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Evaluation of the variance of a particular estimate of indirect power spectrum: Application to spectral analysis of membrane noise

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Fisiologia. — *Evaluation of the variance of a particular estimate of indirect power spectrum : Application to spectral analysis of membrane noise* (*). Nota di FRANCESCO ANDRIETTI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Nel presente lavoro è stata considerata una particolare stima $\hat{S}_2(f)$ dello spettro di potenza di un processo casuale stazionario ed è stata calcolata una espressione analitica della sua varianza, valida nel caso di processi normali. Sono anche state determinate delle espressioni approssimate valide in casi particolari.

I risultati ottenuti sono stati confrontati con quelli della stima $\hat{S}_1(f)$, per cui il valore della varianza era già conosciuto.

Per controllare la validità delle deduzioni teoriche è stato utilizzata una simulazione numerica di un processo stazionario determinato dalle fluttuazioni di conduttanza di un canale del potassio che segue una cinetica di Hodgkin e Huxley.

1. *Theorical analysis.* Let us consider the zero mean random variable $x(t)$. In the present paper we are interested in the estimate of the autocovariance function (see, for example, Bendat and Piersol, 1971, p. 282, [1])

$$\hat{R}_2(r) = (1/T) \int_0^{\infty} x(t)\, x(t + |r|)\, dt$$

and the corresponding power spectrum estimate

$$\hat{S}_2(f) = \int_{-T}^{T} \hat{R}_2(r) \exp(-i\, 2\pi f r)\, dr$$

for which we do not know any analytical evaluation of the variance. It is clear that in this case an extra length of signal must be available on the right of the $(0, T)$ interval.

In particular we want to compare the variance of $\hat{S}_2(f)$ with that of the more commonly used estimate

$$\hat{S}_1(f) = \int_{-T}^{T} \hat{R}_1(r) \exp(-i\, 2\pi f r)\, dr$$

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where

$$\hat{R}_1(r) = (1/T) \int_0^{T-|r|} x(t)\, x(t+|r|)\, dr .$$

To obtain our results we will follow a method similar to that given by Jenkins and Watts, 1968, p. 412, [2] to evaluate the variance of $\hat{S}_1(f)$, for Normal processes.

Let us rewrite the autocovariance function estimate $\hat{R}_2(r)$ in the form

$$\hat{R}_2(r) = \begin{cases} (1/T) \int_{-T/2}^{T/2} x(t - r/2)\, x(t + r/2)\, dt & -T \leq r \leq T \\ 0 & |r| > T \end{cases} \tag{1}$$

and let us assume that the stochastic process $x(t)$ has the property

$$\text{Cov}\,[x(t)\, x(t+r_1)\, ,\, x(v)\, x(v+r_2)] = R(v-t)\, R(v-t+r_2-r_1) + \\ + R(v-t+r_2)\, R(v-t-r_1) + K(v-t, r_1, r_2) \tag{2}$$

where $R(r)$ is the autocovariance function of $x(t)$. This is the case, for example, for zero mean Normal processes ([2], p. 175), in which moreover

$$K(v-t\, , r_1\, , r_2) = 0\, .$$

From (1) and (2) one has

$$\text{Cov}\;[\hat{R}_2(r_1)\, ,\, \hat{R}_2(r_2)] = (1/T^2) \int_{-T/2}^{T/2} \int_{-T/2}^{T/2} \left\{ R\left(v - t - \frac{r_2 - r_1}{2}\right) \right.$$

$$R\left(v - t + \frac{r_2 - r_1}{2}\right) + R\left(v - t + \frac{r_2 + r_1}{2}\right) R\left(v - t - \frac{r_2 + r_1}{2}\right) +$$

$$\left. + K(v - t\, , r_1\, , r_2) \right\} dv\, dt\, .$$

After a first integration one finds

$$\text{Cov}\;[\hat{R}_2(r_1)\, ,\, \hat{R}_2(r_2)] = (1/T^2) \int_{-T}^{T} \gamma(k)\, (T - |k|)\, dk \tag{3}$$

where $v - t = k$, and

$$\gamma(k) = R\left(k - \frac{r_2 - r_1}{2}\right) R\left(k + \frac{r_2 - r_1}{2}\right) +$$

$$+ R\left(k + \frac{r_2 + r_1}{2}\right) R\left(k - \frac{r_2 + r_1}{2}\right) + K(k, r_1, r_2).$$

