distance limits between field and laboratory, and limited only by the availability of mobile phone network and Internet connections. We used a homemade technology that was first described and critically assessed under laboratory conditions by Tran et al. (2003). Lightweight electrodes (60 mg) were attached to the two valves of the *H. hippopus* shell (Fig. 1D). The electrodes were linked by 1-m flexible wires ( $2.14 \text{ g m}^{-1}$ , diameter = 0.9 mm) to a waterproof case containing the first-level electronic control unit, which measured valve activity. The second electronic card (developed by the Society Eukrea Electromatic) was fixed outside the water on the Ioro reef lighthouse (distance from the animals,  $\sim 30$  m). This second card used a Linux operating system to drive the first card and the mobile phone emission protocols. It received the measurements of the distance between electrodes, which arrived every 0.1 s from the pool of the equipped animals. With 16 bivalves, the initial number when the experiment started, information was received from each animal (live or dead) every 1.6 s, and each day a data matrix of 864,000 rows was collected. For each animal, each day, 54,000 pairs of values (one distance between electrodes plus one sampling time) described every single valve-gaping behavior. The total electronic consumption of 2 W was supplied by a solar

panel positioned on the Ioro lighthouse. The measurements are based on the magnetic principle: each laboratory-made electrode encloses a coil; one is a transmitter, the other a receiver. The strength of the electric field produced between the two coils decreases with distance, according to the law  $1:D$ , where  $D$  is the distance between the point of measurement and the center of the transmitting coil. On each clam, the first distance measurements were performed following electrode fixation when the animals' valves were firmly shut in the air, and these were taken as zero values.

*Principle of the mathematical analysis*—To model giant clam behavior, we used a nonparametric regression model based on the kernel estimator (Härdle 1990; Tran et al. 2003; Briollais et al. 2007; Durrieu and Briollais 2009). The relationship between the two electrodes ( $Y_i$ ) and the time of the measurement ( $T_i$ ) is modeled using the following nonparametric regression model:

$$Y_i = m(T_i) + \varepsilon_i, \quad \text{for } i = 1, \dots, n$$

where  $n$ ,  $m$ , and  $\varepsilon$  denote the sample size (total number of paired values), the unknown regression function to be estimated, and the model error term, respectively. The stochastic distribution  $f$  of  $\varepsilon$  is typically unknown and is unlikely to follow any familiar distribution, such as the normal distribution, and is independent of  $T$ ; hence, no distribution is assumed (nonparametric statistics). The distance between the two electrodes is measured at equally spaced measurement points. So, we follow the case of fixed equidistant design that implies that the  $T_i$ 's have a uniform known design density  $f_T$ . The variation  $\varepsilon_i$  is a random variable with a mean value equal to 0, and the stochastic distribution  $f$  of these random variations enables us to characterize the variation of the random variable  $Y$  around:

$$m(t) = E(Y/T=t) = \frac{\int y f_{Y,T}(y,t) dy}{f_T(t)}$$

This regression function  $m$  (definition of the conditional expected value of  $Y$  given  $T$ ) depends only on the unknown joint distribution function of  $(Y, T)$ , denoted by  $f_{Y,T}$ , because  $f_T$  is known. The regression estimator of  $m$  is given by

$$\hat{m}_{h_n}(t) = \begin{cases} \frac{\sum_{i=1}^n K\left(\frac{t-T_i}{h_n}\right) Y_i}{\frac{1}{n} \sum_{i=1}^n K\left(\frac{t-T_i}{h_n}\right)} & \text{if } f_T(t) \neq 0, \\ \frac{1}{n} \sum_{i=1}^n Y_i & \text{otherwise,} \end{cases}$$

where  $h_n$  is the bandwidth (the smoothing parameter) satisfying three conditions: (1)  $h_n$  tends to zero and  $nh_n$  tends to infinity as  $n$  tends to infinity, (2) the kernel  $K$  is a symmetric function satisfying  $\int K(t)dt=1$ ,  $\int tK(t)dt=0$ ,  $\int |t|K(t)dt=0$ , and (3) the distribution function is twice differentiable. Selecting a proper bandwidth parameter  $h_n$  is a critical step in estimating the regression function. Although in practice one can choose the bandwidth subjectively, this can lead to inaccurate estimation. The choice of  $h_n$  is much more important for the behavior of the estimators than the choice of the kernel function  $K$ . Small values of  $h_n$  make the

estimate look uneven and suggest features that do not really exist, whereas large values of  $h_n$  will lead to an estimate that is too smooth in the sense that it is heavily biased and may not reveal important structural features, like a bimodality, for example. Under previous conditions 1–3 and assuming that  $m$  is twice differentiable and  $E(Y^2) < +\infty$ ,  $\hat{m}_{h_n}(t)$  is a consistent and asymptotically normally distributed estimate of  $m(t)$  (Härdle 1990; Sow et al. in press). We used a cross-validation criterion to estimate the bandwidth parameter. This bandwidth is asymptotically optimal and yields the same speed of convergence as other techniques (Härdle 1990). Once the daily activity of each animal was modeled, different parameters specifying openings and closures (duration, number, degree of opening in mm), and also the number of partial or total closures, could be studied for each day of recording by using the modeling approach. The computations were performed under the Linux operating system (version Fedora 12) on a biprocessor xeon workstation DELL using R (<http://www.r-project.org/>).

