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DIFFERENT TOOLS TO OPTIMIZE THE WELFARE AND THE MORPHOLOGICAL QUALITY OF REARED FINFISH LARVAE

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INTRODUCTION

- The morphological quality of species considered as 'consolidated' for aquaculture is far to be “wild-like”, so determining economic losses and inducing diffidence in consumers for aquaculture products.
- Available data in literature are still unsatisfactory: e.g., the same anomalies are ascribed to many different causes.
- Actual knowledge seems to indicate that anomalies are the consequence of so many factors acting and interacting among them that probably multidisciplinary and multilevel studies seem to be necessary.

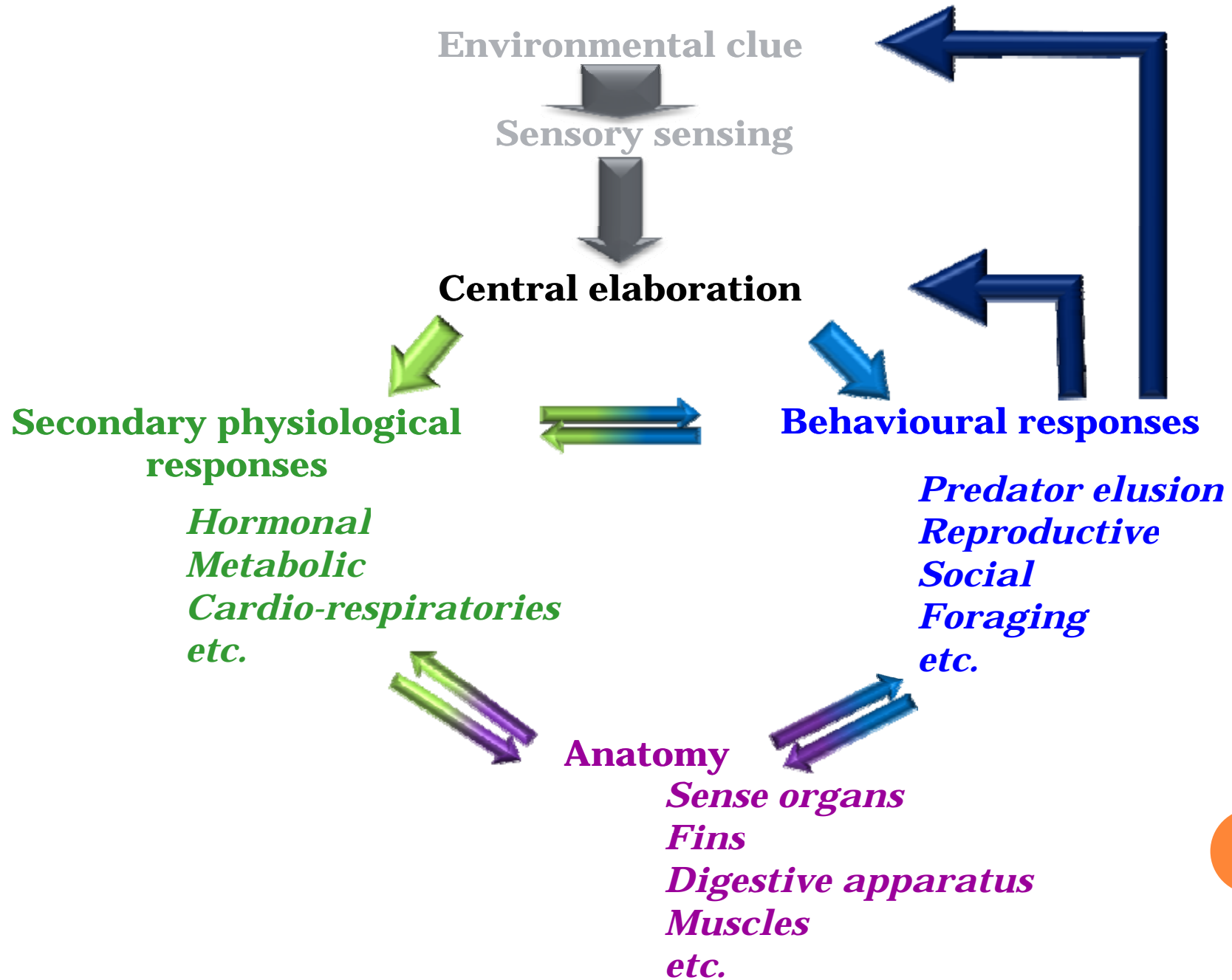
INTRODUCTION

- In particular, if we considered that vertebral malformations may now be the norm in hatchery lots, we should begin to take into consideration that all reared fish in each developmental stage are probably 'distressed' fish, in which epigenetic and genetic factors try to buffer the environmental noises effects.
- The setting up of proper rearing protocols in a framework of a responsible aquaculture should take into account what is the larval behaviour in wild instead of what happens in 'forced' environment.

IN OUR LAB, WE ARE FACING THE PROBLEM OF DEVELOPMENTAL ANOMALIES BY MEANS OF THREE APPROACHES:

- **the widening of knowledge on the development of locomotor, sensorial and digestive organs in the different reared species**, considered as an essential tool to properly fit the rearing methodology to the species' and developmental stages' needs.
- **the monitoring of morpho-anatomic quality in mass produced gilthead seabream, from different Mediterranean hatcheries and larval rearing methodologies.** This activity is actually carried out in the framework of a EU research Project (SSP-2005-44483 "SEACASE - Sustainable extensive and semi-intensive coastal aquaculture in Southern Europe"), and it's directed to the individuation of rearing methodology able to produce *wild-like* juveniles.
- **the development of a model of the occurrence of skeletal anomalies in reared juveniles of different species, both in terms of type and quantity.**

- the widening of knowledge on the development of locomotor, sensorial and digestive organs in the different reared species



- the widening of knowledge on the development of locomotor, sensorial and digestive organs in the different reared species
- May developmental intervals be different in the same species?
- Are developmental intervals different in the same family?
- Do all the larvae actually reared exhibit the same feeding behaviour?
- Do similar rearing methodologies return the same morphological quality of juveniles?

- **Developmental intervals may be different in the same species?**

Evidences available from literature reinforce the idea that in the same species some heterochronies are induced by rearing temperatures

HETEROCHRONY (introduced by Ernst Haeckel in 1875¹)

Bertolini *et al.*, (1986)

Boglione *et al.*, (1989)

Marino *et al.*, (1991)

Seikai *et al.* (1986)

Batty *et al.* (1993)

Fuiman *et al.* (1998)

Koumoundouros *et al.* (2001)

Sfakianakis *et al.* (2004)

¹Horder, T. (2006) *Heterochrony*. In: *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd: Chichester.

So the answer at this question is YES!

○ Developmental intervals/patterns are different in the same family?

| Species | Authors | Sequence of the first appearance of skeletal elements in some Sparids | | | | | | | | | | | |
|------------------------|--|---|---------------|--------|------------------|--------------------------------|------------------------|------------------------|-------------------------------------|-------------------|------------------------|------------------------|-------------------|
| <i>S. aurata</i> | Faustino and Power 1998, 1999, 2001 | mouth , gills and pectoral girdle | | caudal | vertebral column | notochord flexion | vertebrae ossification | dorsal and anal | | pelvic | ribs | | |
| <i>P. major</i> | Matsuoka, 1987 | pectoral | mouth & gills | | | lack data on notochord flexion | | anal | pelvic | dorsal | lack data on ribs | | |
| <i>A. rhomboidalis</i> | Houde and Potthoff 1976 | mouth & gills | pectoral | | | notochord flexion | | dorsal and anal | | pelvic | ribs | | |
| <i>D. puntazzo</i> | Sfakianakis et al., 2005 | lack data on cranium | | | | dorsal and anal | | | | | | | |
| <i>P. erythrynus</i> | Sfakianakis et al., 2004 | lack data on cranium | | | | dorsal | | anal | notochord flexion | | | vertebrae ossification | |
| <i>P. pagrus</i> | Çoban et al., 2009 | mouth , gills and pectoral girdle | pectoral | | | dorsal | | notochord flexion | lack data on vertebrae ossification | vertebral column | anal | pelvic | lack data on ribs |
| <i>D. dentex</i> | Koumoundourous et al., 1999; 2000; 2001a | | | | | notochord flexion | | vertebrae ossification | dorsal and anal | | ribs | | |
| <i>D. sargus*</i> | Koumoundourous et al., 2001b | lack data on cranium | pectoral | | | vertebral column | | dorsal and anal | | notochord flexion | vertebrae ossification | ribs | |

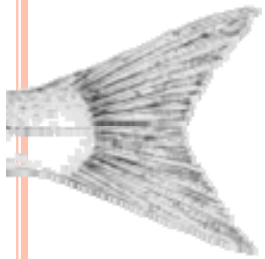
* indicates that some data were extrapolated from figures in the article. when some elements start to differentiate at the same time, they are put together.

- Developmental intervals/patterns are different in the same family?

| <i>Species</i> | <i>Authors</i> | <i>Skeletogenic sequence of final number of lepidotrichia achievement in Sparids fins</i> | | | | |
|----------------------|--|---|-------------------------|-------------------------|----------|---------------------|
| <i>S. aurata</i> | Faustino and Power 1998, 1999 | caudal | dorsal | anal | pelvic | pectoral |
| <i>P. major</i> | Matsuoka, 1987 | | pectoral | dorsal, anal and pelvic | | |
| <i>D. puntazzo</i> | Sfakianakis <i>et al.</i> , 2005 | | anal | pelvic | dorsal | pectoral |
| <i>P. erythrynus</i> | Sfakianakis <i>et al.</i> , 2004 | | dorsal | anal | pelvic | pectoral |
| <i>P. pagrus</i> | Çoban <i>et al.</i> , 2009 | | pectoral | pelvic | | |
| | Machinandiarena <i>et al.</i> , 2003 | | caudal, dorsal and anal | | | pectoral and pelvic |
| <i>D. sargus</i> | Koumoundourous <i>et al.</i> , 2001b | caudal | anal | dorsal | pectoral | |
| <i>D. dentex</i> | Koumoundourous <i>et al.</i> , 2000; 2001a | | | dorsal* | pelvic | pectoral |

* indicates that some data were extrapolated from figures in the article; when some elements start to differentiate at the same time, they are put together.

THE FIN DIFFERENTIATION PATTERN IS LINKED TO BEHAVIOUR/REQUIREMENTS OF LARVAE AND JUVENILES



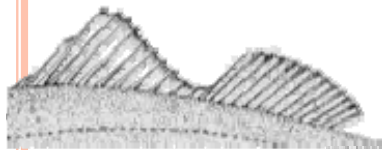
Transmitting muscular force for propulsive tail strokes, used in escape or attack responses (Kohno *et al.* 1984, Hale, 1999)

the precocious caudal fin development is linked to the pelagic larval niche, where the needs for prolonged swimming against water currents dominate

the precocious presence of the caudal fin in Sparids gives to larvae the capability not only of feeding on encountered preys but also of actively capturing (and selecting) preys through fast-start movements.



The differentiation of the **pectoral fin** may facilitate **grazing**
the prolonged and delayed pectoral fin development in *S. aurata* was linked to the **transition of to the demersal juvenile environment** where manoeuvrability is considered an advantage.



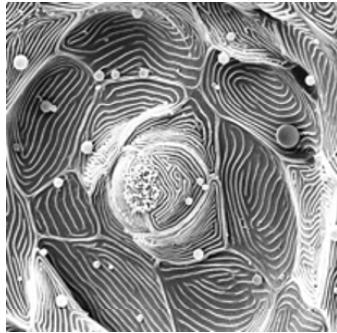
In higher Teleosts, the anterior spinous **dorsal fin** is used to slice through water whilst the soft, posterior, part plays an anti-pitch function during forward movement, or acts in synergy with the anal one, in braking, crawling along the underwater-floor, aiding in propulsion during slow swimming or in backing-up motions, allowing the fish to turn sharply

- Sense organs are similar in fish?

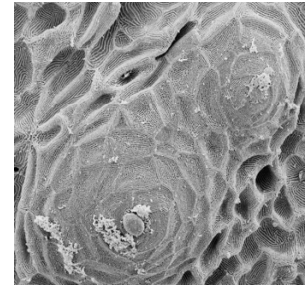
Variety of TB typologies in the oro-pharyngeal cavity

SUPERFICIAL

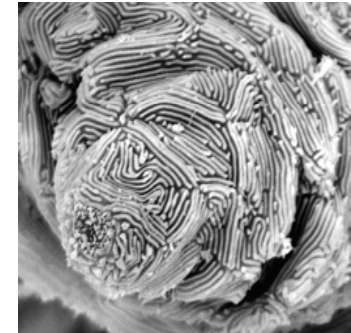
Type I single



Type II single

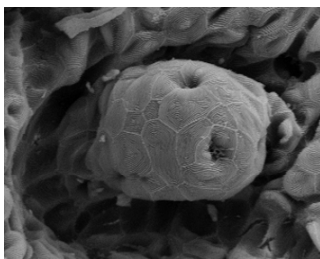
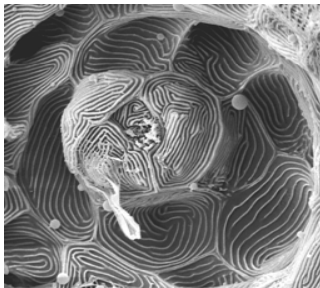


Type III

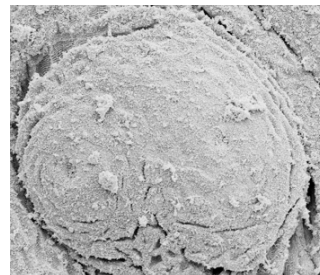
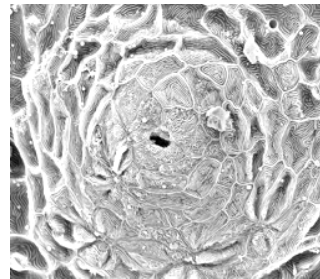