Recalling that

$$R(r) = \int_{-\infty}^{\infty} S(f) \exp(-i\,2\pi f r)\, df$$

and interchanging orders of integration, (3) becomes approximately

$$\mathrm{Cov}[\hat{R}_2(r_1), \hat{R}_2(r_2)] \approx (1/T^2) \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \{S(g_1)\, S(g_2) \exp(i\pi r_1 (g_1 - g_2)) \times$$

$$\times (\exp - (i\pi r_2 (g_1 - g_2) + \exp(i\pi r_2 (g_1 - g_2))\}\, dg_1\, dg_2$$

$$\int_{-T}^{T} (T - |k|) \exp(i\,2\pi k (g_1 + g_2))\, dk.$$

This reduces to

$$(1/T^2) \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \{S^*\} \frac{\sin^2 \pi (g_1 + g_2) T}{\pi^2 (g_1 + g_2)^2}\, dg_1\, dg_2$$

where $\{S^*\}$ stands for the quantity in braces above. This formula is exact for Normal processes.

Substituting $g_1 = f + g$, $g_2 = f - g$, so that $dg_1\, dg_2 = 2\, df\, dg$, the integral reduces to

$$(4) \qquad (2/T^2) \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \{S(f+g)\, S(f-g) \exp(i\,2\pi g (r_1 - r_2)) +$$

$$+ S(f+g)\, S(f-g) \exp(i\,2\pi g (r_1 + r_2))\} \frac{\sin^2 2\pi f T}{4\pi^2 f^2}\, df\, dg$$

By recalling the definition of $\hat{S}_2(f)$, we easily find that

$$\text{(5)} \qquad \operatorname{Cov}[\hat{S}_2(f_1], \hat{S}_2(f_2)] = \int_{-T}^{T}\int_{-T}^{T} \operatorname{Cov}[\hat{R}_2(r_1), \hat{R}_2(r_2)] \times \times \exp(-i\,2\pi(f_1 r_1 + f_2 r_2))\, dr_1\, dr_2 .$$

Substituting (4) in (5), interchanging orders of integration and integrating over r_1 and r_2 one obtains for $\operatorname{Cov}[\hat{S}_2(f_1), \hat{S}_2(f_2)]$

$$\text{(6)} \qquad (2/T^2)\left\{\int_{-\infty}^{\infty} \frac{\sin^2 2\pi f T}{4\pi^2 f^2}\, df \int_{-\infty}^{\infty} S(f+g)\, S(f-g)\, \frac{\sin 2\pi(f_2+g)T}{\pi(f_2+g)} \frac{\sin 2\pi(f_1-g)T}{\pi(f_1-g)}\, dg + \right.$$
$$\left. + \int_{-\infty}^{\infty} \frac{\sin^2 2\pi f T}{4\pi^2 f^2}\, df \int_{-\infty}^{\infty} S(f+g)\, S(f-g)\, \frac{\sin 2\pi(f_1-g)T}{\pi(f_1-g)} \frac{\sin 2\pi(f_2-g)T}{\pi(f_2-g)}\, dg \right\}$$

When the spectrum is approximately constant over the range from f_1 to f_2, the term $S(f)$ may be taken outside the integral, and one obtains

$$\text{(7)} \qquad \operatorname{Cov}[\hat{S}_2(f_1), \hat{S}_2(f_2)] \approx (S^2(f)/T)\left\{\int_{-\infty}^{\infty} \frac{\sin 2\pi(f_1-g)T}{\pi(f_1-g)} \times \frac{\sin 2\pi(f_2+g)T}{\pi(f_2+g)}\, dg + \int_{-\infty}^{\infty} \frac{\sin 2\pi(f_1-g)T}{\pi(f_1-g)} \frac{\sin 2\pi(f_2-g)T}{\pi(f_2-g)}\, dg\right\}$$
$$= S^2(f)\left\{\frac{\sin 2\pi(f_1+f_2)T}{\pi T(f_1+f_2)} + \frac{\sin 2\pi(f_1-f_2)T}{\pi T(f_1-f_2)}\right\}$$

so that

$$\text{(8)} \qquad \operatorname{Var}[\hat{S}_2(f)] \approx 4\, S^2(f)\left\{\frac{\sin 4\pi T f}{4\pi T f} + 1\right\}$$