*H. hippopus growth model using valvometry*—The model allows for estimating the growth vs. time using measurements collected by valvometry. The general form of the model is

$$Y = X\beta + \varepsilon$$

where  $Y$  is the cumulated growth vector (in mm),  $X$  is a known design matrix of time condition,  $\beta$  is the vector of unknown regression parameters to be estimated, and  $\varepsilon$  is the error term. Linear least-squares regression estimators are highly nonrobust to outliers or extreme observations often present in an environmental context. To avoid any distributional assumptions and to protect against outlying measurements, we proposed a robust and nonparametric inferential method by using quantile regression. An overview of robust statistics literature can be found in Huber (2004). Our approach is based on regression quantile estimators of  $\beta$  (Dodge and Jurečková 1995). Instead of focusing on the changes in the mean cumulated growth, the quantile regression approach allows one to test whether there is a change in the  $\theta$ th quantile of  $Y$  for any given  $\theta$  in  $(0,1)$ . When the conditional distributions of  $Y$  are non-Gaussian, the mean might not be the best summary, and a change in distributions may not be detected. Inference for linear quantile regression models has become a subject of intense investigation in the past years. Any solution of the following minimization problem ( $x'_i$  denotes the  $i$ th row of the matrix  $X$ )

$$\hat{\beta}_\theta = \arg \min_{\beta \in \mathbb{R}^p} \sum_{i=1}^n \rho_\theta(Y_i - x'_i \beta)$$

is called the  $\theta$ -regression quantile with  $\rho_\theta(x) = x(\theta - I(x < 0))$ , where  $I(P)$  takes the value 1 or 0 depending on whether the condition  $P$  is satisfied or not. Quantile regression reveals multiple rates of changes (slopes) from minimum to maximum response, providing a more complete picture of the relationships between variables missed by other regression methods. Most regression applications in the

ecological sciences focus on estimating rates of change to the mean of the response variable distribution. For instance,  $\theta = 0.5$  is chosen as an alternative to least squares estimators in the presence of heavy-tailed distribution (median regression). It can be proved that under regularity conditions, this estimator is asymptotically normally distributed (Jurečková 1984; Durrieu and Briollais 2009). The asymptotic variance of  $\hat{\beta}_\theta$  is unknown because it depends on the distribution function  $f$  of the error term in the model, which is itself unknown. So, we estimate this asymptotic variance using a regression-invariant and scale-equivariant kernel-type estimator (Dodge and Jurečková 1995; Durrieu and Briollais 2009).

**Sclerochronology**—Fifteen animals were collected alive on 18 August 2008. Soft tissues were immediately separated from the shells, which were then air-dried. Five animals out of the 15 were selected for sclerochronology based on their representativeness of the whole group's behavior following HFNI valvometry analysis. One valve of each of the five selected specimens (a1, a3, a5, a6, and a15) was cut, using a diamond precision saw (Buehler®) in  $\sim 2$ -mm-thick slices along the maximum growth axis (Fig. 1D,E). Indeed, compared to other portions of the shell, growth patterns are the most easily seen in the inner layer of *H. hippopus* (Aubert et al. 2009). The slices were embedded in epoxy and polished with diamond suspensions with decreasing grain size, the last polishing step being performed with a 0.25- $\mu$ m-grain-size suspension. In between each suspension, the sections were carefully washed in an ultrasonic water bath.

After the polishing steps, the growth increments were not clear enough for accurate measurements of their thicknesses. Consequently, an etching technique was used to reveal the shell growth pattern. The etching was accomplished following the procedure described by Aubert et al. (2009), which uses a slightly modified Mutvei's solution (Schöne et al. 2005) for 45 min. Here, an increment was defined as the amount of shell between each ridge revealed by the etching. The etched sections were photographed under an optical microscope under diffuse light at a magnification of 100 $\times$ , choosing a part of the inner layer where the increments were the most easily seen and where the growth rate was maximal. Then, growth-increment thicknesses were measured using a modified version of Analysis Visilog basic® version 6.841 (Noesis).

**Environmental data**—To analyze *H. hippopus* shell growth variations with respect to its environment, environmental data for the period of growth were collected. The SST, irradiance, wind force, and precipitation data were provided by Météo France Nouméa (<http://www.meteo.nc/>) for the southern island. Tide timetables and ephemerides were produced by the Service Hydrographique et Océanographique de la Marine (<http://www.shom.fr/>).

## Results

**Gaping behavior and growth as revealed by HFNI valvometry**—The typical daily behavior of a permanently immersed *H. hippopus* was mostly characterized, all year

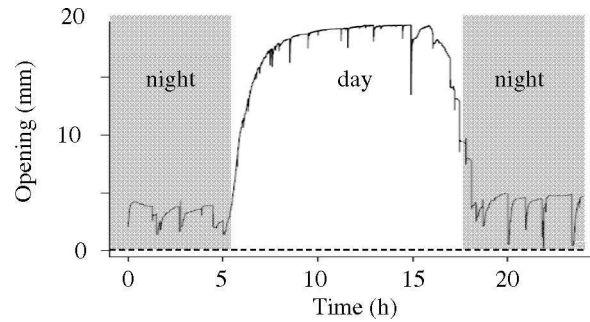


Fig. 2. Typical daily valve behavior in a resting giant clam *H. hippopus* recorded in situ in the southern lagoon of New Caledonia, on the Ioro reef (clam a4, 21 November 2007). The shell is largely open during the day and partially closed at night. Complete shell closures occur briefly at night; they are rare during the day.

long, by a consistent basic pattern (Fig. 2). During daytime, valve opening was large, while at night it was reduced to about 20% of its maximum daily opening value. No relationship between tide (timing and amplitude) and shell closing and opening time was observed, showing that the primary zeitgeber was clearly the night and day cycle. Short-term complete closures regularly occurred at night (Fig. 2), while they were exceptional during daytime. During daytime, most valve movements, micro-closures, or partial closures were of small amplitude (in relative value), characterizing a settled and resting state.

We observed daily the increase of the minimal distance between electrodes. It was regular, demonstrating globally a continuous growth all year round, although exceptions do exist: periods without daily growth are seen, for example, in clam a8 during a few days in June and in July 2008 (see Fig. 3). To visualize the growth of the *H. hippopus* specimens for the studied year, the daily shortest distance between valves was plotted as a function of time (Fig. 3). From 27 August 2007 to 18 August 2008, at the position of the electrodes, the distance between each specimen's valves increased by between 4 and 18 mm (min, max) depending on the individual. The periodic peaks on each curve show that a complete closure every 24 h (more precisely, at night) is not a prerequisite in the species and does not occur every 24 h.

For all individuals and for the whole year, no spawning activity, characterized in oysters by a stereotyped series of rapid valve contractions (Galtsoff 1938), was observed.