HOLLOW

Type I single/multiple



Type II single/multiple

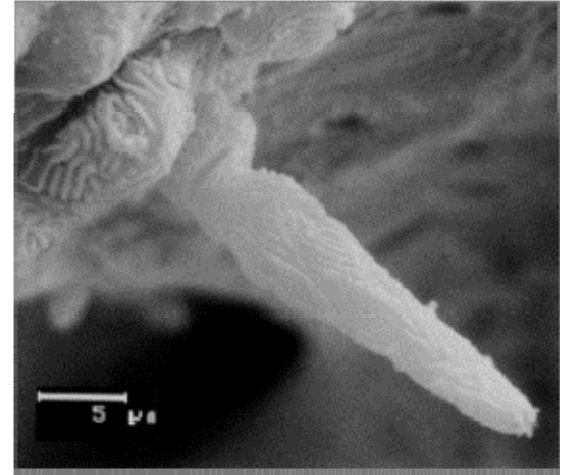
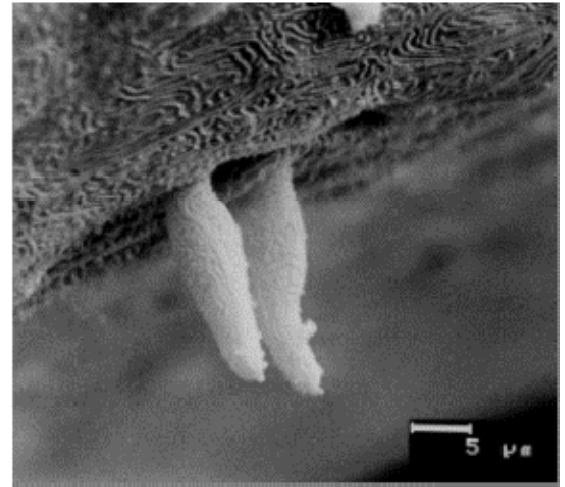
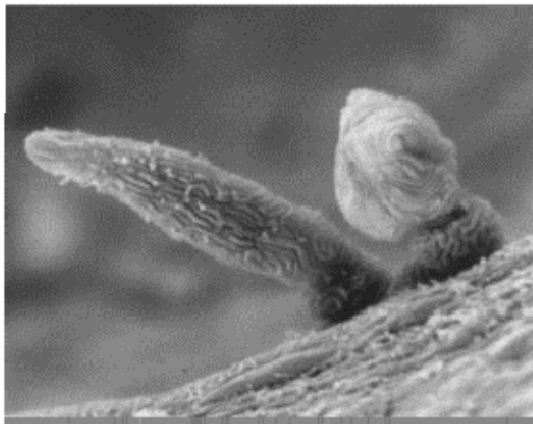
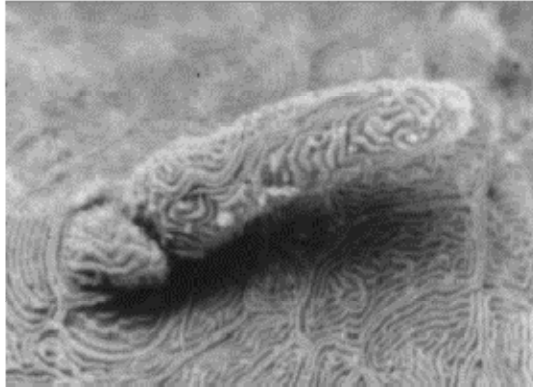
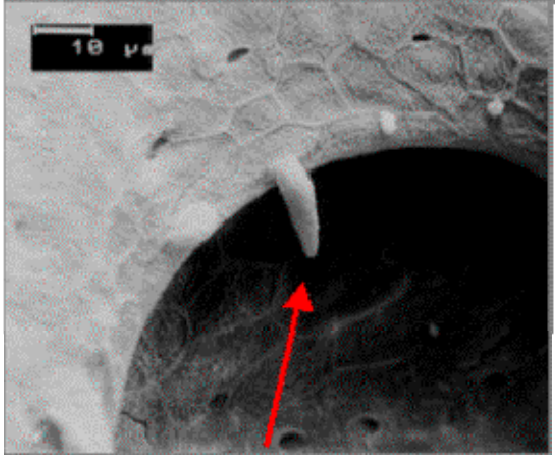
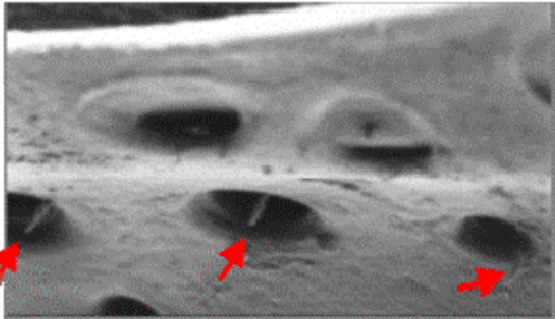
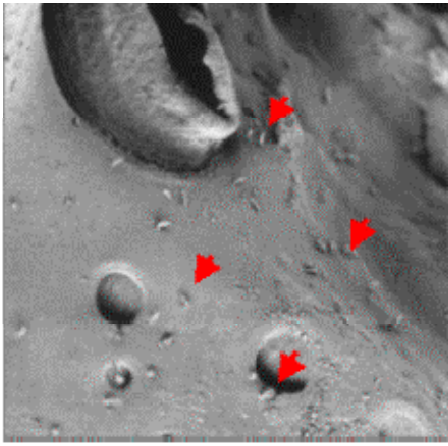


Type III



Type IV



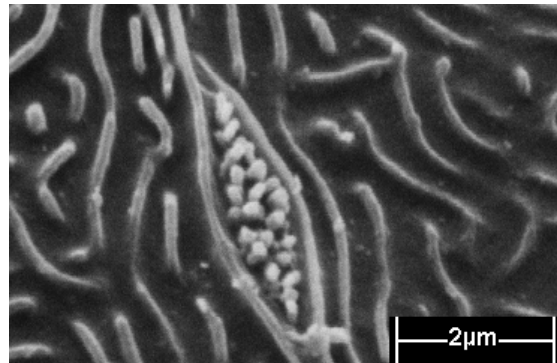


?

Chemoreceptive solitary cells

Salmo trutta macrostigma, Dumeril 1858 (unpublished data)

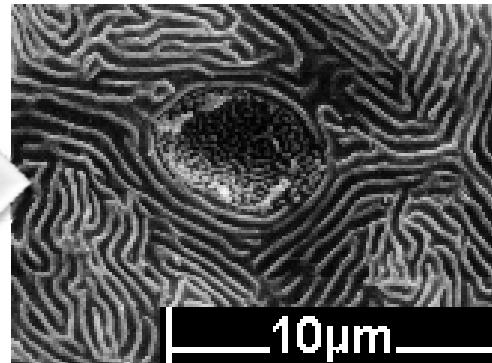
27 days post fecundation



Oligovillous cell



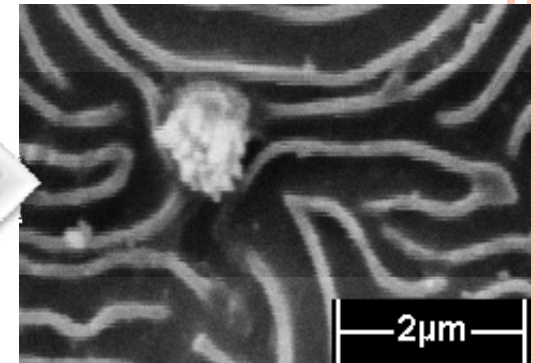
25 days post hatching
(TL = 2,22 cm)



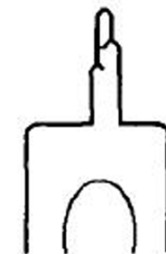
Degeneration



50 days post hatching
(TL = 2,71 cm)

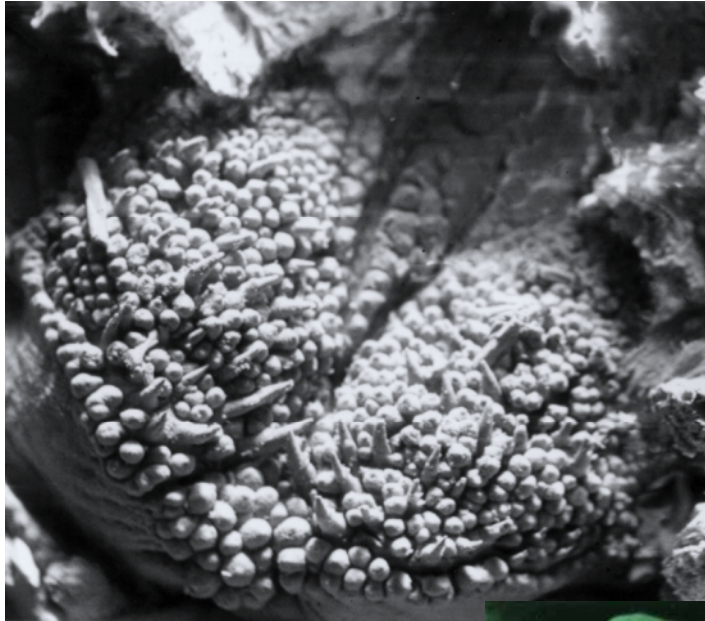


Monovillous
ramified cell

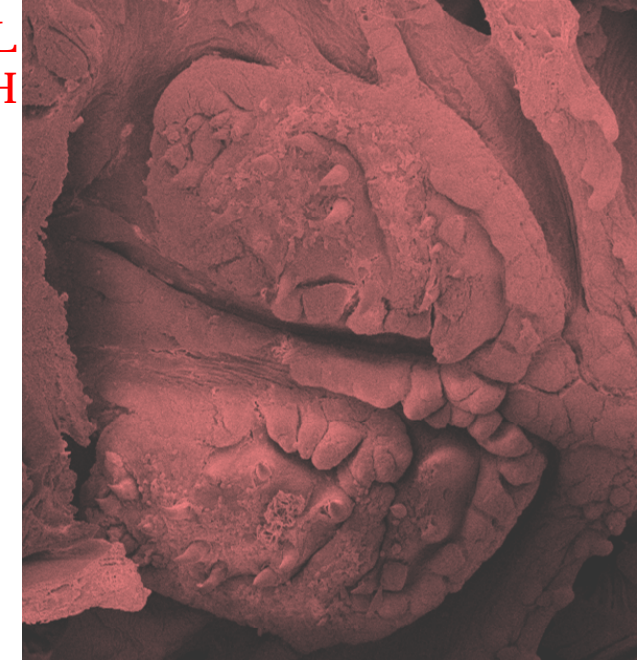
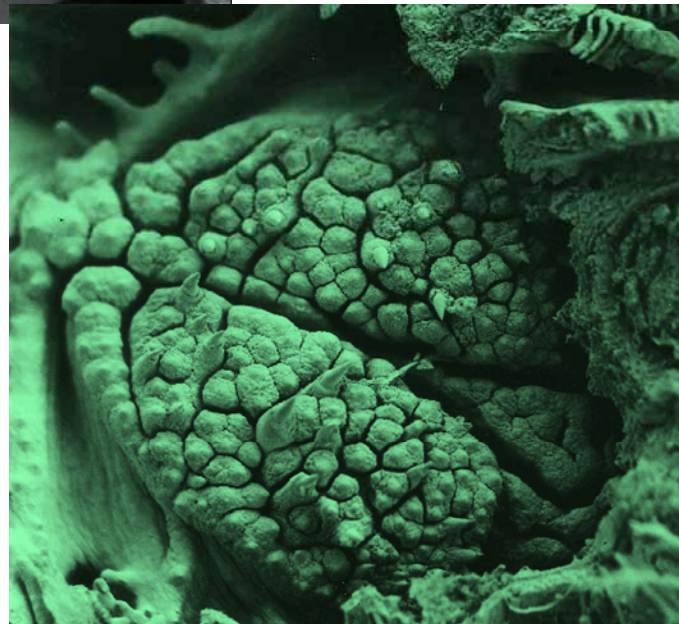


- Recognition and elusion of predators
- Food searching (?)
- Gas-receptors (?)

Morphological and functional differences of the same organ occur among Sparids



P. erythrinus
27 mm SL
80 DPH



S. aurata 17.9 mm SL
89 DPH

DORSAL
PHARYNX

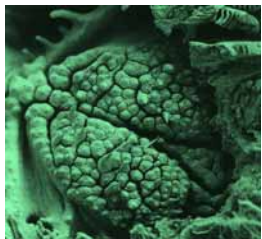
D. puntazzo 12.2 mm SL -
51 DPH

..... and during the development in the same species

 *Aquaculture International* 11: 17-41, 2003.
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Morphoecology in larval fin-fish: a new candidate species for aquaculture, *Diplodus puntazzo* (Sparidae)

C. BOGLIONE*, M. GIGANTI, C. SELMO and S. CATAUDELLA



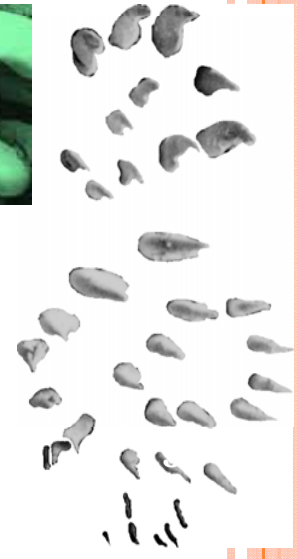
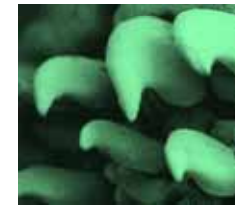
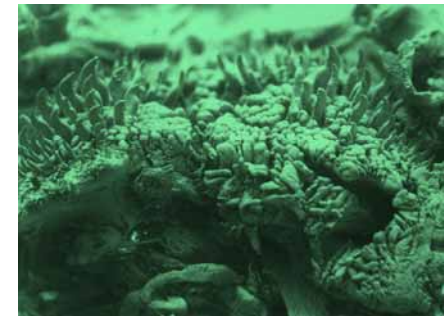
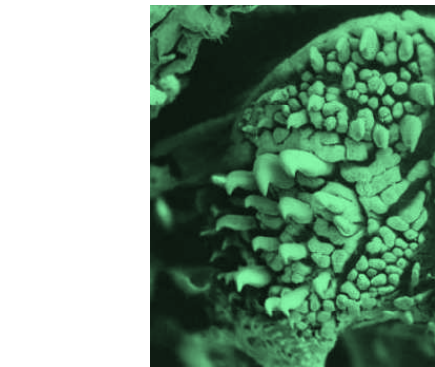
14.2 mm



