Seasonal variation of the circadian activity rhythm of the *Talitrus saltator* population living in the Italian study site of the MEDCORE project

Dr. Mariella Nardi – University of Florence, Italy
Prof. Felicita Scapini - University of Florence, Italy
Dr. Elfed Morgan – University of Birmingham, UK

species:

Talitrus saltator (Crustacea Amphipoda)

study site:

the sandy beach along the coast of the Maremma Regional Park (Tuscany, Italy)

- the environment

the beach at Collelungo

- picture by Claudia Rossano -

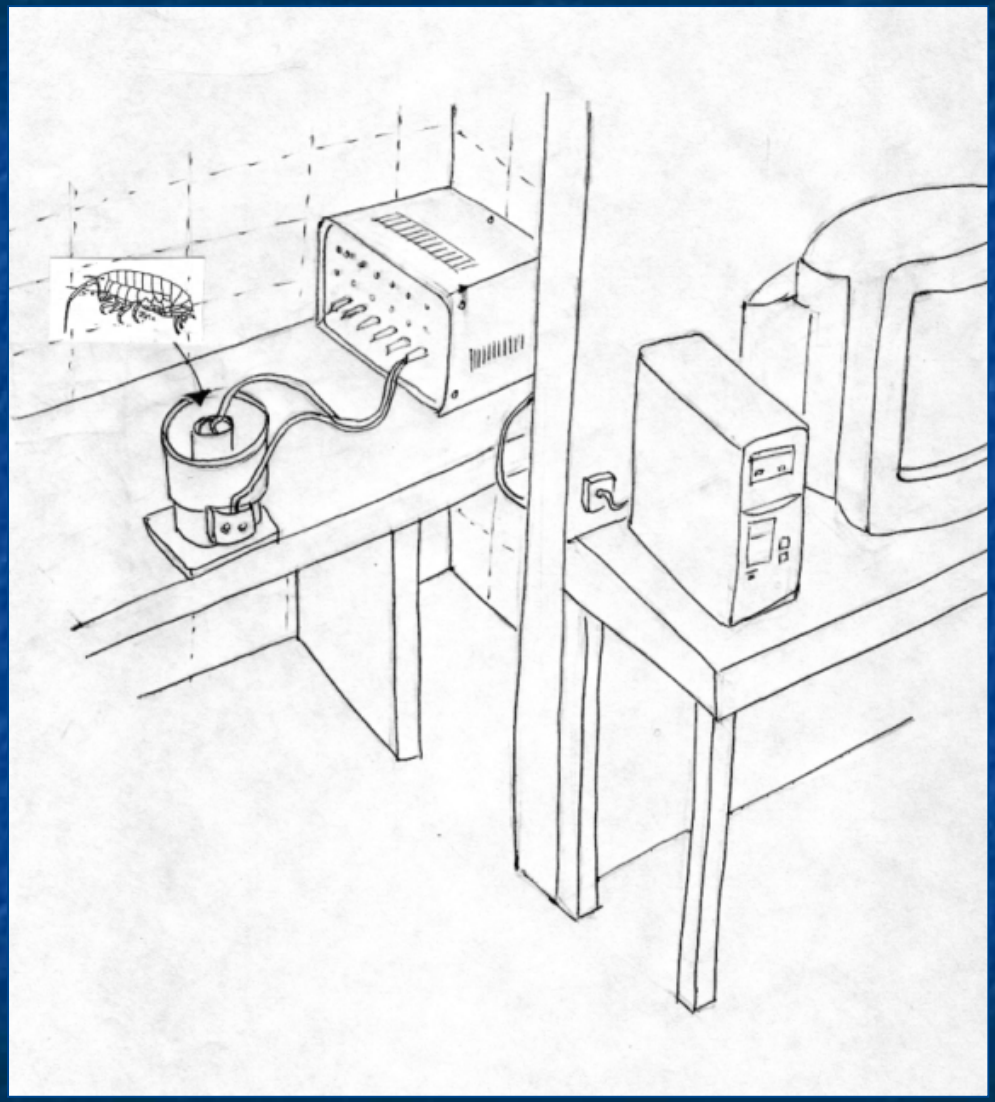
- picture by Simone Gambineri -

- the rationale of the study

an in-depth analysis of the rhythmic behaviour
throughout a year
of a population choosen as a case study,
allowing successive comparisons on a wide range
of different ecological and geographical conditions

→ we highlighted different profiles of rhythmic patterns of circadian activity
→ we analysed the possible causes of the found variability
→ we made qualitative comparisons with other researches carried out in the framework of the MEDCORE Project