**Cumulated growth and comparison between sclerochronology and HFNI valvometry**—Most animals exhibited a similar mean annual growth (Fig. 3). The cumulated growth curves obtained from the sclerochronology and the growth model obtained using HFNI valvometry were similar. This is shown by the significant correlation coefficients (up to 0.998,  $p < 2 \times 10^{-16}$ ) between the results obtained using both techniques (Table 1), although the two sets of measurements were not performed at the same shell position. The increment thickness of the five *H. hippopus* measured by sclerochronology in the inner layer varied from 5.5 to 50  $\mu$ m, with a mean value of  $22.4 \pm$

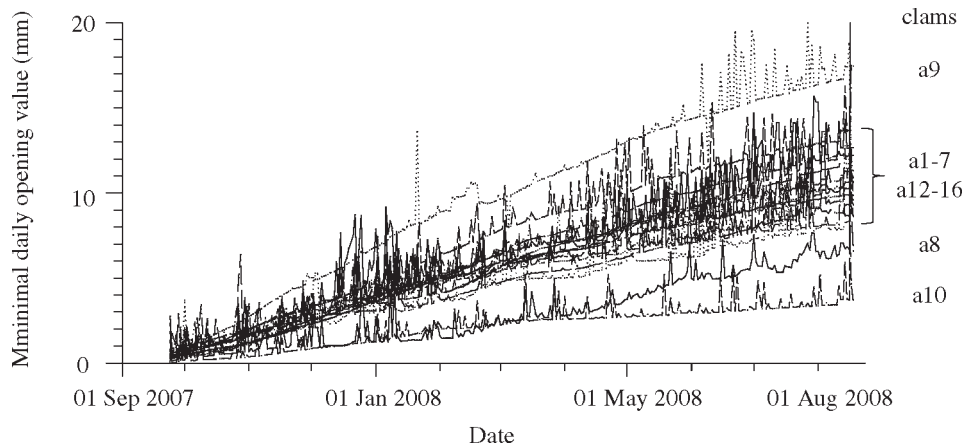


Fig. 3. Growth index measured by HFNI valvometry from September 2007 to August 2008. The index corresponds to the minimum daily opening value measured between the electrodes shown in Fig. 1D in 15 giant clams *H. hippopus* living in the southern lagoon of New Caledonia. The growth is continuous.

8.1  $\mu\text{m}$ . The results given by the HFNI valvometry were in the same range, from 12 to 27  $\mu\text{m d}^{-1}$ .

As the sampling date was known and the HFNI valvometry showed statistically a continuous growth, a date was attributed to each shell growth increment measured by sclerochronology. The last shell increment (cf. asterisk on Fig. 1E) was deposited on 18 August 2008 (sampling date), and the former ones were then dated retroactively.

**Sclerochronological profiles**—The sclerochronological profiles of the five clam shells studied are presented in Fig. 4A, and the mean value in Fig. 4B. As all animals were located on the same platform of  $\sim 1 \text{ m}^2$  on the reef (uniqueness of time and location, i.e., common exposure to environmental stressors), these data illustrate inter- and intra-individual change and variability as a function of time. First, the global pattern of daily thickness changes throughout the monitored year exhibits remarkable similarities for the five clams. This is furthermore illustrated by the mean curve for the five clams (Fig. 4B, daily SE values, mean not shown for clarity). Based on this full data set we suggest defining three periods (see vertical dashed lines in Fig. 4).

Period 1, from September to early January, was characterized by global growth rate acceleration.

Period 2, early January to early April, was the period exhibiting the highest variability. Based on the mean growth pattern shown in Fig. 4B, we divided it into two subparts, 2a and 2b (respectively, 01 January–14 February and 15 February–06 April). Period 2a started with a deep general slowdown, which reached a minimal value by the end of January (Fig. 4B). Then, shell growth rate increased rapidly again until mid-February. The maximum annual growth rate was observed at the end of this period with a peak value at  $42 \pm 5 \mu\text{m d}^{-1}$ . Immediately after, and within 1.5 months, the mean growth rate decreased rapidly down to the minimal annual values (subpart 2b). The analysis of the individual records (Fig. 4A) clearly shows that the intra-individual variability was maximal, defining a very specific period. The maximum and minimum individual daily shell growth values were essentially asynchronous among animals.

In period 3, from April to 18 August 2008 (sampling date), the clams kept on growing but followed a monotone pattern and rate, at minimal annual values and with a minimal variability. The mean daily growth line thickness during this period was quite close to that observed in late September 2007 (respectively,  $17.9 \pm 0.2 \mu\text{m}$  and  $16.2 \pm 0.4$ ,  $p = 0.018 < 0.05$ , nonparametric Mann–Whitney *U*-test).

Table 1. Comparison of cumulated growth obtained by sclerochronology and HFNI valvometry for the period September 2007–August 2008 in five giant clams *Hippopus hippopus*. Correlation data, *r*; bias mean, mean difference between both techniques in  $\mu\text{m}$ ; SE, standard error of the bias mean in  $\mu\text{m}$ ; CI, confidence intervals, minimum and maximum values covering 95% and 99% of the distribution in  $\mu\text{m}$ .

Clam	Correlation ( <i>r</i> )	Bias mean ( $\mu\text{m}$ )	SE	CI 95%	CI 99%
a1	0.994	0.178	0.03	(0.118; 0.237)	(0.099; 0.256)
a3	0.998	0.322	0.035	(0.253; 0.390)	(0.232; 0.411)
a5	0.998	0.024	0.012	(−0.0004; 0.049)	(−0.008; 0.056)
a6	0.974	0.479	0.047	(0.388; 0.571)	(0.360; 0.600)
a15	0.983	0.109	0.031	(0.048; 0.170)	(0.029; 0.189)