28.5 mm



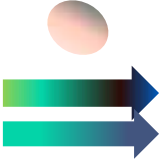



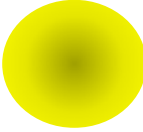

58 mm

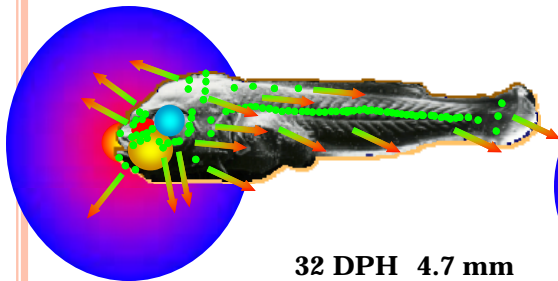
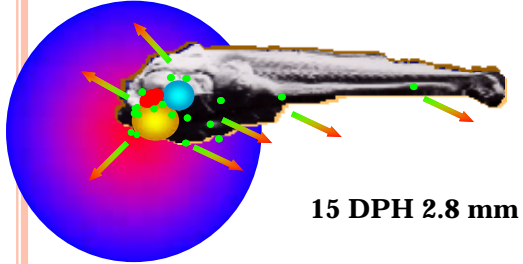
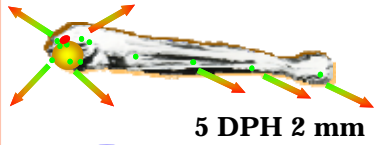
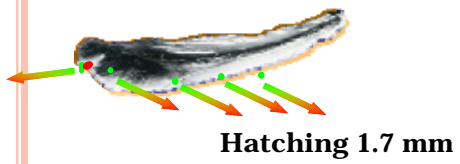


62.9 mm

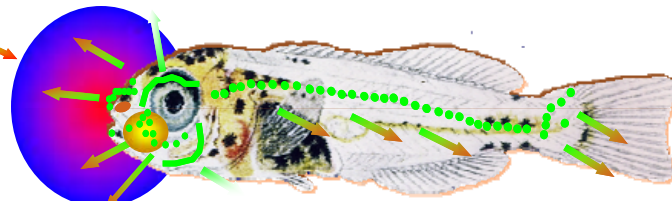
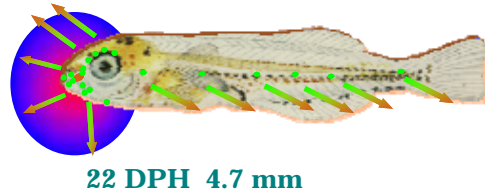
o Do all the larvae actually rear exhibit the same feeding behaviour?

Functional interpretation of sense organs in fish, according to Pavlov and Kasumyan (1990).

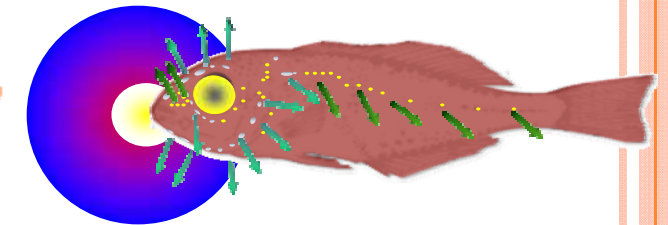
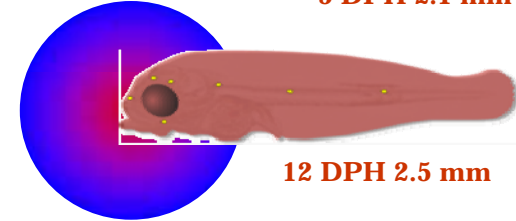
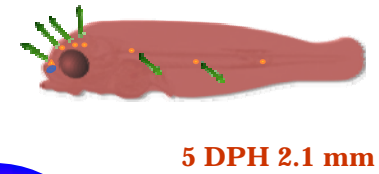
| Graphic symbol | Structure | Function |
|---|--|--|
|  | Free neuromast with cupola (bar = 10µm) | Reotaxis |
|  | Olfactory sensorial cells (bar = 1 µm) | Long range search |
|  | Outset of canalization of the cephalic lateral line (bar = 10 µm) | Perception of water acceleration |
|  | Completion of canalization of the cephalic lateral line (bar = 100 µm) | Perception of water acceleration |
|  | Inner taste buds (bar = 100 µm) | Ingestion and protective reflexes |
|  | Outer taste buds (bar = 10 µm) | Selection and evaluation of food; mechanoreceptive function |



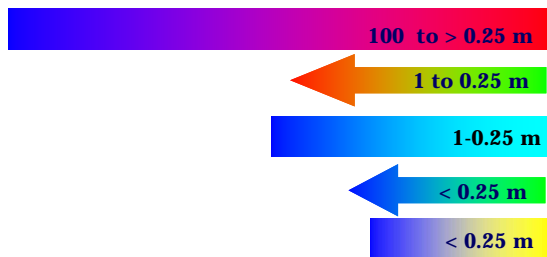
P. erythrinus
Chemo-sensitive larva



D. puntazzo
Mechano-sensitive larva

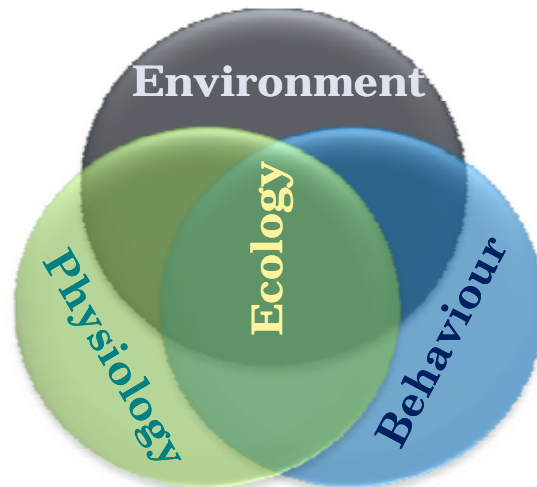


S. aurata
Visual feeder larva



MONITORING AND MODELLING: THE RATIONALE BEHIND OUR APPROACH

- The study of fish larvae comprises several tasks, each one characterized by a different methodology and therefore resulting in different kind of data
- Is it possible to build a model which is able to compare data from these different tasks?
- Is this model useful in investigating both theoretical and applied aspects of larval ecology of fish?



THE TOOLS

There is a new generation of powerful computational tools: the unsupervised artificial neural networks (ANN), have been used in fishery and aquatic sciences for:

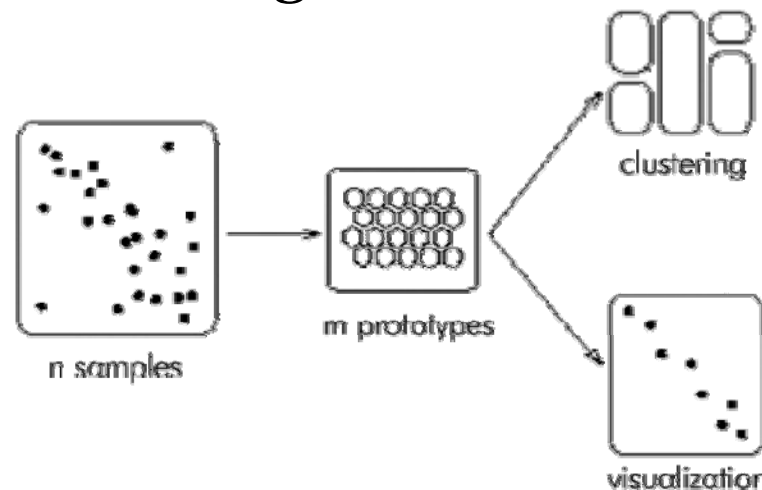
- classification,
- pattern recognition,
- empirical modeling

These tools are particularly effective and sound for analysis of ecological and biological data, which are frequently characterized by non linearity, internal redundancy and noise

A BRIEF INTRODUCTION TO SOM

Kohonen's Self-Organising maps (SOMs) are a data visualization technique which reduces the dimensions of data and generates a model in which the information stored in the original data is exemplified by “prototypes”.

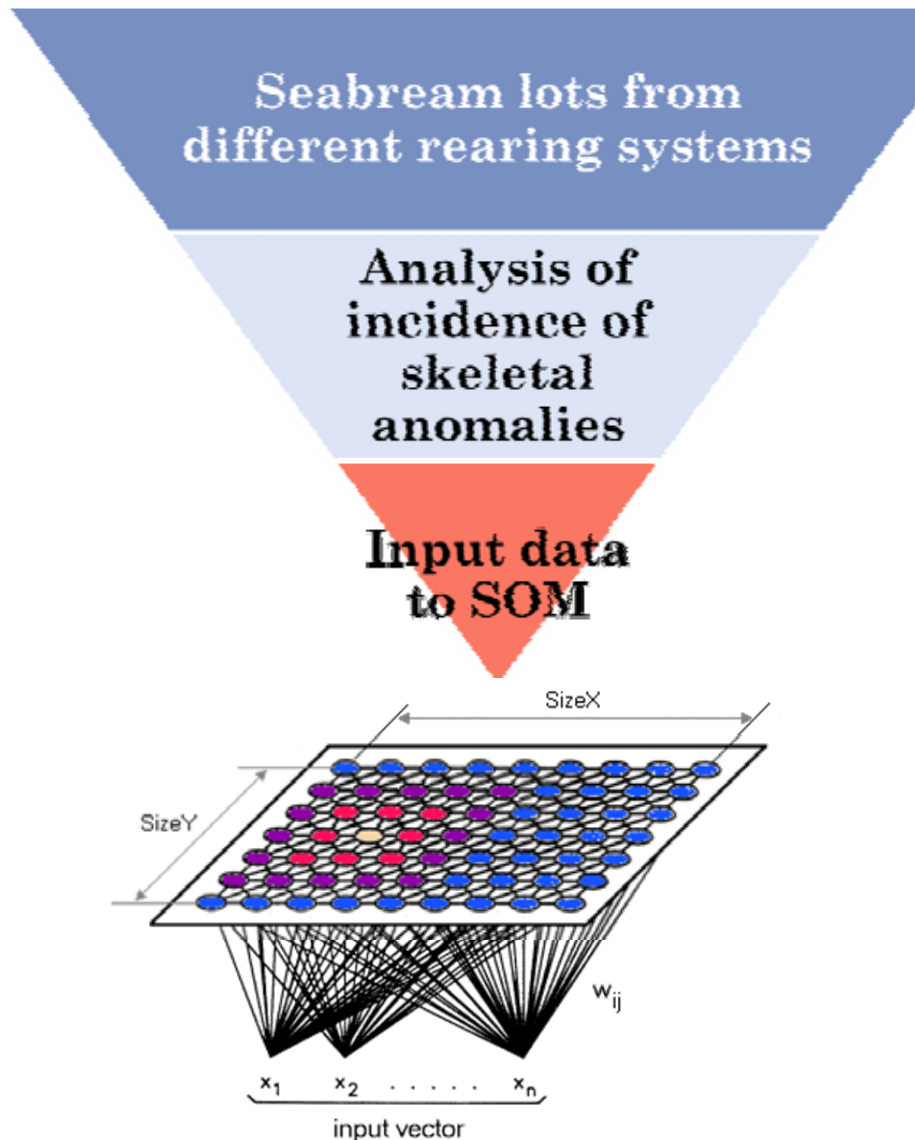
Further, SOMs allow the analysis of correlations among the data used for training with other external sources of information



OUR APPLICATION OF SOM

- to develop a model of the occurrence of skeletal anomalies in reared fish, both in terms of type and quantity. This model can correlate data on deformities and any other external source of information (growth performance, survival rates, densities,...), and having forecast and validation capabilities
- to investigate correspondence between different types of developmental anomalies (skeletal, meristic counts, pigmentation,...);
- to set up a method for an objective evaluation of morphological quality of cultured lots of juveniles