- the equipment



designed and constructed by

Mr. Derek D. Green

and colleagues

in the Workshop of the School of Biosciences of the University of Birmingham, UK

- pencil drawing by my friend Claudia Borgioli

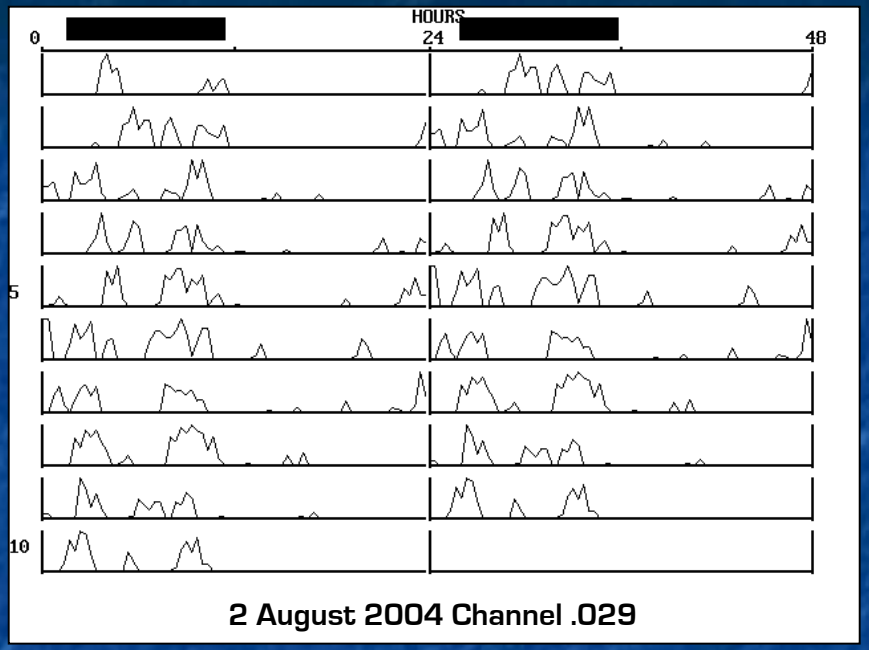
- the experimental protocol

- animals collected by hand on the beach and immediately transferred by car (in boxes containing wet sand) to the laboratory in Florence
- animals put individually in the 39 recording chambers (sand from the beach of origin)
- experiments performed in controlled laboratory conditions: constant temperature = 18 ± 1 °C; continuous darkness (*free-running conditions*)
- recording sessions lasting 10 days
- 1 recording session in each season from summer 2004 to spring 2005

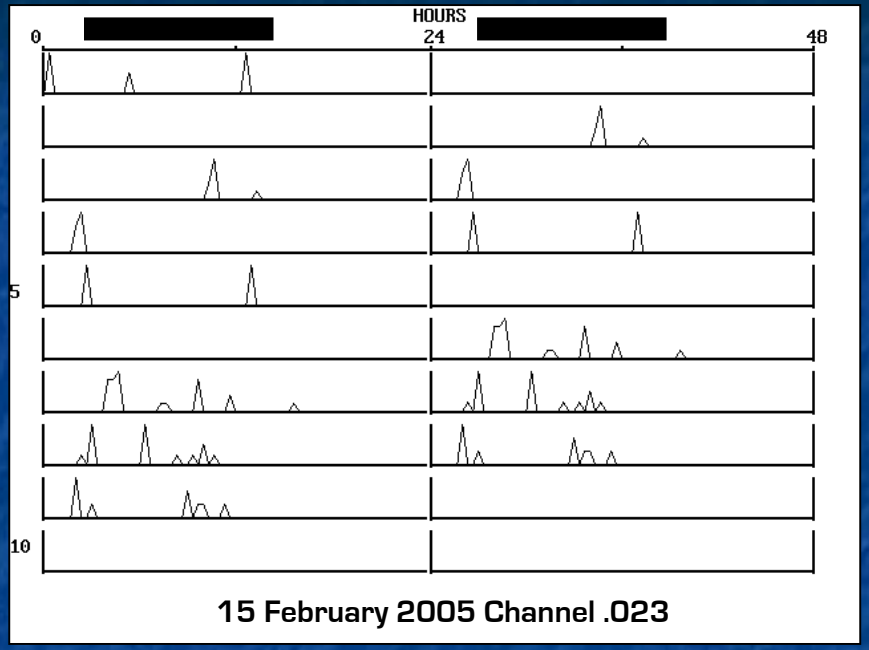
Calendar of the experiments	
summer	*2-12 August 2004*
autumn	*1-10 October 2004*
winter	*15-25 February 2005*
spring	*11-21 May 2005*

- at the end of the experiments each individual was put in alchool 75% → check of the morphometric parameters: *sex, number of the second antennae tagma, cephalic length*