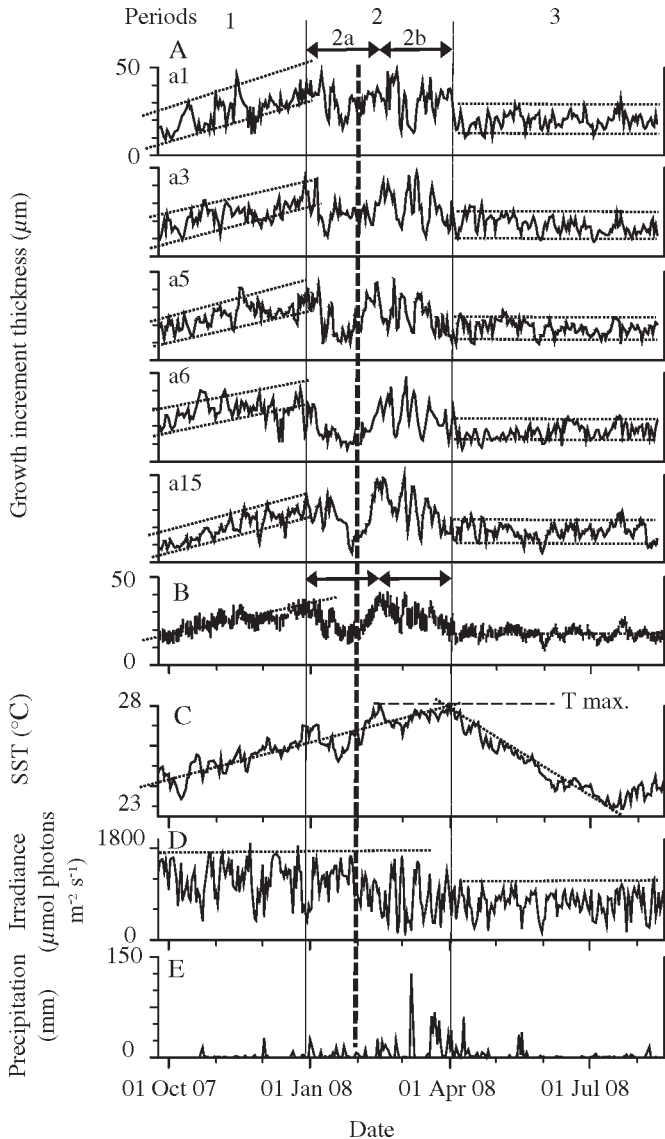


Fig. 4. Change of daily growth-increment thickness at the umbo level in the inner layer in *H. hippopus* as a function of time, from September 2007 to August 2008, sea surface temperature (SST), solar radiation, and precipitation. (A) a1, 3, 5, 6, and 15: giant clams 1, 3, 5, 6, 15. (B) Mean growth-line thickness for the same clams  $\pm 1$  SE; (C) SST; (D) solar irradiance; (E) precipitation. (B–E) Data from Météo France. Vertical large dashed black line: Cyclone Gene was approaching, and the swell amplitude reached 6–7 m in the southern lagoon. The two vertical thin dashed lines separate the three periods: 1, 2a, 2b, and 3. The subperiods 2a and 2b are shown by two horizontal double arrows. The growth-increment thickness became highly variable, and its mean value started to decrease when the SST reached its maximal annual value during period 2b.

To explain these global growth patterns, we then turned to the analysis of the corresponding changes in SST, solar irradiance, and precipitation in the area (Fig. 4C–E).

*Environmental changes*—The New Caledonia climatic regime is characterized by two main periods, an austral rainy summer (December–March) and a drier austral

winter (May–October), each separated by transition phases. The experimental period, September 2007 to August 2008, thus started and ended in austral winter. The SST changes during that period can be divided into two parts (Fig. 4C). A warming period occurred from September until early April, followed by a cooling period from mid-April to August. These two periods can be described by two linear regressions that are, respectively:  $SST = 0.018 \text{ time} + 24.37$ ,  $R^2 = 0.82$ ,  $p < 2.2 \times 10^{-16}$  and  $SST = -0.04 \text{ time} + 24.37$ ,  $R^2 = 0.83$ ,  $p < 2.2 \times 10^{-16}$ . The annual change in solar irradiance can also be described as two main periods separated by an intermediary one (Fig. 4D). Maximum annual solar irradiance values were systematically observed from September to March (mean,  $1031 \pm 40 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ; maximum values,  $1850 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), which corresponds to the SST warming period. Then, from April to August, the solar irradiance was generally lower ( $684 \pm 40 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ; significantly different from the previous period,  $p < 0.001$ ), corresponding to the decrease in water temperature. In early February 2008, Tropical Cyclone Gene passed to the east of New Caledonia (vertical dashed bar on Fig. 4). This climatic event generated, from 31 January to 02 February, sea swell amplitude above 6–7 m in the Havannah channel, where the Ioro reef and the giant clams were located (data Météo France).

*Daily growth changes vs. environmental parameters*—Regarding water temperature, a major drive in poikilotherms, we first looked for a global relationship between mean daily growth line thickness and SST during the whole period September 2007–August 2008. A significant correlation was observed, characterized by the following linear regression equation: growth increment thickness =  $2.33 \text{ SST} + 32.12$ ,  $R^2 = 0.304$ ,  $p = 2.2 \times 10^{-16}$  (Fig. 5A). We turned then to a closer systematic analysis, mainly based on the three periods defined following the sclerochronological study. Considering period 1 (24 September 2007–01 January 2008), the correlation between shell growth and SST was clearly better than for the whole year (growth increment thickness =  $4.54 \text{ SST} - 89.9$ ,  $R^2 = 0.55$ ,  $p = 2.2 \times 10^{-16}$ ; Fig. 5B). Interestingly, not only did this acceleration period of growth rate clearly parallel the SST change, but it also paralleled the slow-down period recorded just afterward, during period 2a (01 January–14 February 2008; Fig. 4, 5C2). Indeed, a virtually day-by-day correlation between growth rate and SST was observed at that time ( $R^2 = 0.45$ ; compare Fig. 5C2 and 5C3). Remarkably, the nature of the correlation during period 2b (15 February–06 April 2008), characterized by SST above  $27^\circ\text{C}$ , was completely different. Indeed, the positive relationship between increment thickness and SST vanished, and the relationship became inverse (Fig. 5D1). Specifically, when SST stayed above  $27^\circ\text{C}$ , individual shell growth became erratic (Fig. 4A, period 2b) and its mean value exhibited a significant and rather monotone slow-down (Fig. 4B, 5D2). During period 2b, the relationship between daily growth increment thickness and SST is described thus: growth increment thickness =  $-7.34 \text{ SST} + 228.8$ ,  $R^2 = 0.27$ ,  $p < 0.003$ . Finally, from April to August