This result may be compared with the variance of $\hat{S}_1(f)$. From Jenkins and Watts' formula (A 9.1.16) ([2], p. 415) one obtains

$$\text{(8')} \qquad \operatorname{Var}[\hat{S}_1(f)] \approx S^2(f)\left\{\frac{\sin 2\pi T f}{2\pi T f} + 1\right\}$$

When the value of T is large, the standard deviations of $\hat{S}_2(f)$ and $\hat{S}_1(f)$ for spectra approximately constant around f, become

$$\sqrt{\text{Var}\,[\hat{S}_2(f)]} \approx 2\,S(f) \tag{9}$$

$$\sqrt{\text{Var}\,[\hat{S}_1(f)]} \approx S(f)\,. \tag{9'}$$

One obtains a different approximation of (6), holding when T is large enough, by substituting $(\sin 2\pi f T)/\pi f$ with a δ function

$$(1/T)\left\{\int_{-\infty}^{\infty} S^2(g)\,\frac{\sin 2\pi(f_2+g)T}{\pi(f_2+g)}\,\frac{\sin 2\pi(f_1-g)T}{\pi(f_1-g)}\,dg\right. \tag{10}$$

$$\left.+\int_{-\infty}^{\infty} S^2(g)\,\frac{\sin 2\pi(f_1-g)T}{\pi(f_1-g)}\,\frac{\sin 2\pi(f_2-g)T}{\pi(f_2-g)}\,dg\right\}$$

that for $S(f)$ constant reduces to the preceding formula (7).

When a lag window $w_1(r)$ is considered, $w_1(r)=0$ for $T \geq r > M$, so that the spectral estimate is given by

$$\hat{S}_2(f) = \int_{-M}^{M} \hat{R}_2(r)\,w_l(r)\exp(-i\,2\pi f r)\,dr\,,$$

one has

$$\text{Cov}\,[\hat{S}_2(f_1)\,,\,\hat{S}_2(f_2)] = \int_{-M}^{M}\int_{-M}^{M} w_l(r_1)\,w_l(r_2)\,\text{Cov}\,[\hat{R}_2(r_1\,,\,\hat{R}_2(r_2)] \tag{11}$$

$$\times \exp(-i\,2\pi(f_1 r_1 + f_2 r_2))\,dr_1\,dr_2$$

When T is large, by approximating $(\sin 2\pi f T)/\pi f$ with a δ function, one has from (4)

$$\text{Cov}\,[\hat{R}_2(r_1)\,,\,\hat{R}_2(r_2)] \approx (1/T)\int_{-\infty}^{\infty} S^2(g)\,\{\exp(i\,2\pi g(r_1-r_2)) \tag{12}$$

$$+\exp(i\,2\pi g(r_1+r_2))\}\,dg\,.$$

Interchanging orders of integrations, one obtains from (11) and (12)

$$\text{(13)} \quad \operatorname{Cov}[\hat{S}_2(f_1), \hat{S}_2(f_2)] \approx (1/T) \int_{-\infty}^{\infty} S^2(g)\, W(f_1-g)\, \{W(f_2+g) + W(f_2-g)\}\, dg$$

where

$$W(f) = \int_{-\infty}^{\infty} w_l(r) \exp(-i\, 2\pi f r)\, dr .$$

Let us assume that the spectrum S (f) is smooth over the width of the spectral window W (f), which is narrow, so that the terms in the right hand side of (13) do not overlap very much. In this case, taking $f_1 = f_2 = f$, we may neglect the first term of the right hand side of (13), and obtain

$$\text{(14)} \quad \operatorname{Var}[\hat{S}_2(f)] \approx (S^2(f)/T) \int_{-\infty}^{\infty} W^2(g)\, dg = (S^2(f)/T) \int_{-M}^{M} w_l^2(u)\, du$$

by Parseval's theorem.