- some examples of our results

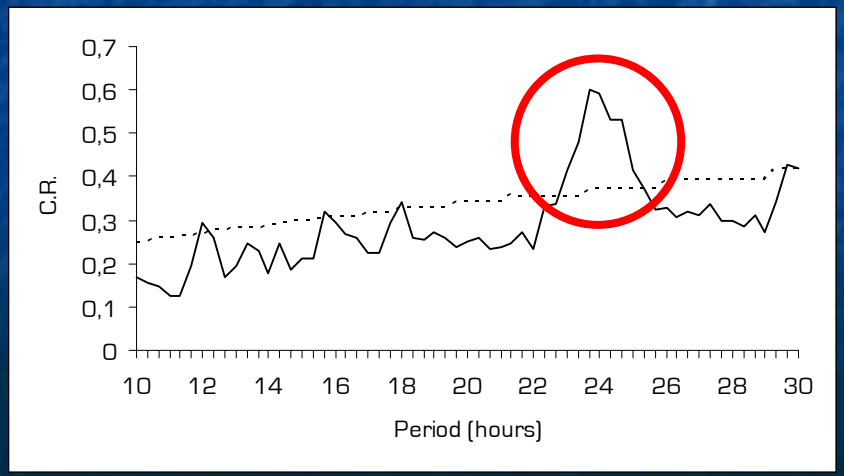


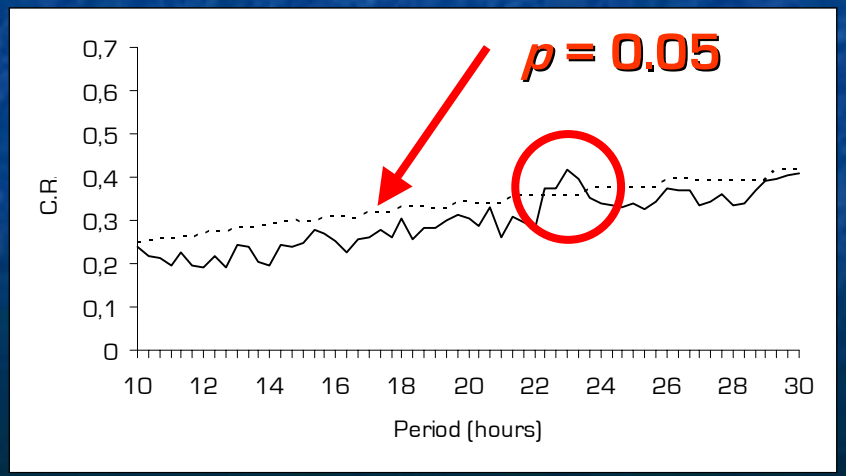
2 August 2004 Channel .029



15 February 2005 Channel .023

periodogram analysis





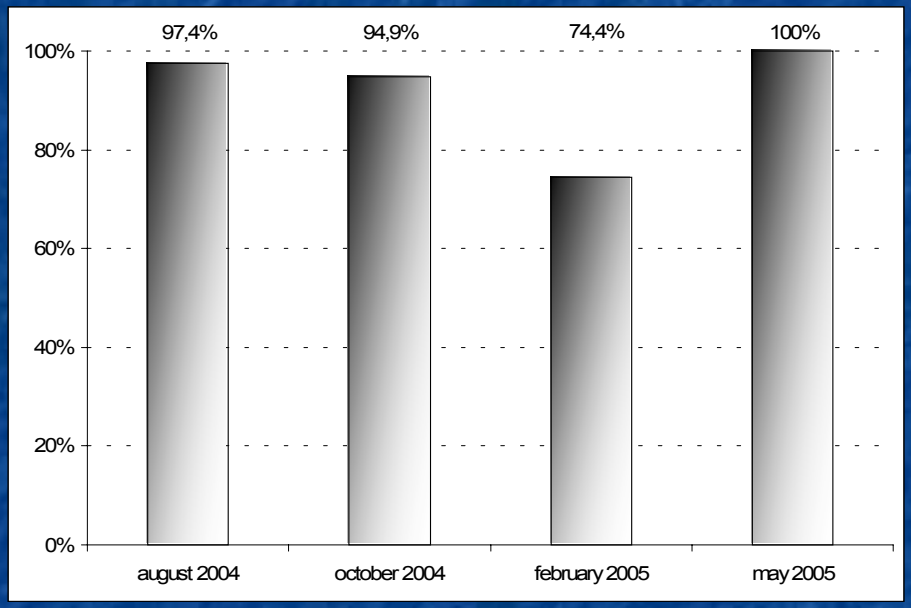
RESULTS - seasonal differences

Parameters	August 2004	October 2004	February 2005	May 2005
Survival (N_{exp} = 39)	38	37	29	39
Rhythmicity	18	18	17	23
Mean circadian period	24h 5' ± 18'	24h 10' ± 34'	24h 8' ± 44'	24h 8' ± 27'
Definition of the circadian period (*snr*)	0.79 ± 0.22	0.38 ± 0.22	0.30 ± 0.24	0.44 ± 0.23