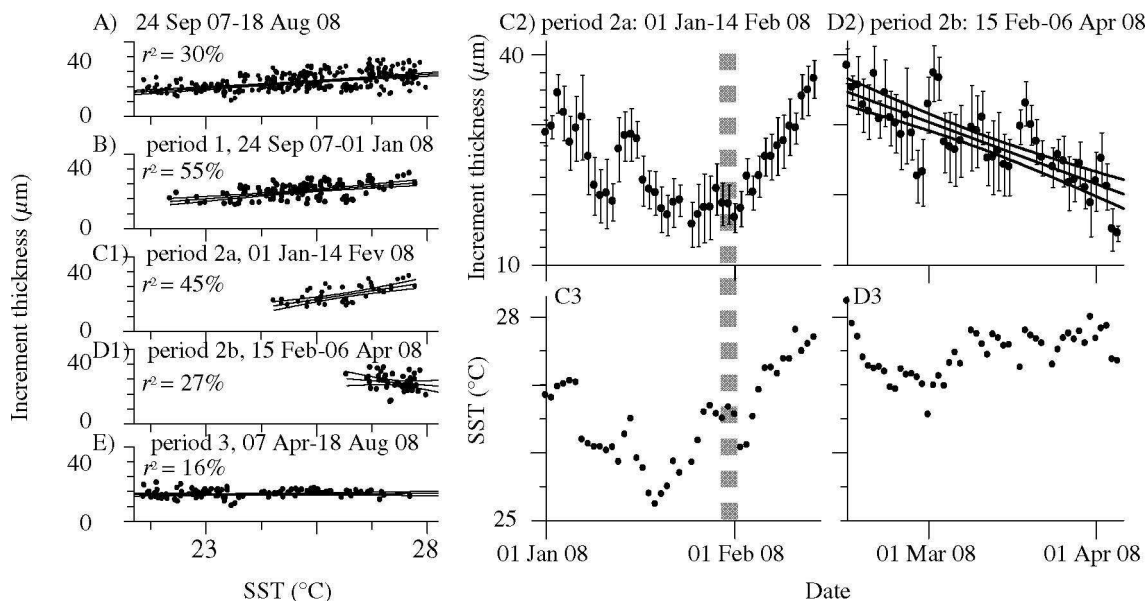


Fig. 5. (A, B, C1, D1, E) Relationships between mean daily growth-increment thicknesses measured by sclerochronology and sea surface temperature (SST) and time. The sampling periods are mentioned in each panel. (A) Global relationship between growth rate and temperature (B, C1, D1, E) relationship for specific periods. Warmest periods 2a and 2b: (C2, C3) parallel changes of (C2) growth-increment thickness and (C3) SST as a function of time from 01 Jan to 14 Feb 2008. (D2–3) Parallel changes of (D2) growth-increment thickness and (D3) SST as a function of time from 15 Feb to 06 Apr 2008 (C2–3, D2–3, enlarged views of Fig. 4B and 4C). Vertical dashed gray line: Cyclone Gene, which generated sea swell amplitude of 6–7 m around the clams' location. See text for regression.

2008 (i.e., period 3), there was a complete change of pattern. While SST progressively decreased (Fig. 4C), the clams kept on growing, but at a low and constant rate. Daily shell growth was independent of SST (growth increment thickness =  $0.25 \text{ SST} + 11.76$ ,  $R^2 = 0.016$ ,  $p = 0.13$ ; Fig. 5E). For that last period, the shell growth pattern paralleled the solar irradiance regime that was at its lowest annual values.

**Related behavioral changes**—The above data set showed that shell growth variations were not a direct function of the water temperature and, more specifically, that growth was highly variable, even erratic, by the end of the hottest period (period 2b). We next addressed the existence of behavioral signs associated with this altered growth pattern. Specifically, we examined whether the erratic and slowing-down growth period 2b was associated with valve behavior revealing either a protective behavior or a stress reaction. Consequently, we focused our attention on the changes of maximum opening status of the group of clams.

Figure 6 shows the change of maximal daily openings from September 2007 to August 2008 in the whole group. For clarity, data were combined with the mean growth line thickness from Fig. 4B (Fig. 6A). Besides the general growing pattern that confirms the continuous growth as illustrated in Fig. 3, two striking events of major interest for our purposes were observed in Fig. 6B. The first was the valve behavior associated with the presence of Tropical Cyclone Gene, obviously a stressful condition for clams living at ~3–4-m depth. Clam behavior was characterized by a sudden decrease of maximum opening state. This behavior abruptly started on 30 January 2008 and had returned to reference values on 05–06 February 2008. The

second striking event was that the maximum valve opening status at the end of period 2b (large arrow) shows a similar change, although its time course was different. The maximum opening values decreased progressively during early March and abruptly recovered at the beginning of period 3. Interestingly, the recovery occurred at precisely the same time as the water temperature started to decrease (Fig. 4C). Figure 7 presents two typical records of daily valve activity during these two periods of reduced values of maximal daily openings. They are perfectly representative of the whole animal group and must be compared to the steady reference situation shown in Fig. 7A (redrawn from Fig. 2). These behaviors (Fig. 7B,C) are typically unsteady and disturbed. They are both characterized by numerous partial or nearly complete valve closures during the day- and nighttime. In late January to early February, the stress signature was clearly associated with the presence of Tropical Cyclone Gene (Fig. 7B). In late March to early April, at the end of the warmest period in the lagoon, the same disturbed pattern was observed (Fig. 7C).