So we may conclude this theoretical section by observing that the standard deviation of $\hat{S}_2(f)$, given by (9), is twice that of $\hat{S}_1(f)$, given by (9′), when no windows are used. This seems to be a new result, and is contrary to what could be conjectured on the base that $\hat{R}_1(r)$ (but not $\hat{R}_2(r)$) has meaningless values at lags proximal to T. (Bertora *et al.*, 1973, p. 67 [3]). On the other hand, in the case of windows the same formula (14) holds also for $\hat{S}_1(f)$ ([2], p. 418). Observe that analytical expressions for the variance of $\hat{S}_1(f)$ and any lag window may be generalized to non-Normal processes (Parzen, 1967 [4]).

2. *Application of the theoretical results to the spectral analysis of nerve membrane noise.* In order to test our theoretical results and their implications in the spectral analysis of membrane noise, we will consider a well-known model of the electrical activity of excitable membranes (Hodgkin and Huxley, 1952 [5]). In this model one assumes that the potassium channel permeability depends on the presence of four statistically independent subunits. Each of them exists in an excited or in a non-excited state, with probability p_1 and p_0 respectively. When all subunits of the channel are in the excited state, the channel is open and its conductance is g. Otherwise it is closed, and its conductance is zero.

In steady-state conditions p_0 and p_1 are time-independent, and

$$p_0 = \beta/(\alpha + \beta) \quad , \quad p_1 = \alpha/(\alpha + \beta) \, ,$$

where α and β are experimentally determined.

Let us indicate with $P_{01}(t, \Delta t)$ the probability of finding a channel closed at time t and open at time $t + \Delta t$, and $P_{11}(t, \Delta t)$ that of finding a channel open at time t and $t + \Delta t$. In steady-state conditions the process is stationary and $P_{01}(\Delta t)$ and $P_{11}(\Delta t)$ will depend only on the value of Δt. It will be

$$P_{01}(\Delta t) + P_{11}(\Delta t) = P_1 = p_1^4 \, ,$$

where P_1 is the probability of finding the channel in an open state. Since $P_{11}(\Delta t) = p_1^4 \, p_{1/1}^4(\Delta t)$, where $p_{1/1}(\Delta t)$ is the conditional probability that one subunit be in an excited state at time Δt, provided that it is in an excited state at time 0, one has

$$P_{01}(\Delta t) = p_1^4 - p_1^4 \, p_{1/1}^4(\Delta t) \, .$$

From the Bayes' formula we have the conditional probability that a channel is open at time t, provided that it is closed at time 0

$$P_{1/0}(\Delta t) = P_{01}(\Delta t)/P_0 = (p_1^4 - p_1^4 \, p_{1/1}^4(\Delta t))/(1 - p_1^4)$$

and $p_{1/1}(\Delta t)$ is given by the Hodgkin and Huxley equation

$$p_{1/1}(\Delta t) = \alpha/(\alpha + \beta) + (1 - \alpha/(\alpha + \beta)) \exp(-(\alpha + \beta)\, \Delta t) \, .$$

The conditional probability $P_{0/1}$ is

$$P_{0/1}(\Delta t) = 1 - P_{1/1}(\Delta t) = 1 - p_{1/1}^4(\Delta t) \, .$$

The spectrum of a single channel may be calculated according to the previous model. In fact, letting $g(t)$ a random variable taking on the two states g and 0, the autocovariance function of the zero mean random variable $x(t) = g(t) - g\,P_1$ is

$$R(r) = E\{x(t)\, x(t + |r|)\} = g^2 \, P_1 \, P_{1/1}(r) - g^2 \, P_1^2 \, .$$

Letting $P_{1/1}(r) = p_{1/1}^4(r)$, one finds after some straighforward calculations

$$R(r) = g^2 \, P_1^4 \sum_{j=1}^{4} \binom{4}{j} P_1^{4-j} (1 - P_1)^j \exp(-jr/\eta)$$

where

$$\eta = 1/(\alpha + \beta) .$$

The power spectrum $S(f)$ is then

$$S(f) = \int_{-\infty}^{\infty} R(r) \exp(-2\pi i f r) \, dr = \tag{15}$$

$$= g^2 \sum_{j=1}^{4} \binom{4}{j} P_1^{4-j} (1 - P_1)^j \frac{2\eta/j}{1 + [2\pi f \eta/j]^2} .$$