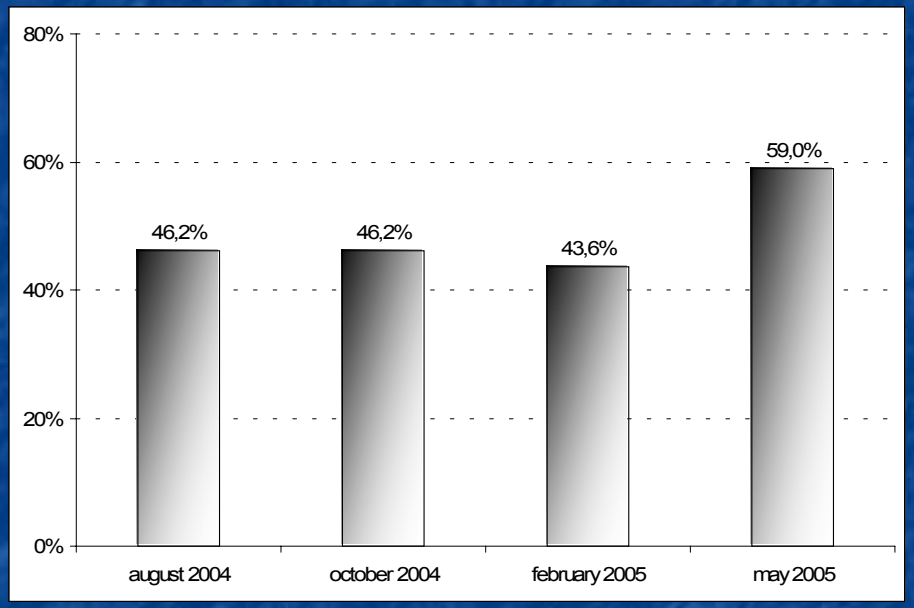
each recording session: N = 39

RESULTS - seasonal differences

survival



rhythmicity

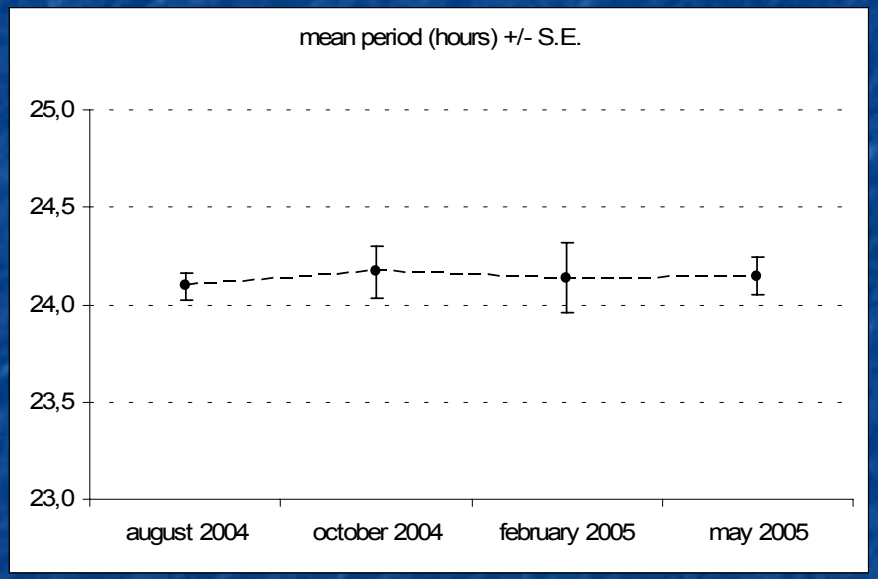


i.e. animals showing
a significant circadian rhythmicity

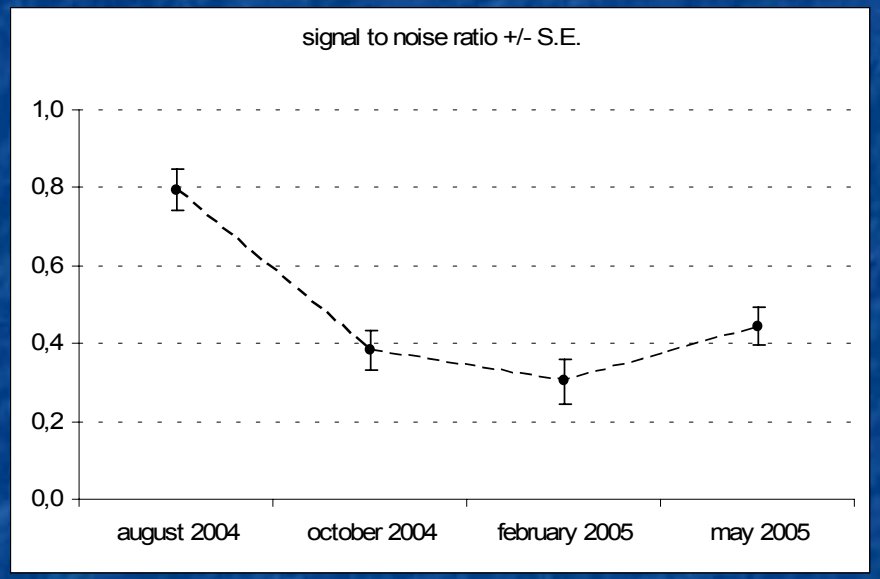
each recording session: N = 39

RESULTS - seasonal differences

mean circadian period
([*in hours*])



signal to noise ratio
([*snr*])