## Discussion

In this 1-yr study of growth rate and gaping behavior in giant clams *H. hippopus* in the southern lagoon of New Caledonia, HFNI valvometry, a new remote sensing system that records molluscan bivalve gaping behavior and shell growth, and classical sclerochronology were used for the first time in combination. Our data show that the relationship between growth rate and temperature is not linear. We present evidence that at the end of the summer period increasing SST (summer values are above  $27^\circ\text{C}$ ) together with elevated solar irradiance deeply affect *H.*



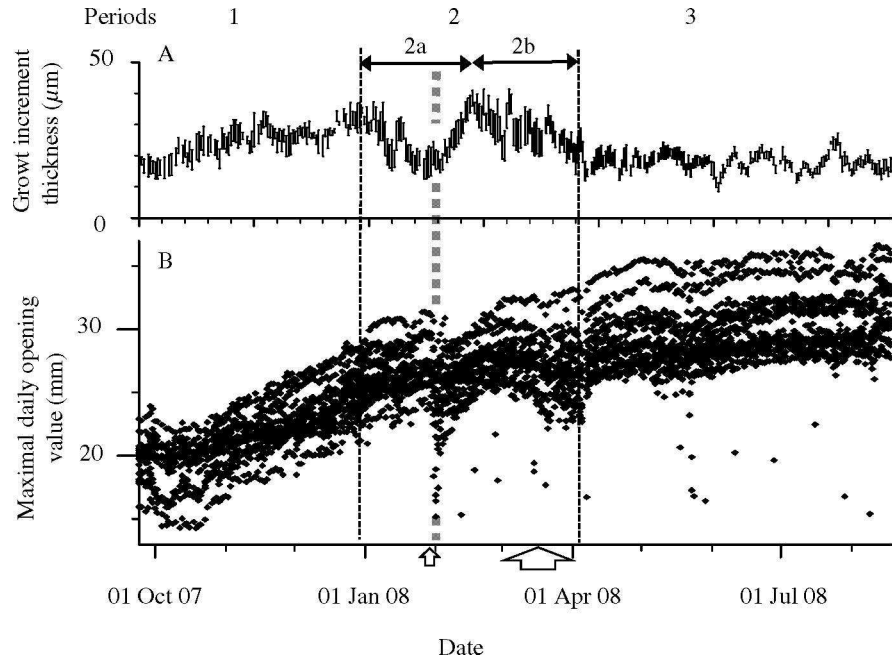


Fig. 6. Change of maximum valve opening values in the whole group of 15 *H. hippopus* during the three time periods defined in Fig. 4. (A) Mean growth-line thickness in five giant clams (same data as in Fig. 4B,  $\pm 1$  SE); (B) Maximum valve openings. Vertical dashed gray line: Cyclone Gene; the two thin vertical dashed lines separate the three periods defined in Fig. 4. Horizontal double arrows defined subperiods 2a and 2b. Arrows, periods during which the maximum shell-opening values decreased during period 2. The size of the open arrows is related to the disturbance duration. See text for further explanation.

*hippopus* daily growth and shell gaping behavior. This strongly suggests that the *H. hippopus* giant clam species could, at 27–28°C, already be living close to its upper thermal limits.

*History of the technique*—Oceanographers rely heavily upon in situ sensing observations to better understand oceans and how marine animals live in the field (Dickey et al. 2008). There is also a compelling need for using remote online sensors to distribute information instantly and widely, on a daily basis, specifically in a marine environment (Kröger et al. 2009). Initially, HFNI valvometry was

developed to solve fundamental questions in respiratory physiology in molluscan bivalves (Massabuau 2001; Chambon et al. 2007), ecotoxicology (Tran et al. 2010), and marine ethology (Tran et al. 2011; Sow et al. in press). However, the study of bivalve gaping behavior has a long history, dating back to the early 20th century (Tran et al. 2010). Since then, various systems have been developed with variable success. The approaches to measurement of valve behavior have been quite diverse, and many different types of recording apparatus have been proposed. During the last 20–30 yr, “valvometry” techniques have been studied as an aquatic pollution biosensor and various

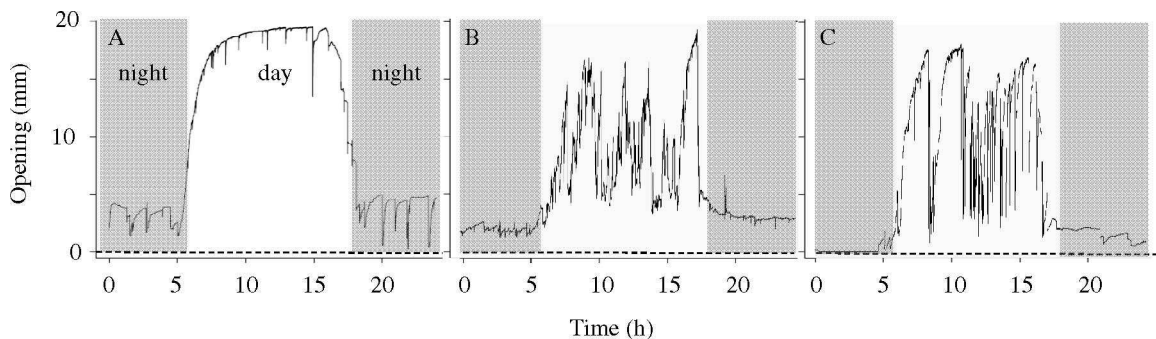


Fig. 7. Typical disturbed daily valve behaviors in giant clams *H. hippopus* from the southern lagoon of New Caledonia. (A) Typical daily valve behavior in a resting giant clam (clam a4, redrawn from Fig. 2). (B) Clam a6 during the swell associated with Cyclone Gene (31 January 2008). (C) Clam a5 after 3 weeks at temperatures ranging from 27°C to 28°C (02 April 2008; sea surface temperature = 27.7°C). Compare (B and C) with the resting pattern shown in (A) and note the analogy between the numerous transient closures occurring during the daytime under (B) the stressful cyclonic conditions and (C) after 3 weeks at 27–28°C.

devices have been developed. More recently, systems using magnetic fields (Wilson et al. 2005), fiber optics (Frank et al. 2007), video recordings (Riisgard et al. 2006; Saurel et al. 2007), and magnetic switches (Garcia-March et al. 2008) have been presented. Some systems are commercially available, such as the Mussel Monitor (Kramer et al. 1989) and the Dreissena Monitor (Borcherding 2006). Our group alone, however, has developed the ability to monitor gaping behavior in marine environments from thousands of kilometers away, accessed the behavior via the Web (of exchange data and programs from the lab to the field and vice versa), and deployed the system for up to 1–2 yr in full autonomy, without in situ human intervention. Our method, therefore, differs from previous efforts in a number of significant ways. First, it can work for long periods even in the high sea. Second, as we use light electrodes and thin cables, it allows bivalves to behave without constraint in their natural environment, including burrowing into the sediment. Third, our technology allows us to monitor bivalve behavior anywhere in the world using cellular, Internet, or satellite networks to transmit information.