One of the many reasons for studying the ionic conductances fluctuations of excitable membranes is that of determining the number ot (potassium) ionic channels (see, for example, Neher and Stevens, 1977 [6]). In fact, if n is the number of channels present in a given area of membrane and if moreover all

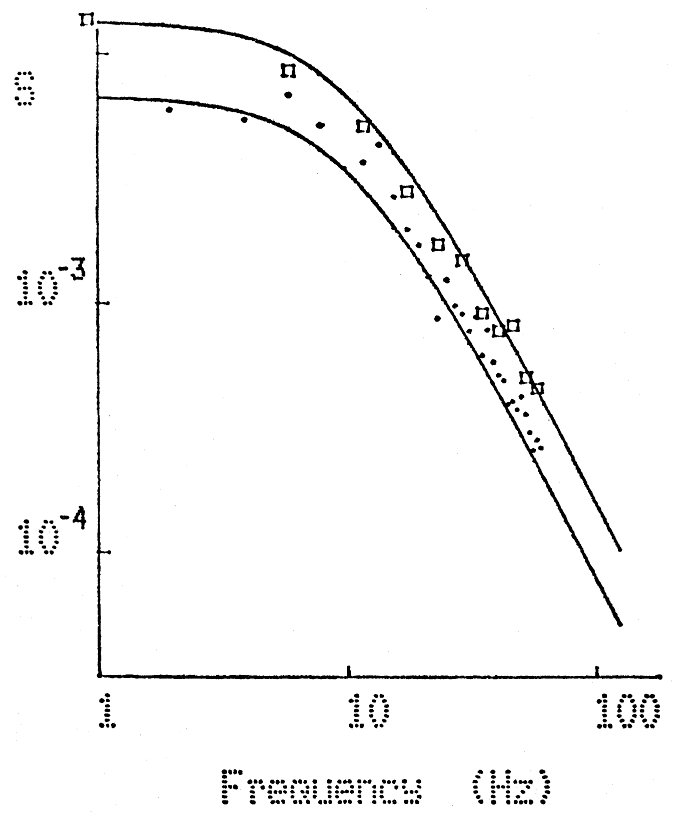

Fig. 1. – (·) Average value of 80 computed spectral estimates $\hat{S}_2(f)$ for N = 7; (□) stantard deviation of (·). The lower continuous line represents $S(f)$ and the upper one $2\,S(f)$.

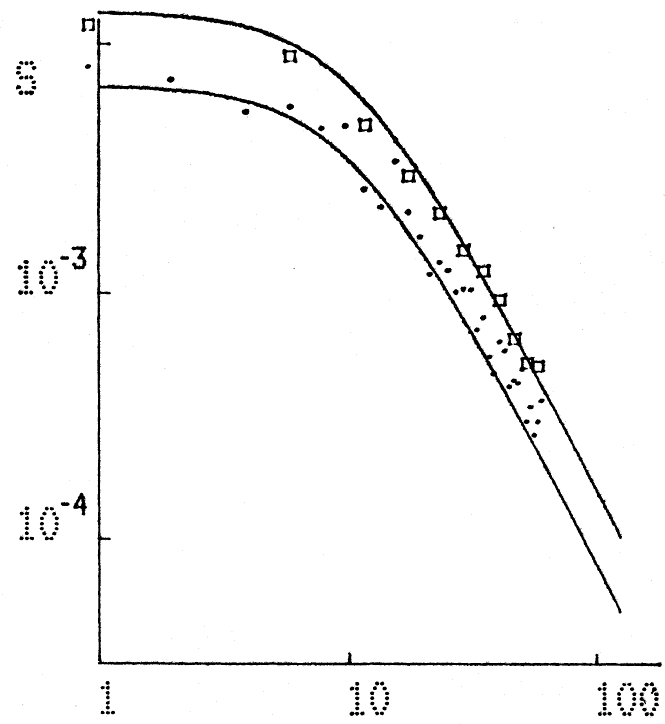

Fig. 2. – As fig. 1 for N = 9.