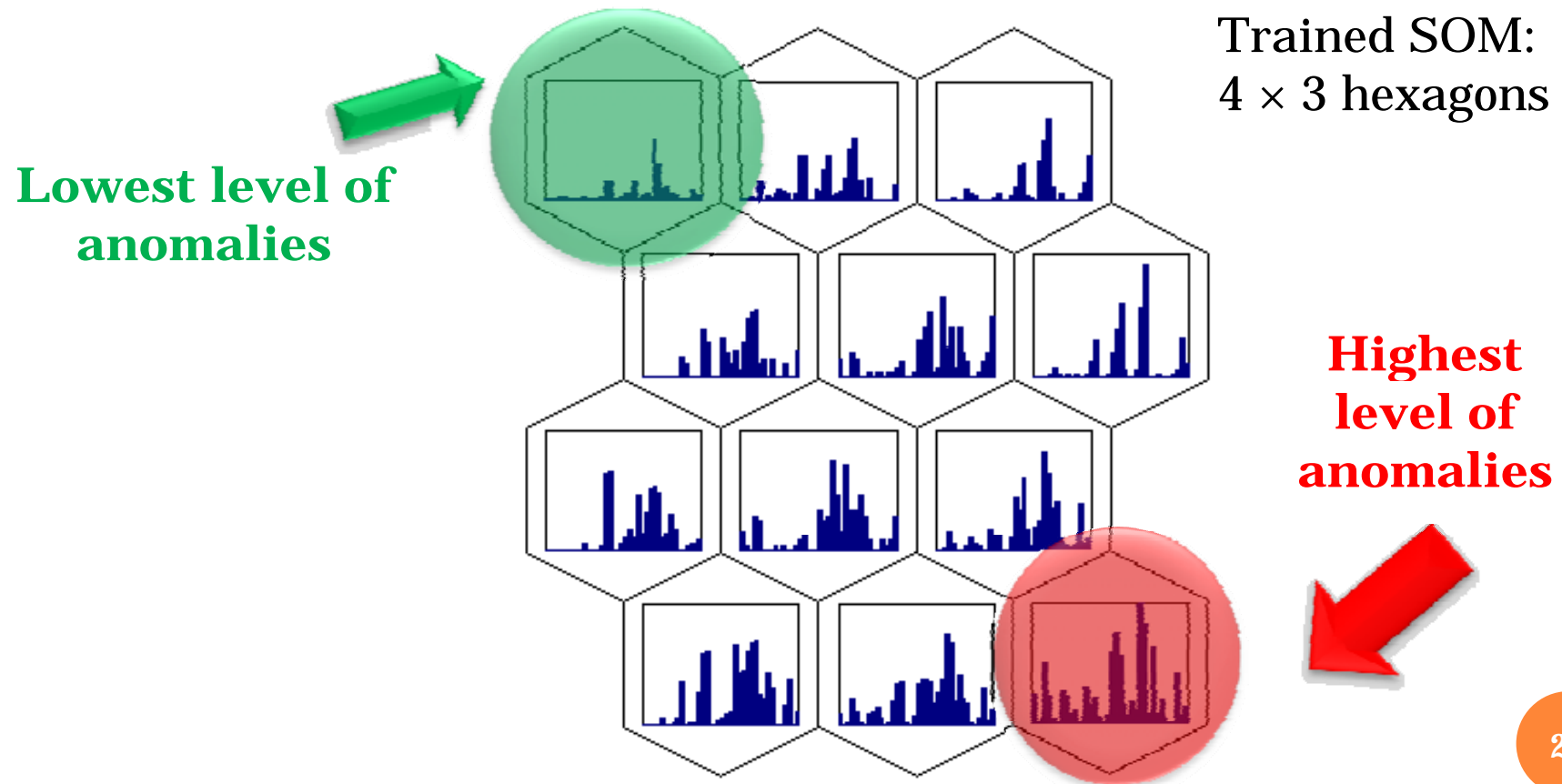
MONITORING THE INCIDENCE OF SKELETAL ANOMALIES IN GILTHEAD SEABREAM



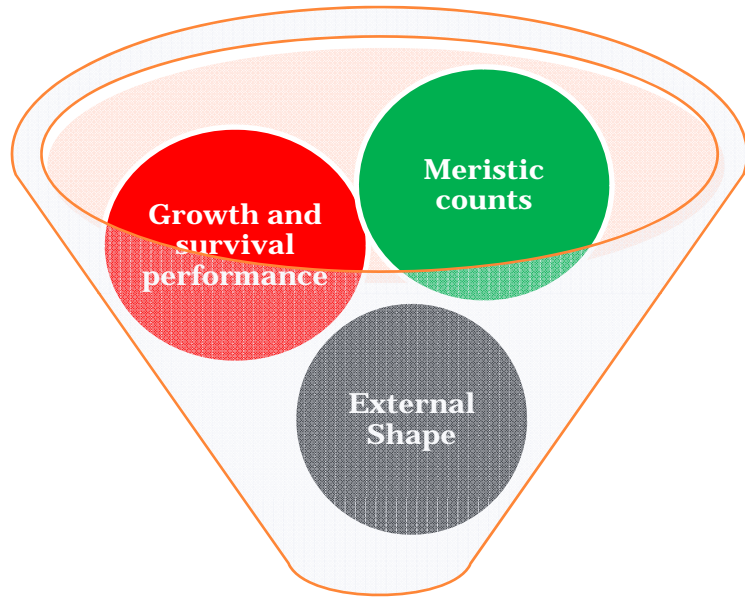
SOMs were applied to a large dataset containing data on skeletal anomalies inherent to 58 lots of both wild and cultured sea bream juveniles (total n. = 3,942). Lots origins: Wild, Intensively and Semi-intensively reared (mesocosms *sensu* Divanach and Kentouri, 2000 - Large Volumes *sensu* Cataudella et al., 2002)

Construction of a model for the incidence of skeletal anomalies

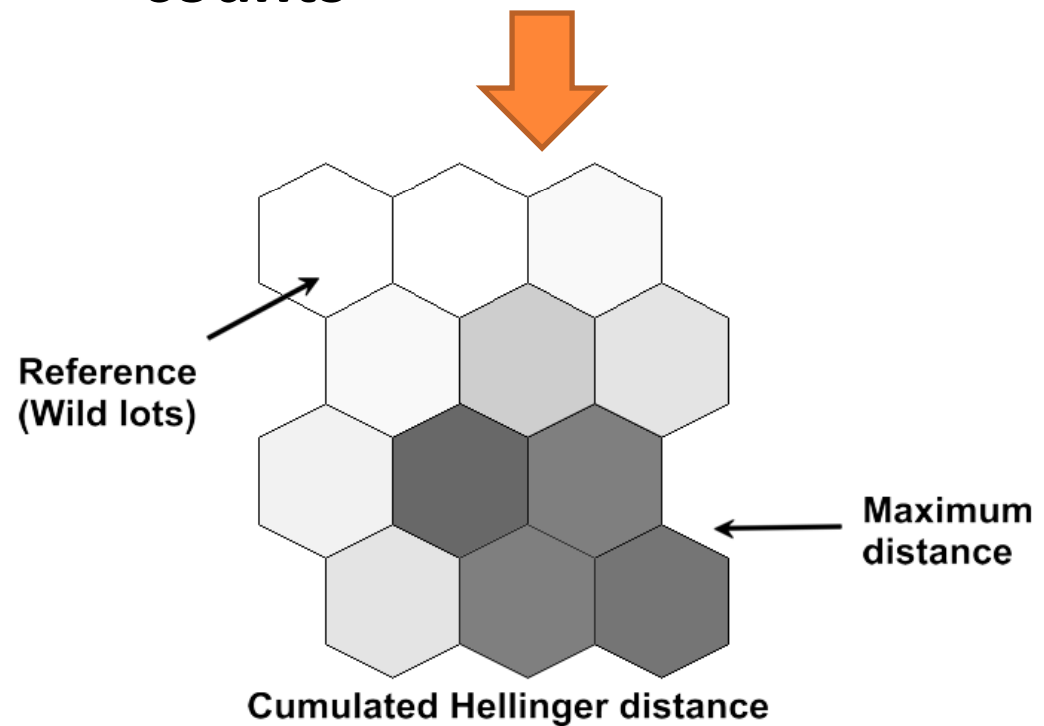
1. The **weights** of each map unit, that is the virtual profiles of frequency for the skeletal anomalies considered, are plotted in an histogram for each unit of the map



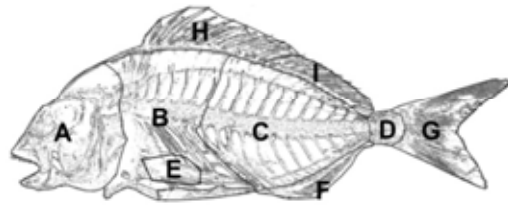
BEYOND THE MODEL: SUPERIMPOSITION OF DATA FROM OTHER SOURCES



Example: Hellinger distances (from the wild) for meristic counts



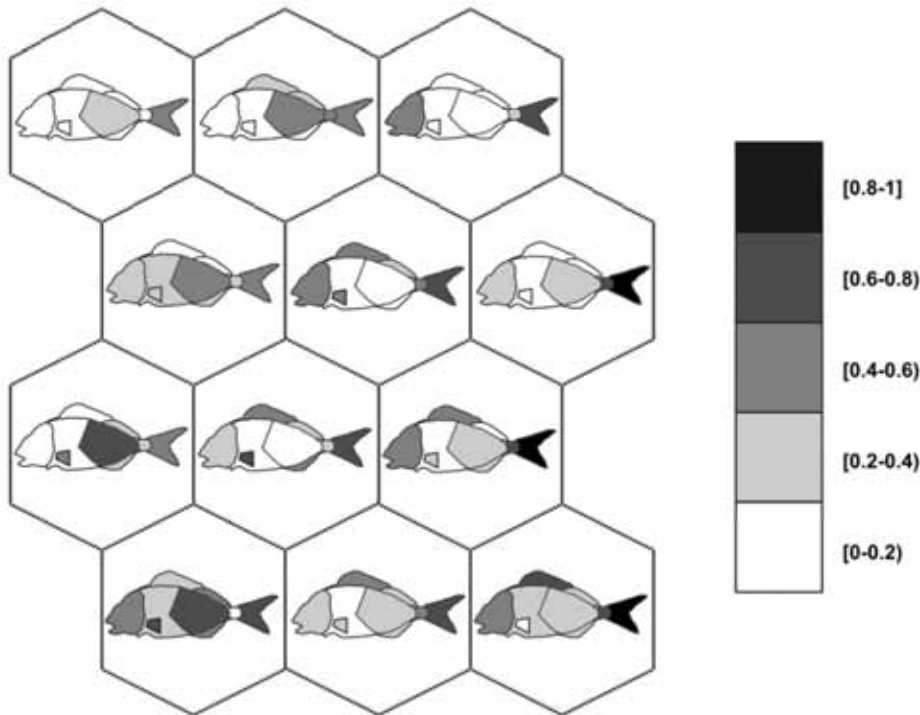
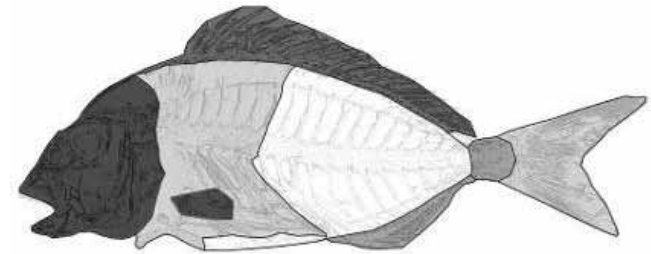
BEYOND THE MODEL: CONSTRUCTION OF A TOOL FOR THE ASSESSMENT OF QUALITY



Regions
A: Cephalic
B: Pre-hemal
C: Hemal
D: Caudal

Fins
F: Pelvic
G: Caudal
H: Antero-dorsal
I: Postero-dorsal

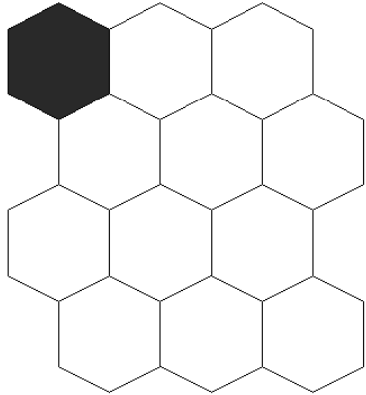
The anomalies were named accordingly to the anatomical region in which they occur



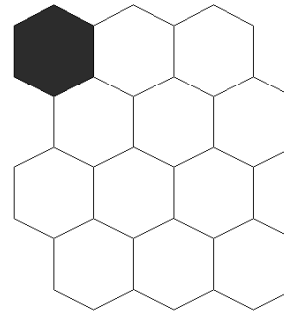
The model can be visually understood by looking at the theoretical distribution of anomalies by region

- **Similar rearing methodologies return the same morphological quality of juveniles?**

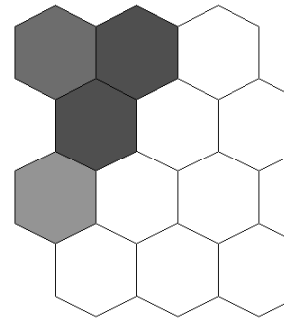
Wild caught lots



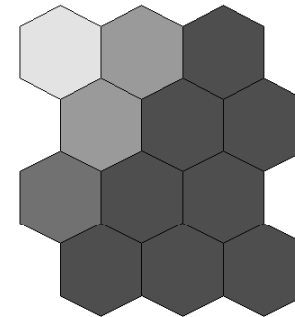
The answer is
sometimes NO!



Mesocosms



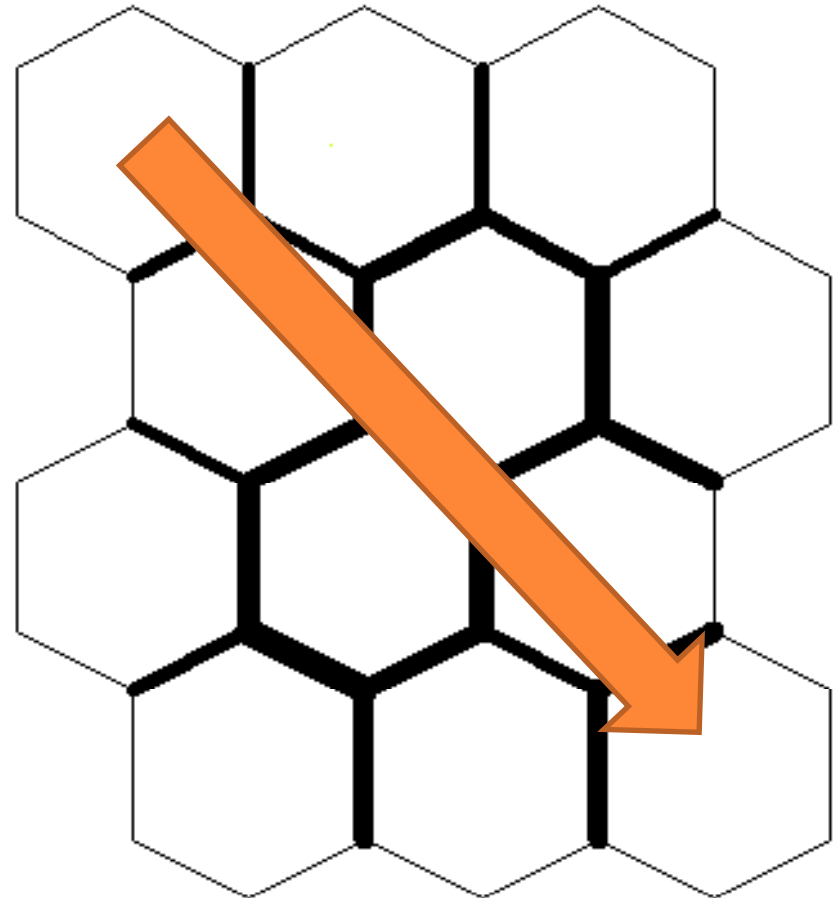
Large Volumes



Intensive

Evaluation of quality (use of the model to assess the quality of a new lot)