The present data demonstrate continuous growth in the giant clam *H. hippopus*, although exceptional periods without growth do exist (compare individual data in Fig. 3 with a particular focus on clam a8); and it is quite likely that an occasional absence of growth increment deposition of 1–2 d could have been missed due to technical limitations. Thus, strictly speaking, over 1 yr of recording, it is not possible to guarantee that an exact date has been attributed to each growth increment; but, statistically speaking, the clams had a continuous shell growth. Quantifying the error on increment date attribution remains to be specifically studied (e.g., using dendrochronology-like cross-dating methods), but this was out of the scope of the present work. Increments could also be marked by fluorescein at the beginning of the experiment, and the number of increments could then be directly compared to the number of days in the experiment. Finally, the comparison between measurements using sclerochronology and HFNI valvometry shows a main mean bias of 0.2  $\mu\text{m}$  over 1 yr. This is not to say that the accuracy of each technique independently is 0.2  $\mu\text{m}$  but that the errors of both methods are identical. The occurrence of daily increments in *H. hippopus* shells has already been proposed (Watanabe and Oba 1999; Aubert et al. 2009); but, to our knowledge, this is the first empirical demonstration of daily growth band production in *H. hippopus*.

*Relationship between growth rate, temperature, and irradiance*—The present annual temperature pattern was representative of the 10-yr-long survey reported by Le Borgne et al. (2010) in the same area. In the southern lagoon of New Caledonia, where SST ranges from 22°C to 28°C, we show that the relationship between growth rate and water temperature in *H. hippopus* is not linear. A similar nonlinearity was shown in a 4-yr sclerochronological record of one *H. hippopus* specimen from the Southwest Nouméa lagoon (Aubert et al. 2009). Particular growth decreases in the course of the year were interpreted as reflecting either physiological or nonclimatic external stress

or upwelling-related environmental stress. The growth scheme observed here, based on 1 yr of growth of five specimens located more northeastward in the lagoon, is quite different. Based on the 2007–2008 record, we suggest the existence of three different periods, and we show that, by the end of the summer period, *H. hippopus* could already be living close to its upper thermal limits. When the water temperature started to rise following austral winter, the growth rate continuously accelerated. When it surpassed 27°C, and up to 28°C, the sclerochronology demonstrates that growth rates became erratic, which we suggest is the first sign of temperature-induced physiological disorder (Fig. 4A). Subsequently, the mean value of the daily growth increment started to decrease, which is the second sign of negative interaction between high temperature and growth rate (Fig. 4B). During period 2b, before the SST started to decrease and the daytime irradiance fell below its maximum value, mollusk behavior was typically deeply disturbed and no longer tranquil (compare Fig. 7A,C). In fact, the behavior was as disturbed as during a very high sea record obtained during a cyclonic alert with a swell amplitude estimated to be 7–8 m by Météo France (Fig. 7B). This is the third sign of negative interaction. Importantly, we also recorded a progressive decrease in daily maximum valve-opening values, a sign of protective behavior in bivalves (Fig. 6B). This behavior started to develop in the whole group in early March, during period 2b, the production period of shell growth increments with erratic thicknesses (compare Fig. 6B and 6A). This is the fourth sign of deep behavioral disturbance that we observed. We strongly suggest that the local changes of SST and local irradiance (Fig. 4C,D) are the most likely explanatory environmental parameters for the reported growth-rate disturbances because (1) the animals we studied were located on an isolated reef in a clean area, that is, in an open lagoon of high natural value and diversity (a UNESCO World Heritage Site; Andréfouët and Wantiez 2010); (2) there was no possibility of contaminant surge at the time of the experiments; and (3) there was no other particular climatic event (Fig. 4E). Thermally related growth reduction and/or cessation have been widely reported in bivalve shells, including summer SST-related stress on shell growth (Rhoads and Lutz 1980; Lazareth et al. 2006). Nevertheless, to our knowledge, this is the first time that perturbations of shell growth (erratic increment thicknesses, slowdown of growth rate at high temperature) without growth cessation have been empirically connected to perturbed shell-gaping behavior, and the first time that both of these observations have been linked with elevated SST and solar irradiance.

Finally, from a sclerochronological point of view, it is interesting to discuss how well the individual shells are synchronized at high (daily) frequency. Indeed, at “low” frequency (the scale of weeks or months: periods 1, 2 [2a and 2b], and 3) the individual shells appear quite well synchronized (Fig. 4). It is clear that all shells obey the same external drive(s) or forcing variables in parallel. Specifically, the comparison between Fig. 5C2 and 5C3 illustrates that specific point during period 2a (45 d). On the contrary, at high (daily) frequency, we have no

experimental data, no evidence of a close or tight daily correlation of increment widths between *H. hippopus* shells in the absence of a strong external drive. Based on a physiological point of view, this would neglect the inter-individual variability in any animal group, i.e., the physiological differences existing between animals of the same species. In the group of *H. hippopus* we studied, the individual shells are thus not rightly synchronized at high (daily) frequency as shown in Fig. 4A.