of them are statistically independent, then the total power spectrum due to the contribution of all channels is

$$S^{tot}(f) = n\,S(f)\,. \tag{16}$$

Given that the value of $n\,g$ is that of the total conductance, as experimentally determined (see [6]), fitting the results of the spectral analysis to $S^{tot}(f)$, (see, for example, Bevan *et al.*, 1979 [7]), and taking into account (15) and (16), one obtains an estimate of n and g, provided that all assumptions are satisfied.

3. *Comparison between the theoretical and the simulated results.* In the simulation we have taken $\alpha = 0.05\ \mathrm{ms}^{-1}$, $\beta = 0.01\ \mathrm{ms}^{-1}$. These are the rounded values experimentally found in our laboratory (Dr. Peres, personal communication) for the semitendinous muscle of *Rana Esculenta* at a membrane potential of $-20\ m\mathrm{V}$, and they are not far from those given in the literature for the same potential and temperature (about 3° C) of similar fibres (Adrian, Chandler and Hodgkin, 1970 [8]).

In order to avoid aliasing effects in the use of fast Fourier transforms algorithms, we have taken a sampling interval $\Delta t = (2/5)\,f_d = 4$ ms ([1] p. 321), where $f_d = 100$ Hz is the cut-off frequency. This because the range of interest of spectral analysis, as appears from the current literature, lies between 0 and 100 Hz. The input was generated by a stochastic routine according to the value of $P_{1/0}(\Delta t)$ and $P_{0/1}(\Delta t)$ of the model, and was represented by 2^N points spaced by Δt. N ranged between 7 and 9. The upper limit was due to the limited storage capacity of the personal computer. The output was represented by 2^N points spaced by $1/(2^N \Delta t)$ in the frequency domain. Our figures will show only a 32 points output, from 0 to 60.5 Hz.

In figs. 1 and 2 the validity of (9), is tested when no lag windows are used. One sees that the theoretical results agree fairly well with the computed ones. Here and in the following figs. the y-axis is in arbitrary units, so that g may be any constant.

In figs. 3 and 4 one may see the decrease of standard deviation when a boxcar window, $w_1(r) = 1$, and an algebraic window, $w_1(r) = (1 - (|r|/M)^8$, are used. For comparison the standard deviation of $\hat{S}_1(f)$ is also shown. We observe that the decrease in the variance is less than that predicted by (14), and is about the same for both $\hat{S}_1(f)$ and $\hat{S}_2(f)$. According to the reduction of the