Using this model is possible to compute the distance of each lot (yet available or coming from future observations) from the “wild-like” morphology represented by the first neuron of the trained SOM



CASE #2: CONSTRUCTION OF A MODEL FOR THE DETECTION OF ORIGIN IN DUSKY GROUPER

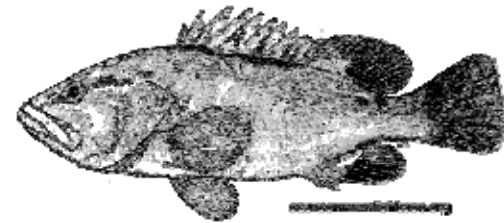
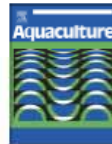
Aquaculture 291 (2009) 48–60



Contents lists available at ScienceDirect

Aquaculture

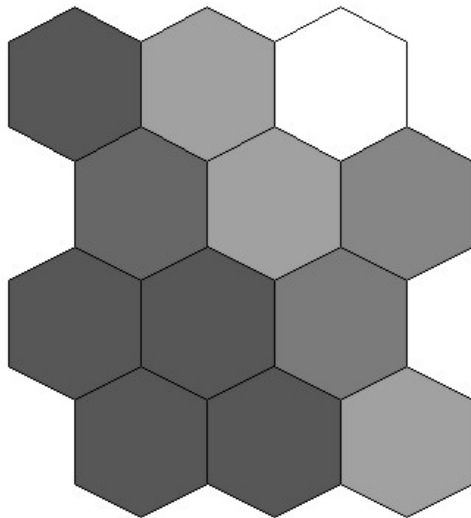
journal homepage: www.elsevier.com/locate/aqua-online



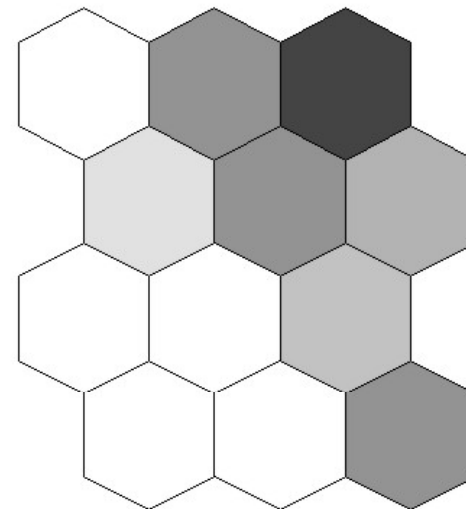
Skeletal anomalies in dusky grouper *Epinephelus marginatus* (Lowe 1834) juveniles reared with different methodologies and larval densities

Clara Boglione ^{a,*}, Giovanna Marino ^b, Maurizio Giganti ^a, Alessandro Longobardi ^b, Paolo De Marzi ^a, Stefano Cataudella ^a

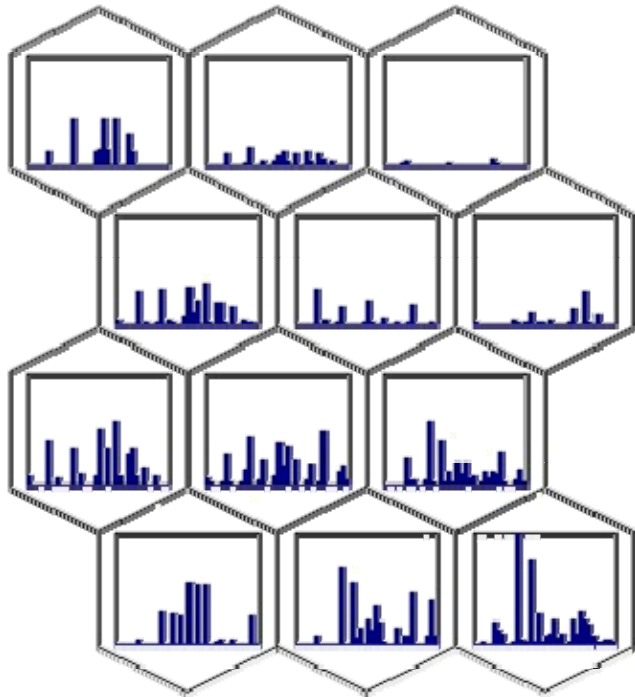
Green water



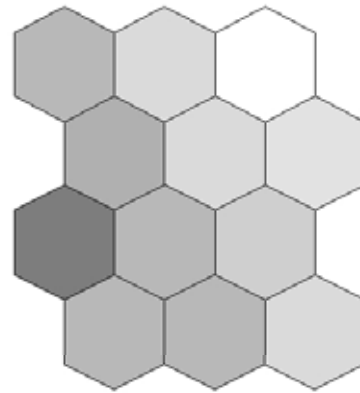
Large volume



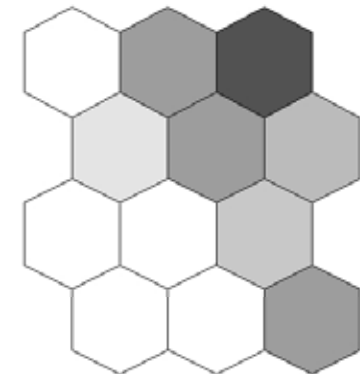
EFFECTS OF LARVAE DENSITIES AND REARING METHODOLOGIES ON SKELETAL ANOMALIES



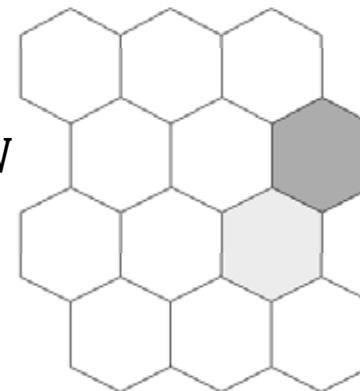
Density (overall)



Low Density LV

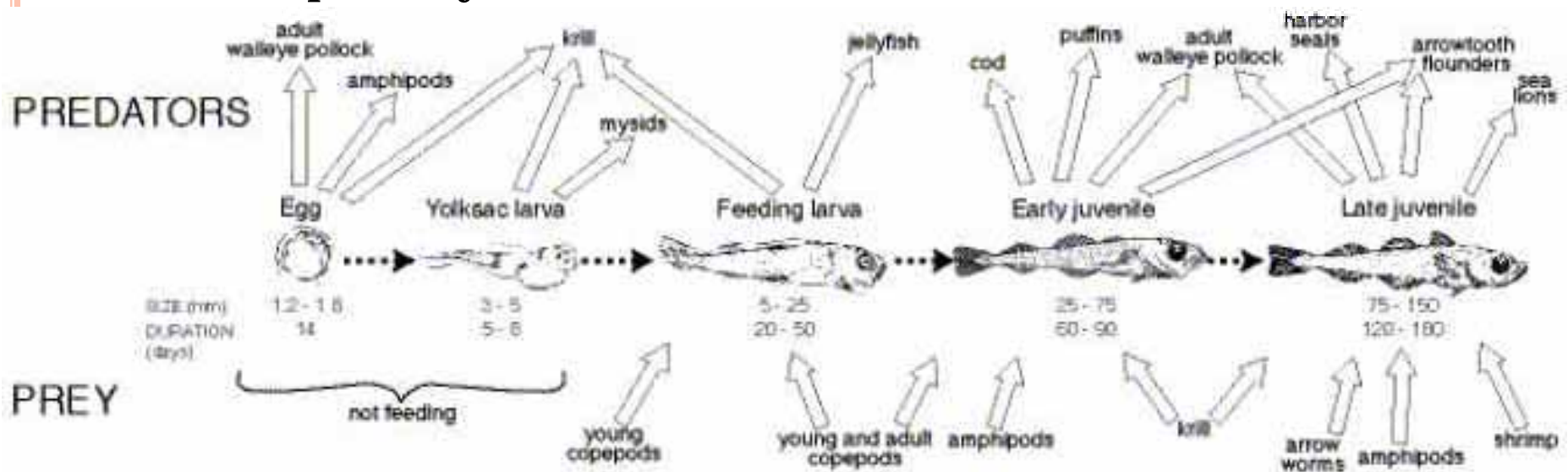


Low Density GW



Our results confirm that:

- ecological interactions, feeding behaviour, requirements change with life stage, species, and environmental conditions
- it is now possible to integrate and to analyse multidisciplinary information with sound tools



We are dealing with complexity and we have not to forget it if our goal is to rear healthy, wild-like fish

ACKNOWLEDGEMENTS

- Publication benefits from participation in LARVANET COST action FA0801'
- This study has been carried out in part with the financial support from the Commission of the European Communities, specific RTD programme “Specific Support to Policies”, SSP-2005-44483 “SEACASE - Sustainable extensive and semi-intensive coastal aquaculture in Southern Europe”, and does not necessarily reflect the European Commission views and in no way anticipates the Commission’s future policy in this area”

THANK YOU!



MATERIALS AND METHODS

Materials & Methods: **Skeletal anomalies inspection**

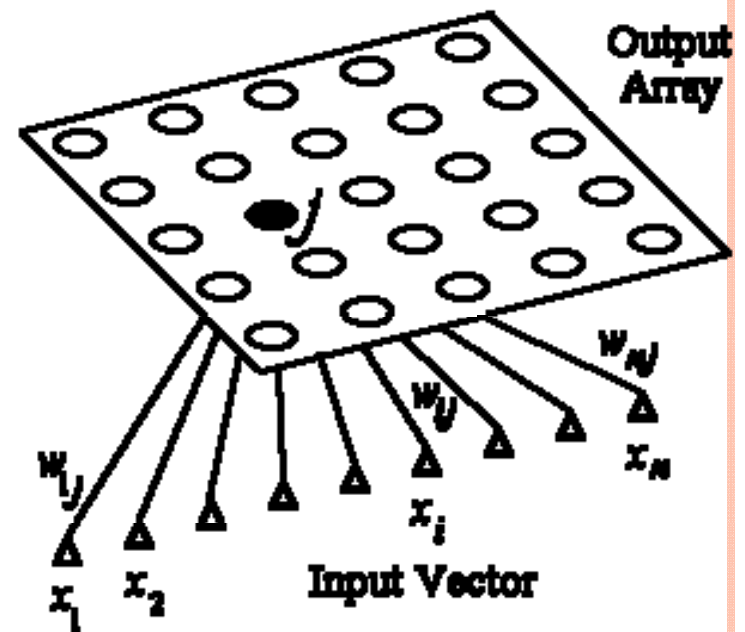
Table 1: list of considered anomalies

| Region | Type |
|--------|---|
| A | <u>Scoliosis</u> |
| B | <u>Lordosis</u> |
| C | <u>Kyphosis</u> |
| D | <u>Complete and Incomplete vertebral fusion</u> |
| E | <u>Malformed vertebral body</u> |
| F | <u>Malformed neural arch and/or spine</u> |
| G | <u>Malformed hemal arch and/or spine</u> |
| H | <u>Malformed ray</u> |
| I | <u>Malformed pterygophore</u> |
| | <u>Malformed hypural</u> |
| | <u>Malformed epural</u> |
| | <u>Swim-bladder anomaly</u> |
| | <u>Calculi in the urinary ducts</u> |
| | <u>Prognatism of dental</u> |
| | <u>Reduced dental</u> |
| | <u>Dislocation of glossohyal</u> |
| | <u>Deformed or reduced opercle</u> |

MATERIALS & METHODS: **KOHONEN'S SELF-ORGANISING MAPS (SOMs)**

SOMs

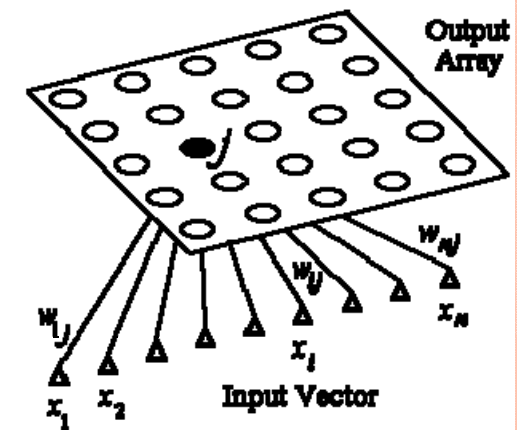
- are a particular type of artificial neural networks
- were used to display the high-dimensional datasets of skeletal anomalies in a two-dimensional space: this implies a non-linear projection onto a lattice of hexagons
- consist of two layers: the **input layer**, connected to each vector of the dataset, and the **output layer**, consisting of a two-dimensional network of neurons (the units of the map).



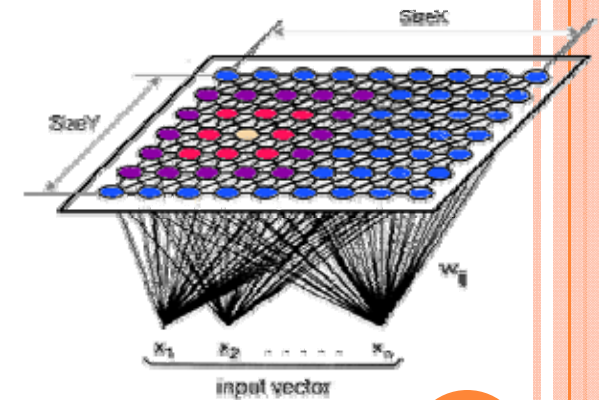
MATERIALS & METHODS: SOMs – LEARNING PROCEDURE

INPUT VECTORS:

Mean frequencies of
38 skeletal anomalies data
for each lot (n individuals = 3,942
grouped in 58 lots)



The learning procedure is
an iterative sequence of
instructions repeated for
a fixed number of times.