i.e. definition of the rhythm with respect to the random noise

RESULTS - seasonal differences

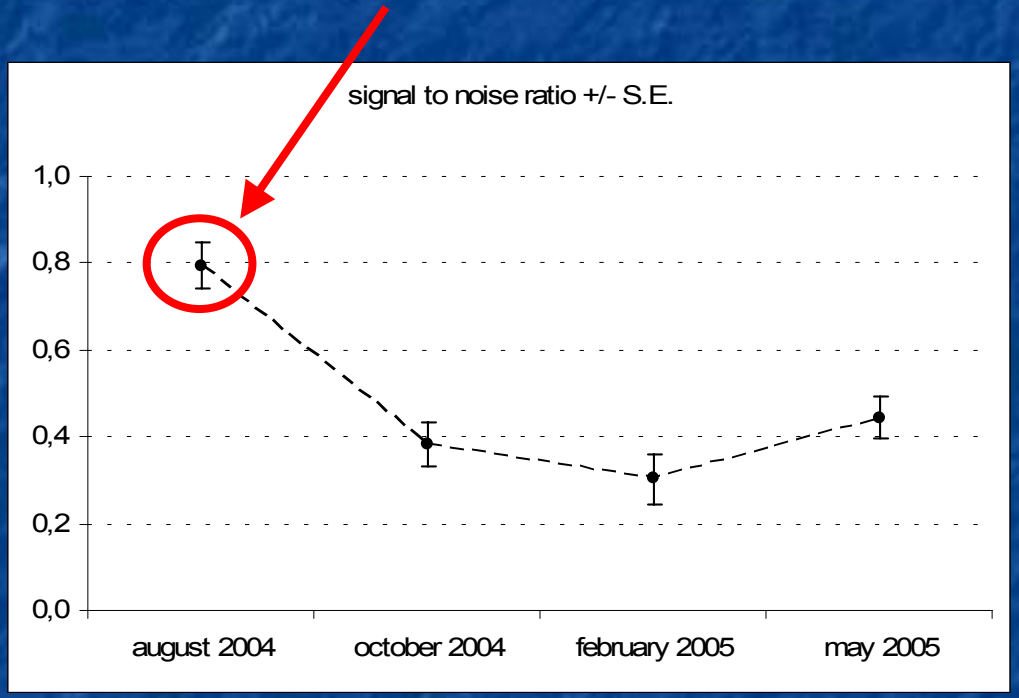
- **NO** significant difference in the survival (χ^2 = 2.120,
- df = 3, p > 0.05)
- **NO** significant difference in the frequencies of the rhythmic animals (χ^2 = 3.755, df = 3, p > 0.05)
- **NO** significant difference in the mean circadian period (Kruskall-Wallis χ^2 = 0.7498, df = 3, p > 0.05)

....but at last....

there was a **SIGNIFICANT** difference in the signal to noise ratio *snr* (Kruskall-Wallis χ^2 = 22.5948, df = 3, p < 0.001)

- *let's focus on the snr...*

signal to noise ratio "expresses" the clearness (*i.e.* the definition) of the rhythm with respect to the random noise



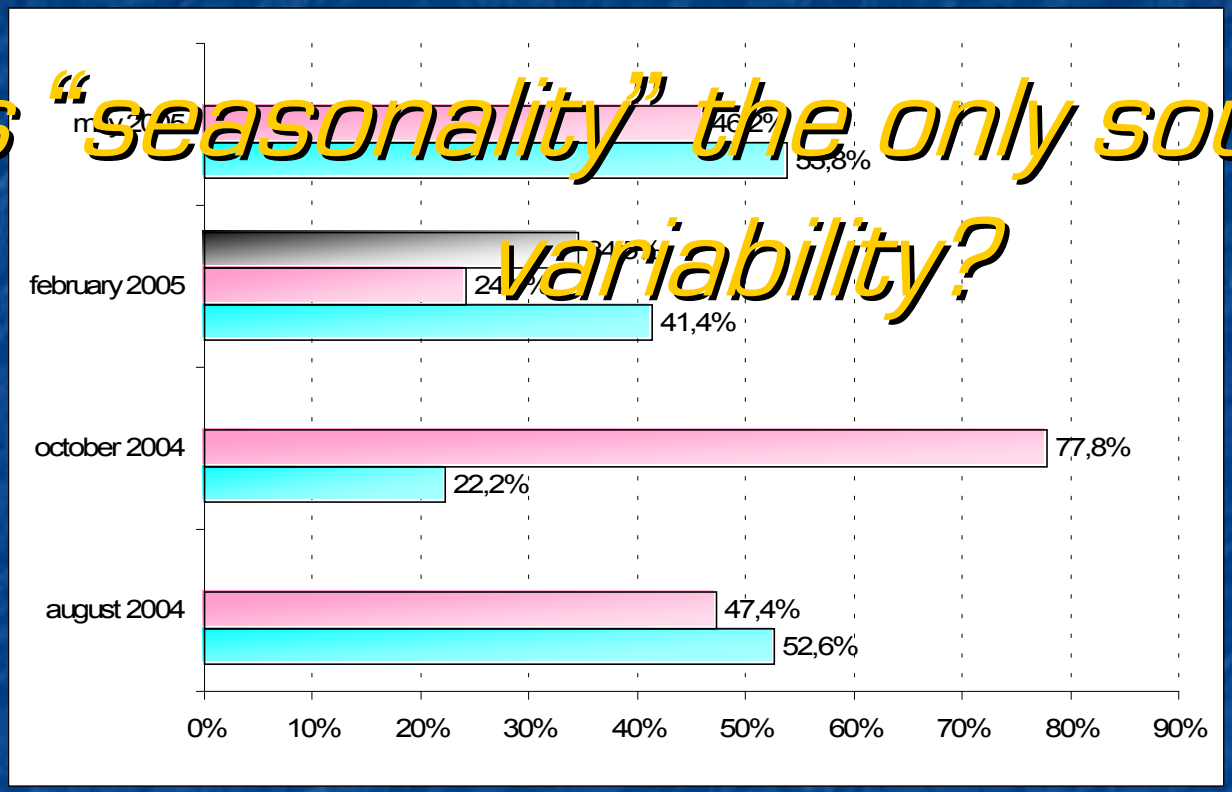
animals showed a *more defined circadian period in summer*