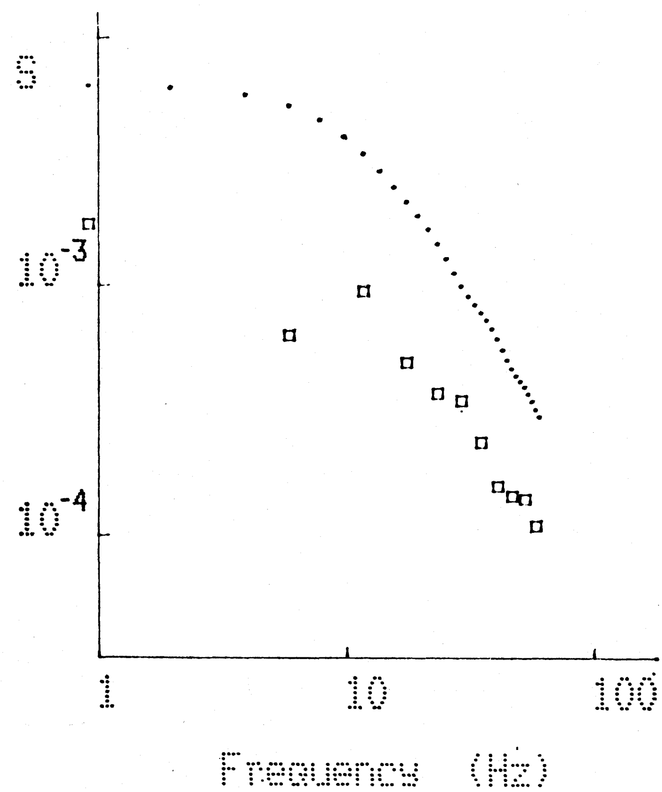

Fig. 3. – (·) Average value of 80 computed spectral estimates $\hat{S}_2(f)$ with a boxcar window, N = 9, M = 16 Δt; (□) standard deviation of (·).

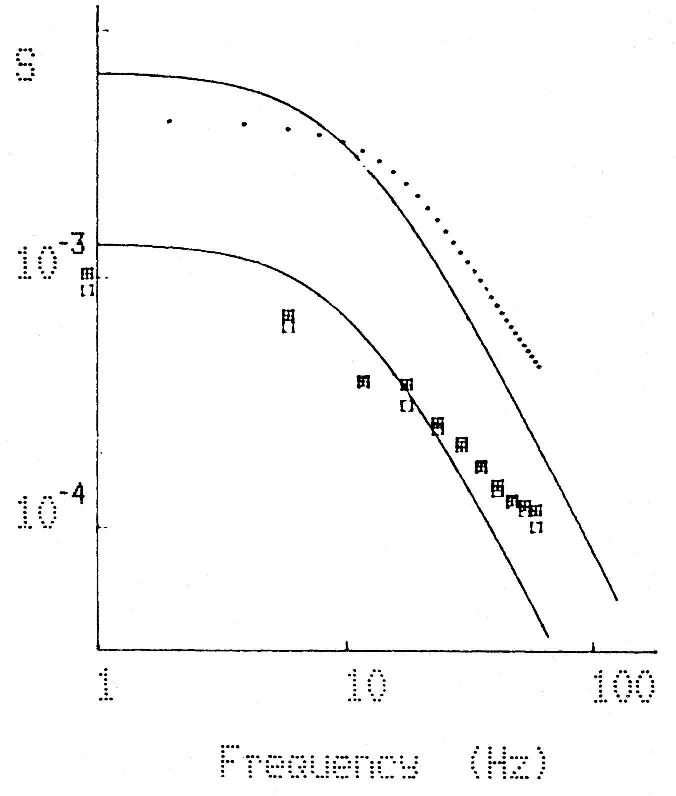

Fig. 4. – Effect of an algebraic window with $\delta = 2$, $N = 7$, $M = 16\, \Delta t$ on the average value of 80 computed spectral estimates $\hat{S}_1(f)$ (·), and on their standard deviation (□). Standard deviation of the same algebraic window for the estimate $\hat{S}_2(f)$ (▪). The lower continuous line represents the predicted value of (□) and of (▪). The upper line represents the unbiased spectrum $S(f)$.

variance due to the use of the windows, one sees that the average values of the computed spectral estimates of figs. 3 and 4 are much smoother than those of figs. 1 and 2.

As a conclusion, for what concerns which spectral estimate should be preferred, $\hat{S}_1(f)$ or $\hat{S}_2(f)$, we do not see any reason in the choice of $\hat{S}_2(f)$, when the analysis is digitally performed. In fact, when no windows are used, the variance of $\hat{S}_2(f)$ is twice that of $\hat{S}_1(f)$. When lag windows are used, the variance of $\hat{S}_2(f)$ is the same of $\hat{S}_1(f)$, and the slight improvement of the bias is too low to justify the use of the first estimate. We recall that for the computation of $\hat{S}_2(f)$ we are not allowed to use fast Fourier transforms algorithms in convolutions, and this fact enormously increases the computation time required for the autocovariance function estimate, when N is large. Instead the estimate $\hat{S}_2(f)$ may become of interest in analogue data analysis procedures (see, for example, [1], p. 282).

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