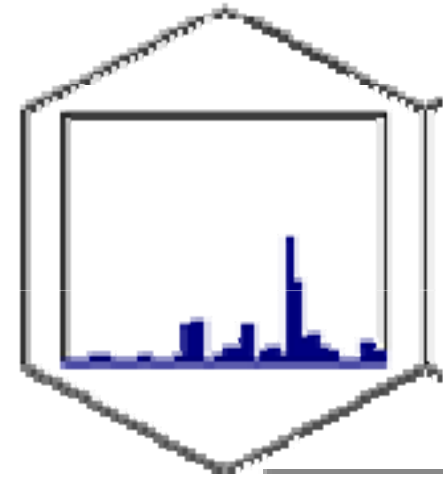
During SOM learning, only the input layer is used, so that this procedure is defined as “unsupervised”.

MATERIALS & METHODS: SOMs - LEARNING

PROCEDURE

virtual shape units (VU - the element of output layer) for each hexagon of the map are computed in order to put the **sample units (SU** - the shape of each specimen which constitutes the input layer) on the map and preserve the neighbourhood, so that similar shapes map close together on the grid.

- 1) $t=0$, when the VUs are initialised with random samples drawn from the input dataset;
- 2) a sample unit SU_j is randomly chosen as an input unit;
- 3) the distance between SU_j and each VUs is computed using some distance measurement;
- 4) the VU $_c$ closest to the input SU is chosen as the best matching unit (BMU);
- 5) the VUs are updated
- 6) $t=t+1$ and steps from 2 to 6 are repeated until $t=t_{max}$.



MATERIALS & METHODS: SOMS – LEARNING PROCEDURE

The neighbourhood function defines the extension of the VU range that was updated in step 5 and, in this study, was chosen to be Gaussian.

Moreover, neighbourhood shrinking and learning rate decay were chosen to be exponential.

The City-Block (Manhattan distance) was chosen as distance measure and several parameters, such as the number of training epochs times and map sizes (number of output units, distributed in rows and columns), were optimized by choosing an optimum based on the minimum values of quantization and topographic errors (Park et al. 2003, 2004).

The number of epochs was set to 1,000 in all cases.

Subsequently, the fine-tuning epochs were set to 500.



G1 che sono le iterazioni successive, quando si aggiungono altri dati?

No, sono delle iterazioni finali in cui vengono sistemati solo gli esagoni periferici, cioè quelli che stanno al contorno. Volendo, questa frase "tecnica" la puoi pure levà

Grishnack; 27/04/2009

MATERIALS & METHODS – SOMs – 2. REPRESENTATION OF THE OUTPUT OF THE SOM LEARNING PROCEDURE

SOMdraw options

(1) Plain SOM with object labels only

(2) SOM with info about variables

(3) SOM with interpolated external variable

(4) SOM with color coded virtual units (max=16) M. Scardi, 2009

.bmu file name

.bmp file name

(2)

.wgt file name Outline

color coded variable (.wgt file column #) Histogram

Distance

(3)

data file name

color coded variable (data file column #)

(4)

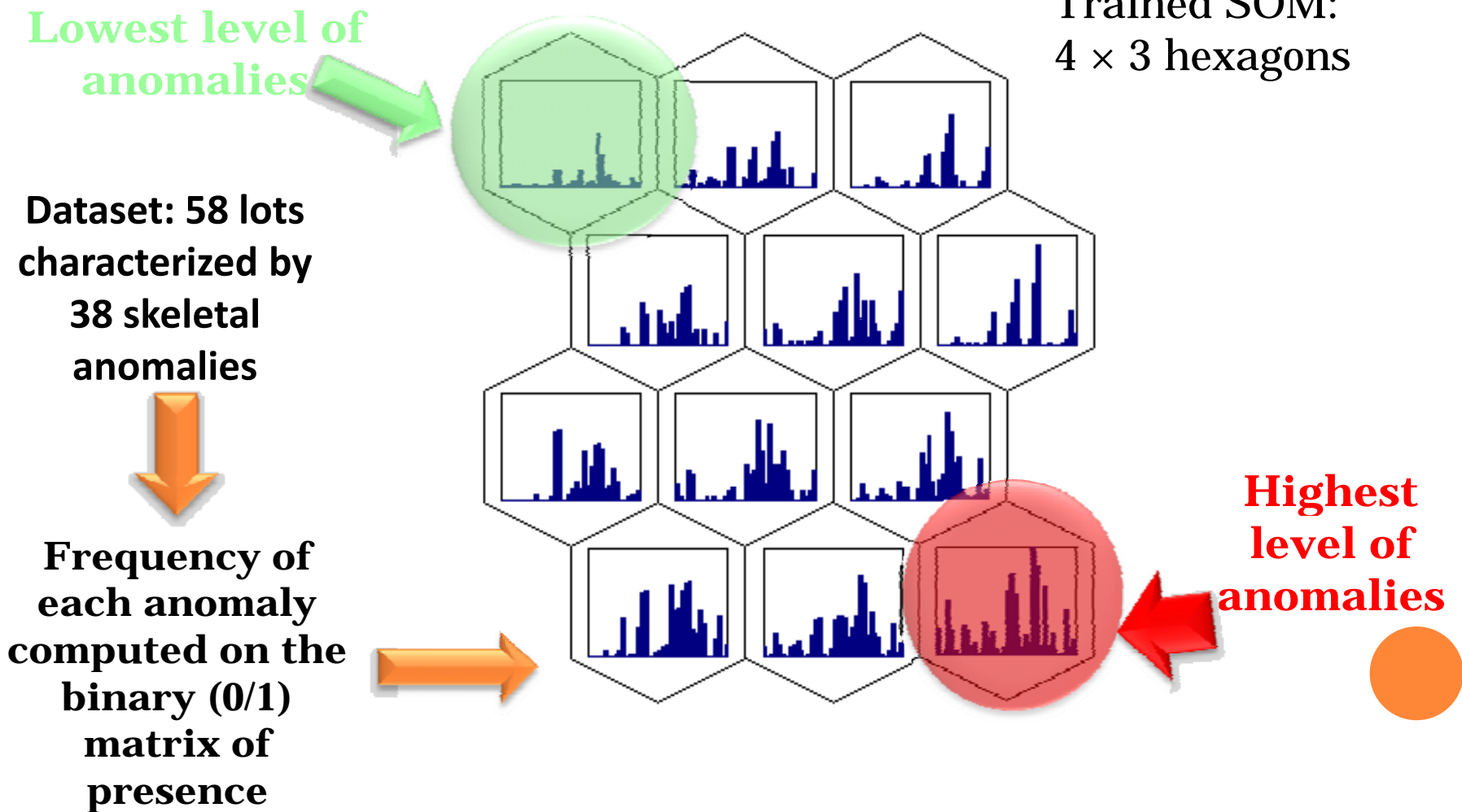
class file name



Results: Construction of a model for the incidence of skeletal anomalies

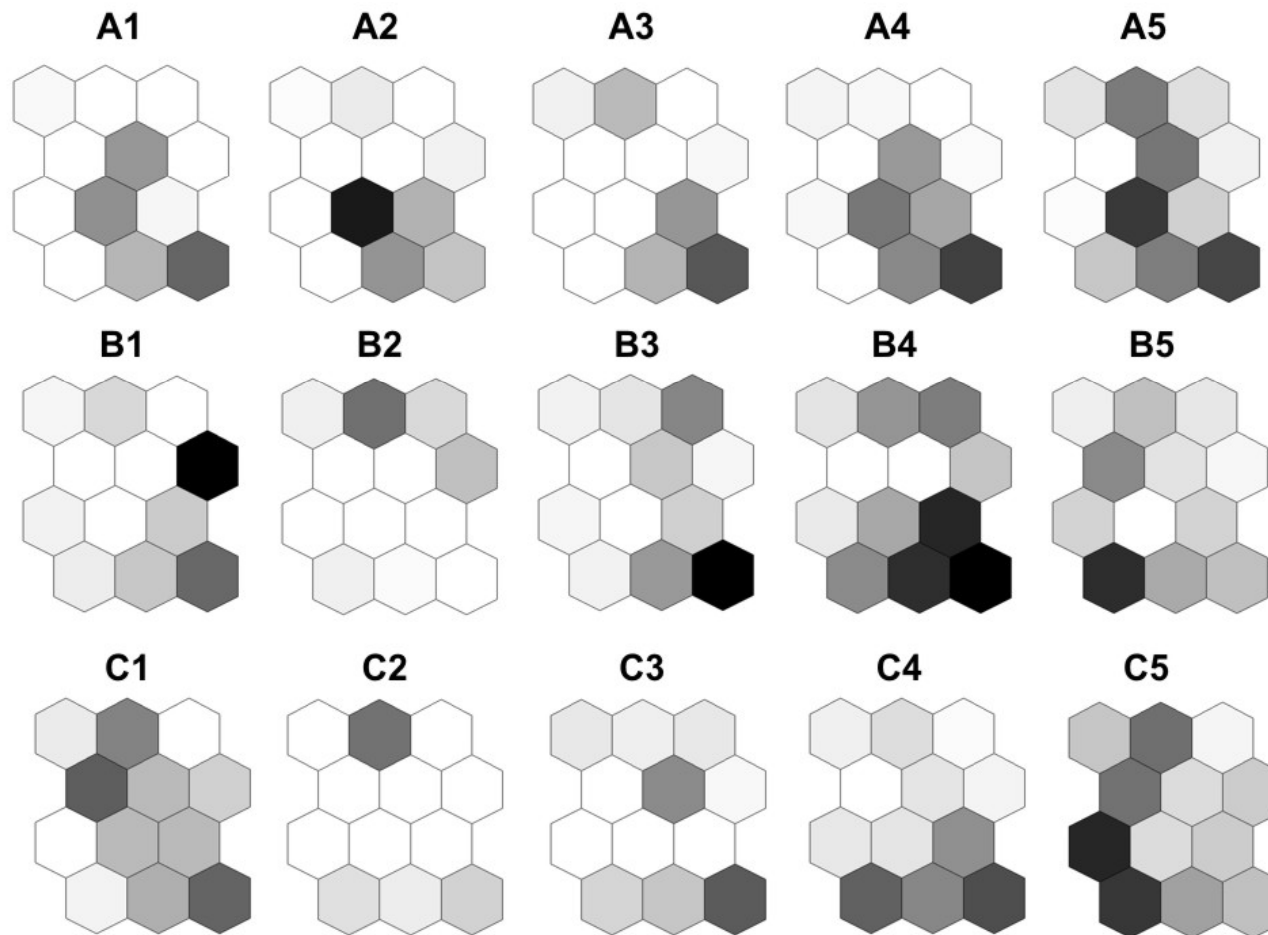
1. The **weights** of each map unit, that is the virtual profiles of frequency for the skeletal anomalies considered, are plotted in an histogram for each unit of the map

Trained SOM:
4 × 3 hexagons



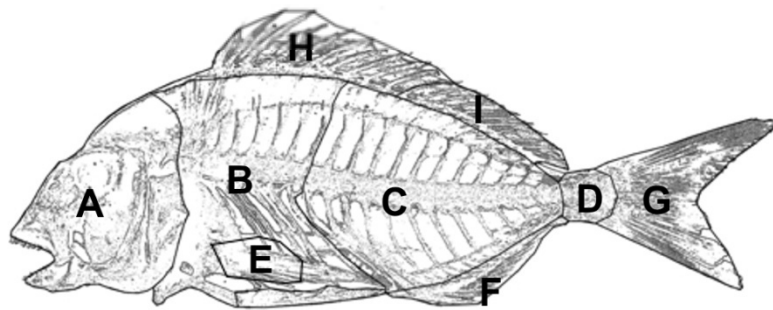
***Results:* gradients for each type of skeletal anomalies used for SOM training**

2. To analyze the contribution of each skeletal anomalies to the pattern obtained by SOM, each input variable was visualized in each neuron in grey scale



2. To analyze the contribution of each skeletal anomalies to the pattern obtained by SOM, the input variables were grouped in **body regions**

The anomalies were named accordingly to the anatomical region in which they occur