these data CONFIRM previous results

obtained with other Italian populations

- let's focus on samples composition

is "seasonality" the only source of variability?

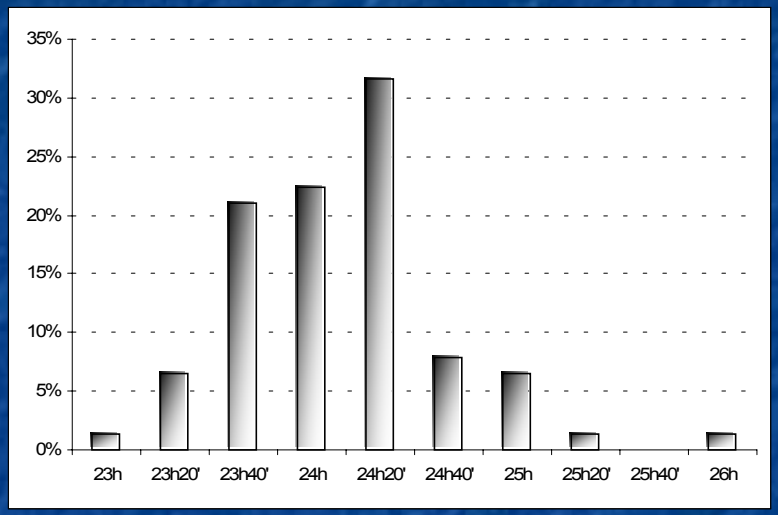


MALES **FEMALES** **JUVENILES** *(found only in February)*

each recording session: N = 39

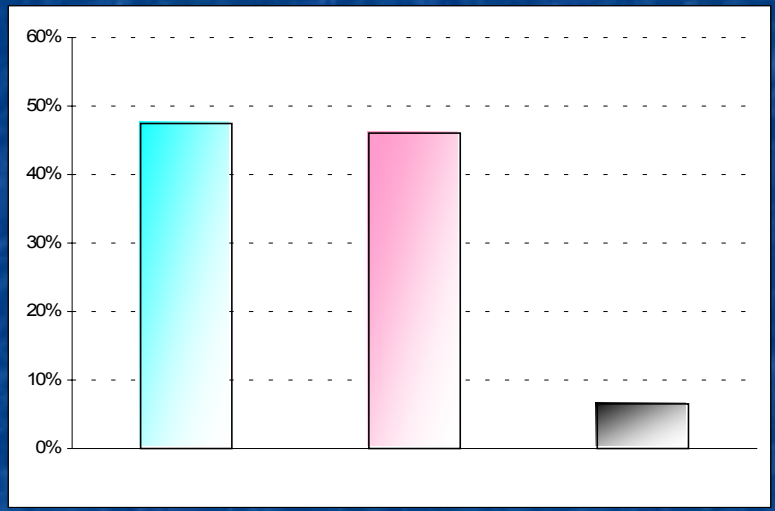
RESULTS – differences between males and females