*Underlying physiological mechanisms*—The disturbances we report were observed during the end of the warm period, which strongly suggests a mechanism based on damage induced by excess light energy and photooxidative stress and/or imbalance between O<sub>2</sub> production by zooxanthellae, O<sub>2</sub>-supply by ventilatory activity, and O<sub>2</sub>-consumption in the clam tissues. Indeed, the irradiance values we report were clearly in the upper range of the maximum required values in giant clams (Klump and Griffiths 1994). These authors showed that gross photosynthetic rates approach saturation at  $723 \pm 75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in *H. hippopus*,  $488 \pm 51 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in *Tridacna gigas*,  $644 \pm 35 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in *Tridacna crocea*, and  $535 \pm 76 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in *Tridacna squamosa*. Here, the mean summer value we reported was  $1031 \pm 40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Interestingly, we also report physiological alterations at temperatures ranging from 27°C to 28°C at the end of the summer period. Lesser (1996) showed that cultures of a symbiotic dinoflagellate, grown in an environment that attempts to simulate in situ light and temperatures at ecologically relevant levels, with and without ultraviolet (UV) exposure, exhibit a significant decline in maximum photosynthetic capacity and cellular growth rates at 25°C and 27°C (fig. 1 in Lesser 1996). Symbiotic animals routinely experience elevated O<sub>2</sub> partial pressures (hyperoxic PO<sub>2</sub>) within their tissues because of photosynthetically produced oxygen (Dykens and Shick 1982). Physiological hyperoxia and UV exposure act with sublethal temperature perturbations to induce oxidative stress (Asada and Takahashi 1987; Lesser et al. 1990). This has been proposed as a mechanism that could lead to bleaching in symbiotic invertebrates (Lesser et al. 1990). The absorption of excitation energy in the presence of excess O<sub>2</sub> produces active oxygen, superoxide radicals, and hydrogen peroxide, compromising cell integrity (Asada and Takahashi 1987). Both elevated temperatures and photo-dynamic production of reactive oxygen species (ROS) induce cellular alteration that further disrupts primary photochemistry (Ludlow 1987). An additional increase in the rates of ROS production, associated with temperature-induced metabolic enhancement, occurs primarily in the mitochondria of host and symbionts (Burdon et al. 1990). In extreme situations, the increase in the cellular production of O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> is not counterbalanced by the increased activities of antioxidant enzymes in zooxanthellae (Dykens and Shick 1982). In addition to the occurrence of hyperoxic blood PO<sub>2</sub>, it has been reported that the oxidative stress in the host tissues also occurs because of direct H<sub>2</sub>O<sub>2</sub> diffusion from algal symbionts. ROS affect cellular proteins and numerous physiological mechanisms,

such as oxidation of deoxyribonucleic acid (DNA), lipids, and proteins. In giant clams, this may lead to the loss of symbiotic dinoflagellates via expulsion or complete loss of gastrodermal cells as described by Gates et al. (1992). Superoxide dismutase, a specialized enzyme aimed at protecting cells against O<sub>2</sub> toxicity, is thought to have appeared very early in evolution. It is an efficient tool to protect the cell against the deleterious effect of excessively high blood PO<sub>2</sub> and excess of free radicals. However, without doubt, preventing high PO<sub>2</sub> is also the simplest and most efficient means of limiting ROS production. It is also fundamental to note that O<sub>2</sub> requirement is not only a matter of adenosine triphosphate (ATP) production, even if 90% of the O<sub>2</sub> supply is devoted to matching the mitochondrial demand. Organisms use O<sub>2</sub> and its derived forms as messengers, modulators, and even neuromodulator-like substances. Even small deviations from precise PO<sub>2</sub> set points can have significant effects, for example, on the lobster neural networks; this highlights the necessity of well-tuned tissue PO<sub>2</sub> status (Clemens et al. 2001). As a whole, the *H. hippopus* story appears in complete opposition to the low PO<sub>2</sub> strategy that is successfully performed in numerous water breathers. Indeed, many aquatic animals have a strategy that enables them to regulate PO<sub>2</sub> in their arterial blood, independent of many oxygenation conditions in the environment, to low levels of between 1 and 3 kPa (Massabuau 2001). The PO<sub>2</sub> in water equilibrated with air is 21 kPa (for reference 1 kPa = 10<sup>-2</sup> bar; in seawater when PO<sub>2</sub> = 1 kPa, it corresponds to an O<sub>2</sub> fraction of 1% and an O<sub>2</sub> concentration of 0.32 mg L<sup>-1</sup> at 25°C). This ability to regulate respiration has been demonstrated in crustaceans, fish, and mollusks; and the strategy appears to be a very ancient one, as it is already present in the primitive ostracods, which existed 420–450 million yr ago (Corbari et al. 2004). By the end of the summer period (when the abnormal behaviors are observed), it is very likely that *H. hippopus* did not follow the low PO<sub>2</sub> strategy. On the contrary, we suggest that they should have a clearly over oxygenated milieu interieur, suffering from oxidative stress triggered by the zooxanthellae symbionts.

While we extensively discussed until now the relationship with temperature, it is important to discuss the possibility that growth could have been also influenced by changes in salinity and/or plankton concentration. Their variability has been reviewed (*see* figs. 7 and 10 in Le Borgne et al. 2010) for the southwest lagoon of New Caledonia. They reported a steady increase of salinity from February to March (mean minimum salinity, 35.4‰) to October (mean maximum value, 35.8‰) for the 1979–1989 period, half of the variance explained by the precipitation. This is certainly too small to induce any measurable physiological change at the timescale we were working on, but it also does not fit with precipitation records (Fig. 4E). As regards the plankton temporal variability, based on a 5-yr-long chlorophyll *a* time series, the same authors reported that the maximum concentration occurs from mid-May to mid-July (0.6 μg L<sup>-1</sup>) and the minimum from October to March–April. Consequently, a low concentration of plankton could contribute to weaken the giant clams

during the warmest period in the lagoon. However, Fig. 4 shows that growth characteristics in October and March were drastically different, thus clearly strengthening the key role of temperature.

*Online recording of effects of climate change on invertebrates of coral reefs*—With tropical reefs around the world threatened by warming oceans, most research is focused on corals and fishes. The present work researches the effect of environmental conditions on giant clams, focusing on a particular species that we believe to be more amenable than others to an online analysis of behavioral activity. It is clear that tropical vertebrates and invertebrates show species-specific sublethal responses to environmental changes, but, based on the present results, we suggest that *H. hippopus* could be an interesting sentinel species. This research was also intended to show how a control framework might be developed to monitor the behavior of benthic invertebrates and eventually predict their vulnerability or adaptability in response to warming tropical oceans on a long-term timescale.

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