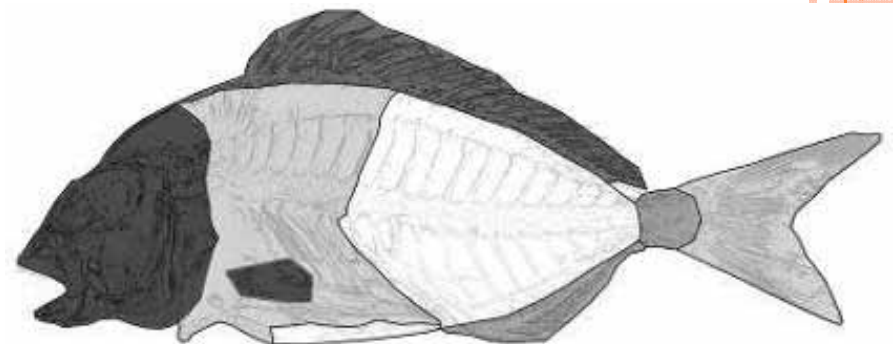


Regions

- A:** Cephalic
- B:** Pre-hemal
- C:** Hemal
- D:** Caudal

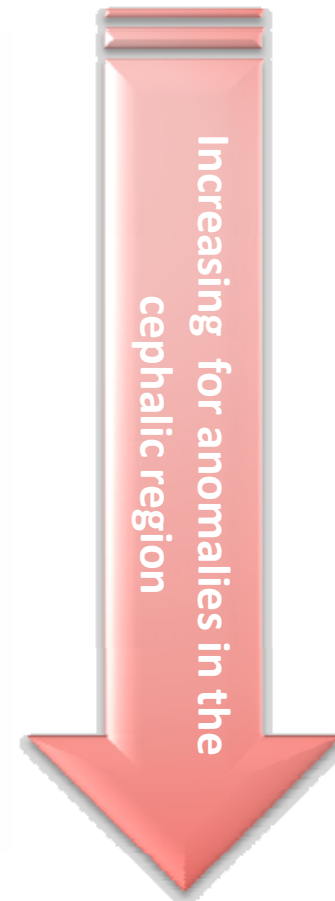
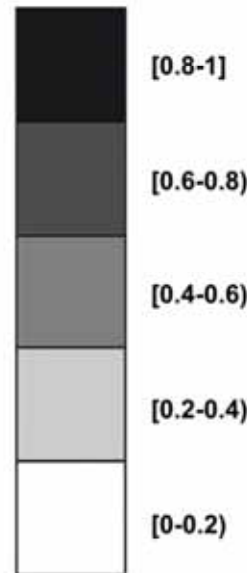
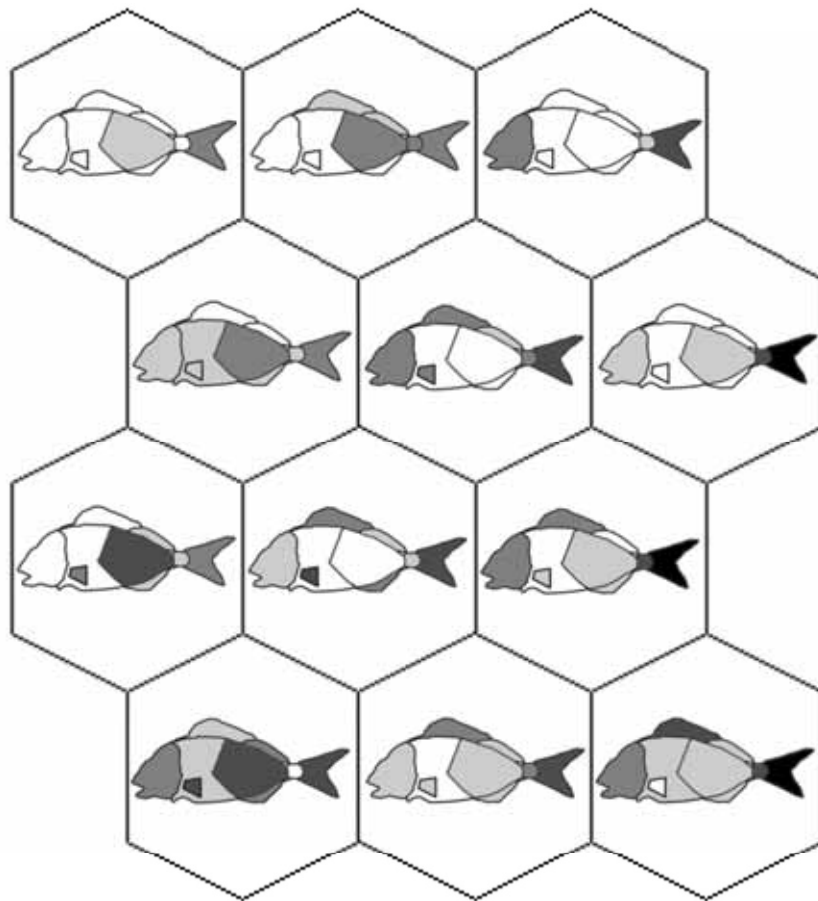
Fins

- F:** Pelvic
- G:** Caudal
- H:** Antero-dorsal
- I:** Postero-dorsal



Results: Simplified pattern of occurrence of skeletal anomalies

The model can be visually understood by looking at the theoretical distribution of anomalies by region

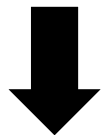


A series of trend can be recognized...

Results: Position of the lots into the trained SOM

3. Labels were used to mark the SOM unit corresponding to each lot

1) Different number of lots assigned to each virtual units



high heterogeneity

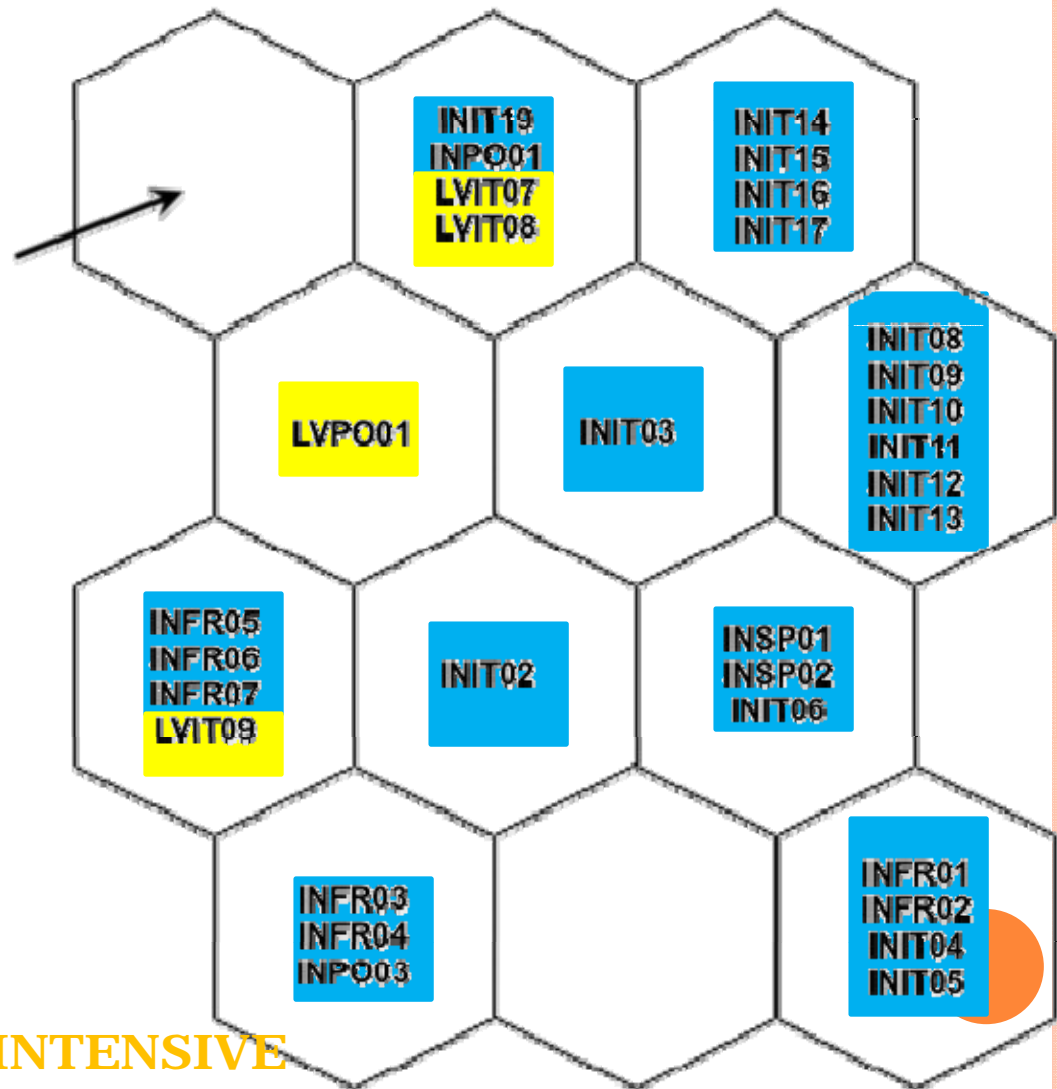
2) Some theoretical conditions were never observed (empty hexagon)

3) Higher heterogeneity in intensive lots

4) Mesocosms, Large Volumes and Wild largely coincide

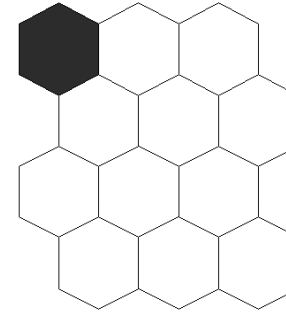
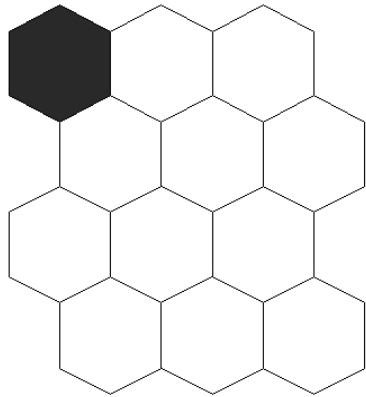
INIT01
INIT07
INIT18
LVIT01
LVIT02
LVIT03
LVIT04
LVIT05
LVIT06
LVIT10
LVPO02
LVPO03
MEGR01
MEGR02
MEGR03
MEPO01
MEPO02
MEPO03
WIIT01
WIIT02
WIIT03
WIIT04
WIIT05
WITU01

WILD
SEMI-INTENSIVE
INTENSIVE

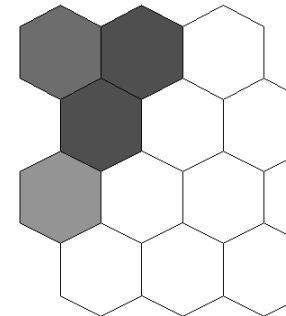


Results: Origin of lots (with respect to the rearing approach)

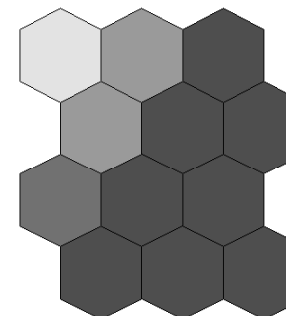
Wild caught lots



Mesocosms



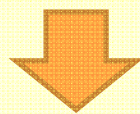
Large Volumes



Intensive

Grey levels gives the number of lots in each hexagon

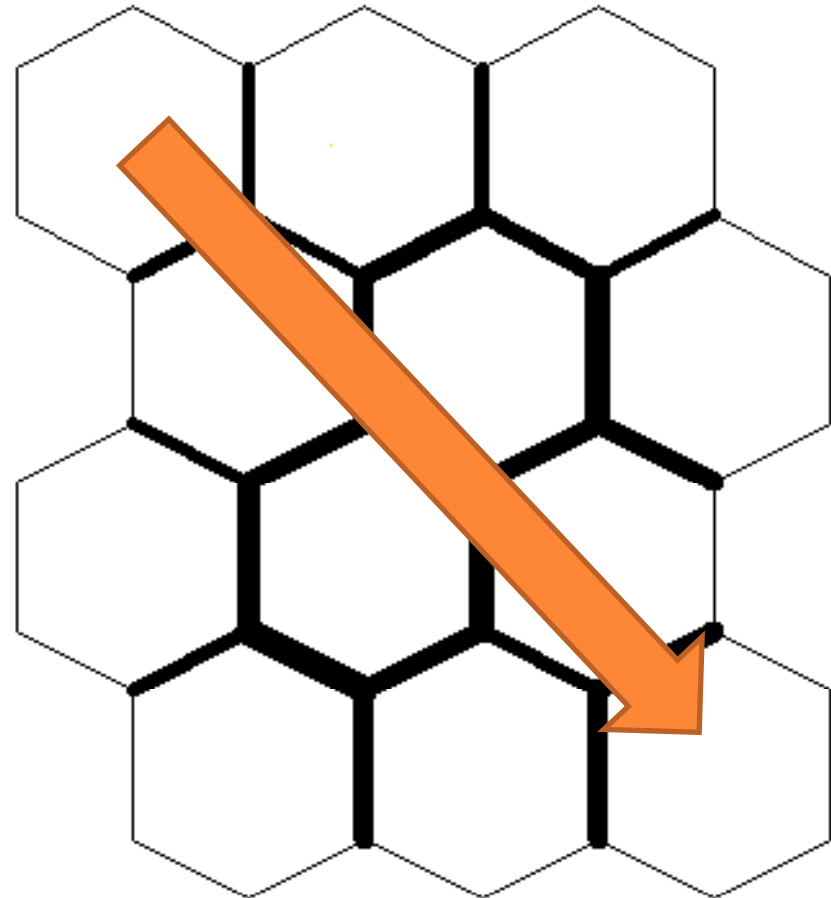
The differences between origin of lots in terms of skeletal anomalies were validated using ANOSIM



$$p=7*10^{-3}$$

Results: Distances among different SOM units (that is among lots assigned to different units

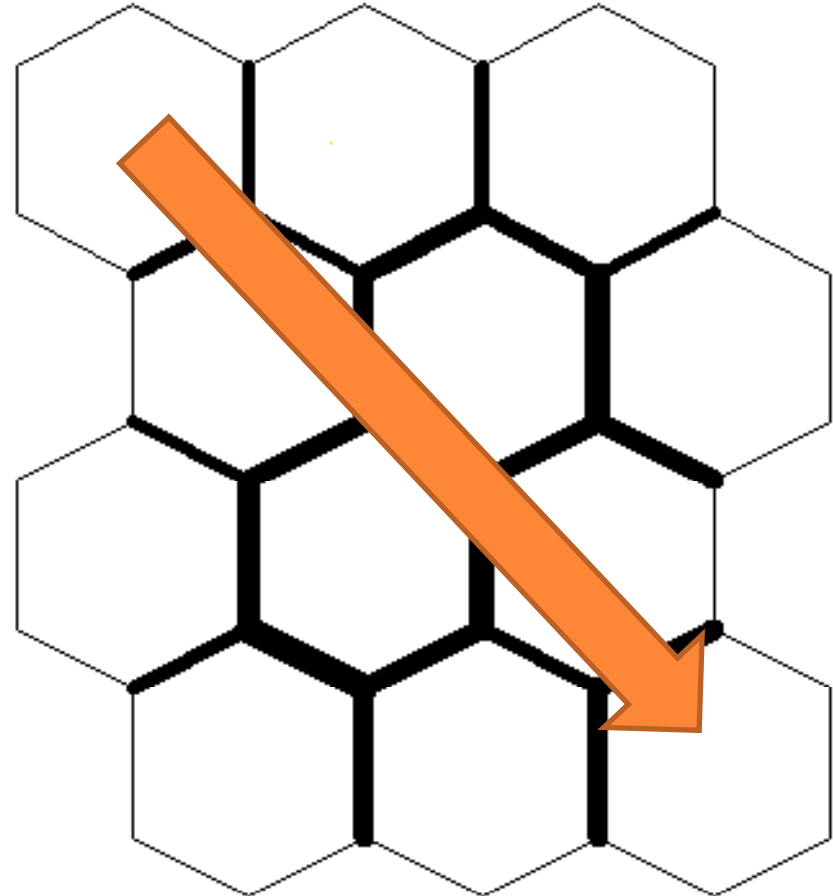
- 1) The Manhattan distance was computed between each pair of neighbour hexagons and then represented by differential thickness of hexagon borders;**
- 2) A progressive increase of inter-hexagon distances can be observed along the main diagonal**