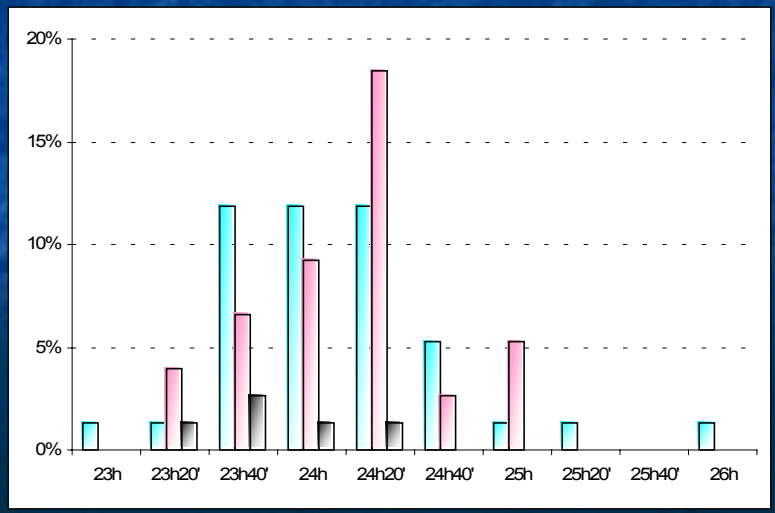
distribution of period



N_{TOTAL} = 76

differences between sexes





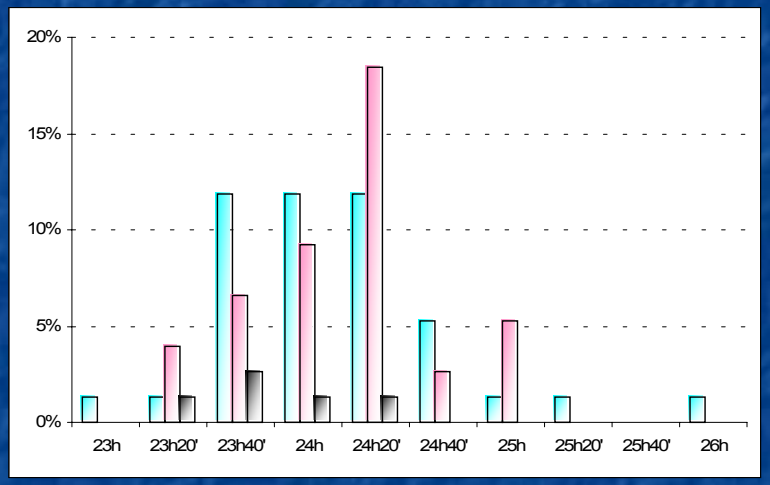
MALES N=36

FEMALES N=35

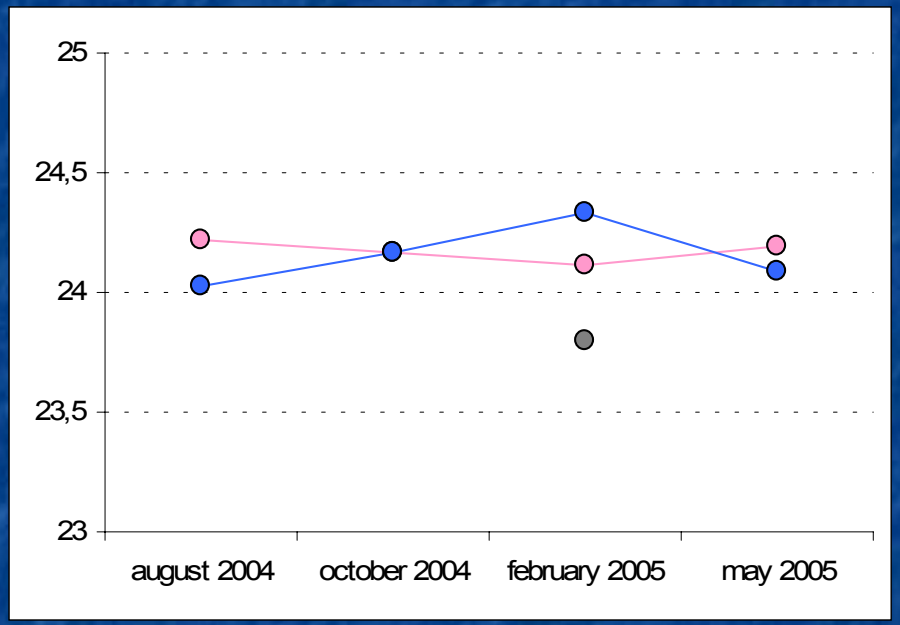
JUVENILES N=5

RESULTS – differences between males and females

frequency of period:
differences between sexes



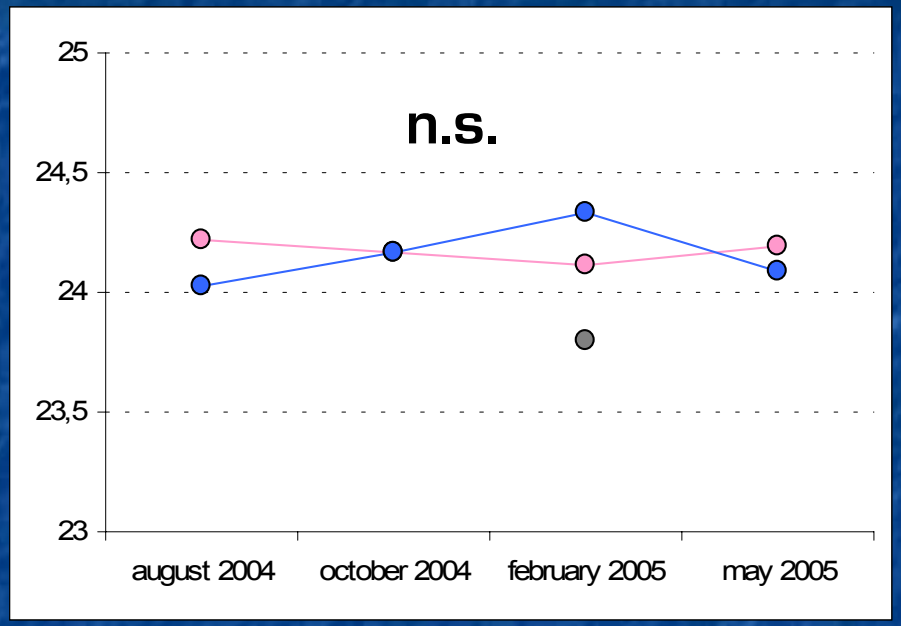
mean circadian period: seasonal
trend in the 2 sexes



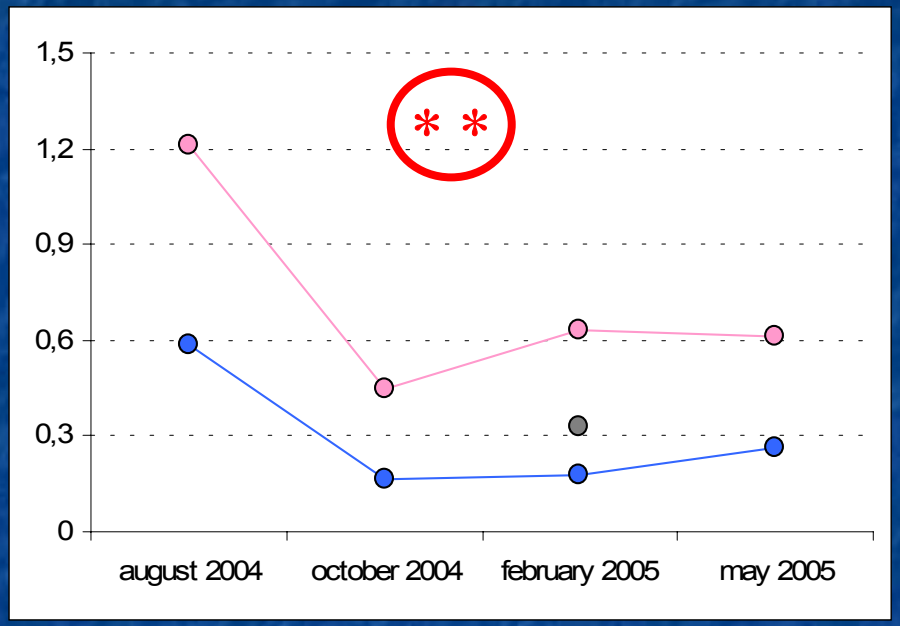
mean period **MALES** 24h8' ± 6'
mean period **FEMALES** 24h10' ± 5'
mean period **JUVENILES** 23h48' ± 10'

RESULTS – differences between males and females

mean circadian period



snr



males *females* *(juveniles were not considered in the statistical analysis)*

Friedman test: $F_r = 1.22$, df = 3, $p > 0.05$

Friedman test: $F_r = 12.01$, df = 3, $p < 0.01$

females showed a more defined circadian period than males

these data CONFIRM the results obtained by Lucia Fanini and collaborators on the orientational choices of the two sexes

RESULTS – differences along the coastline

a study has been started to analyse the eventual differences of the rhythmic behaviour in animals coming from different points along the coastline (*different coastal dynamics*), as it had already been done with orientation

up to the moment, two points only were tested in summer 2004 (point 4000m and 5000m from the mouth of the river Ombrone), and no significant difference was found

CONCLUSIONS

- the population of *Talitrus saltator* living at Collelungo in the Maremma Regional Park is a healty population, being abundantly present on the beach all year long and showing a good circadian rhythm of activity
- our results confirmed the seasonal trend of the circadian rhythm in this population, as already demonstrated in other Italian populations
- the clearness of the circadian rhythm expressed by the signal to noise ratio was the parameter of the rhythm significantly different in the four seasons (*while the period was not*)
- our results showed for the first time the differences between males and females in the definition of the circadian rhythm, as already demonstrated in the precision of orientation in the same species → females (*which carry juveniles in early stages of development in the pouch*), show a "seasonally changing" precision in the activity rhythm in order to better adapt to the environmental ecological conditions

Thank you

and all the people who helped me
carrying out my experiments and researches throughout all these years
offering me their support, suggestions and friendship

Francesco, Carla, Nedo, Felicita, Lucia, Fulvia, Claudia, Francesca, Laura, Nunzia,
Marco Claudio, Marcela, Andrea, Fausto, Paola, Elfed, Derek, Camilla, Claudia,
Piero, Maria Novella, Sara, Lucia, Simone, Karima
and all the others