Ticker lines represent larger distances

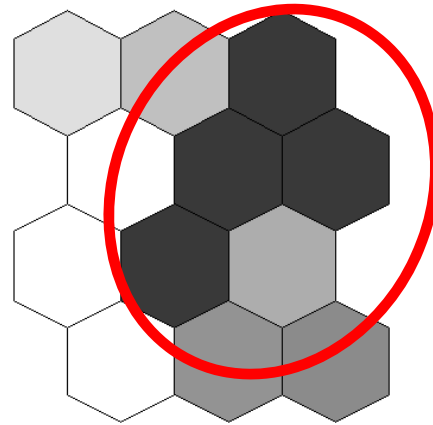
Results: Evaluation of quality (use of the model to assess the quality of a new lot)

Using this model is possible to compute the distance of each lot (yet available or coming from future observations) from the “wild-like” morphology represented by the first neuron of the trained SOM

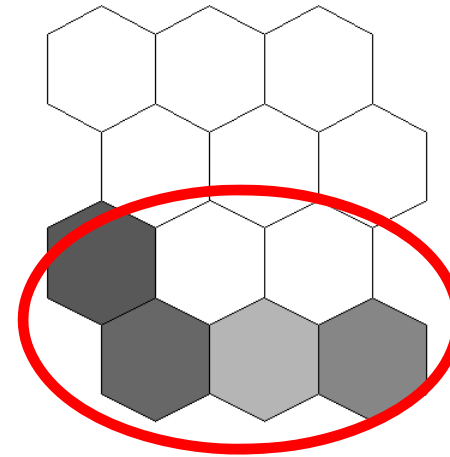


Results: Geographic origin of lots (within the Intensive group)

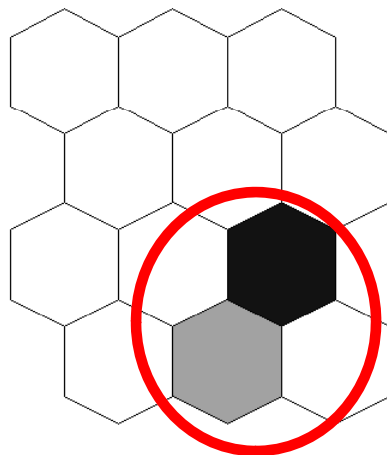
Italy



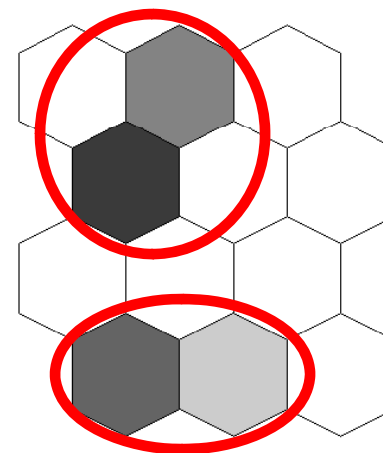
France



Spain



Portugal



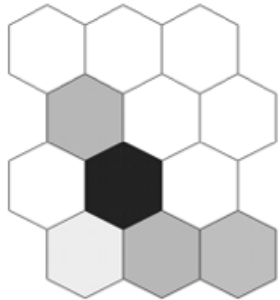
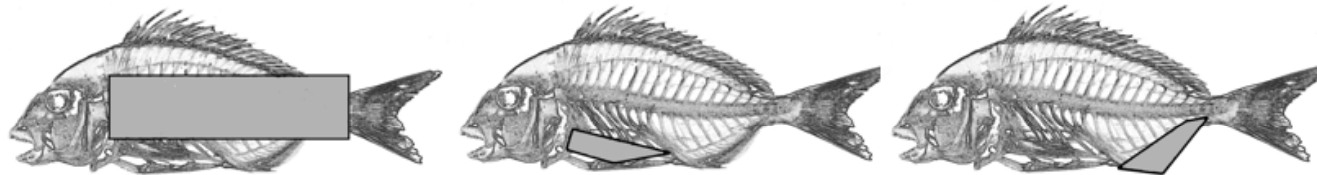
Results: Superimposition of data from meristic counts

1. Range and median values were computed for each meristic character obtaining m discrete classes for each meristic character;
2. The value of frequency of individuals was then calculated for each class of each meristic character, for each lot
3. **Hellinger distance** was computed between the wild condition, used as reference, and each lot from captive conditions, for each meristic character

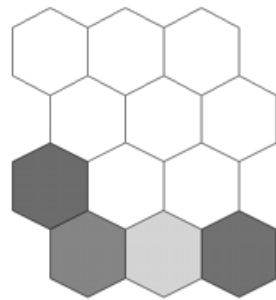
$$H(l_1, l_2) = \left[\sum_j^J (\sqrt{l_1} - \sqrt{l_2})^2 \right]^{1/2}$$



Results: Hellinger distances (from the wild) for meristic counts



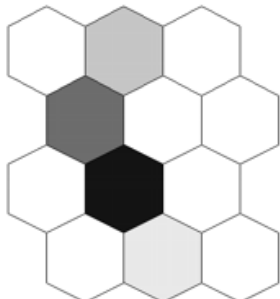
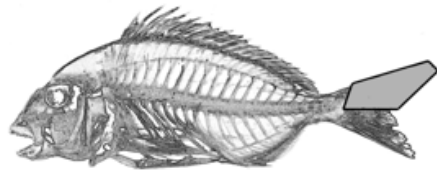
Number of vertebrae



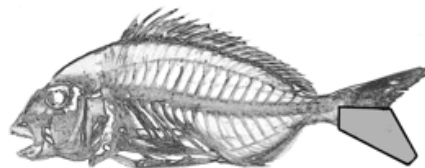
Number of rays of the left pectoral fin



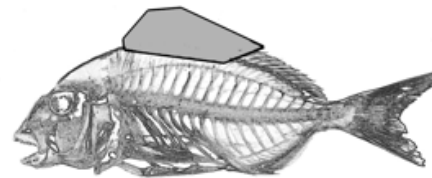
Number of rays of the anal fin



Number of upper caudal lepidotrichia



Number of lower caudal lepidotrichia



Number of dorsal soft rays

A correspondence can be observed between intensive conditions (and then high level of skeletal anomalies) and Hellinger distances from the wild

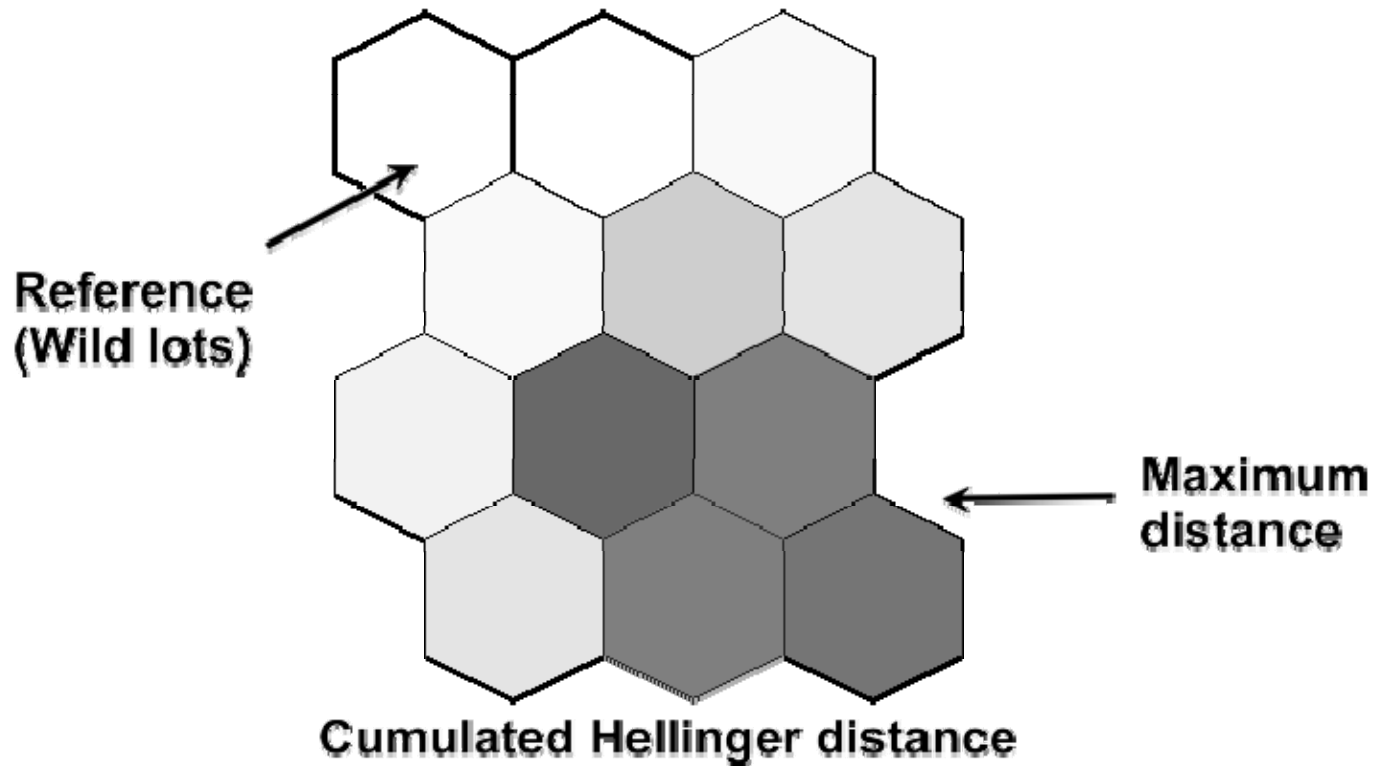


Results: Hellinger distances (from the wild) for meristic counts

A cumulated Hellinger distance was obtained by summing the single distance

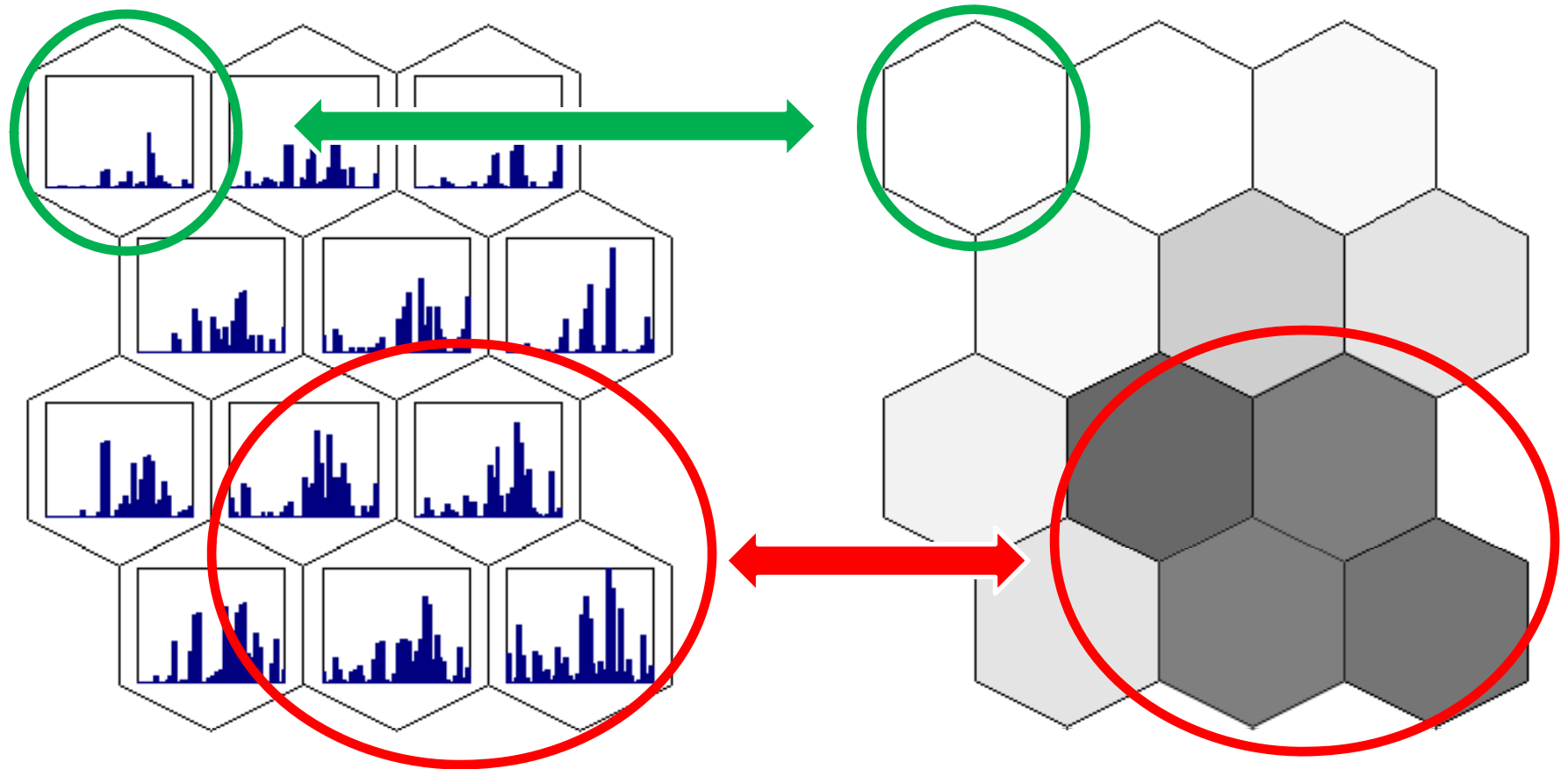


The global signal is very clear



It should be stress that meristic data were NOT used for SOM training

Results: Correspondence between pattern of occurrence of skeletal anomalies and Hellinger distances for meristic counts sovrapporre





Questionnaire about rearing approach and performance

| SECTION 1: TECHNICAL ASPECTS | |
|--|--|
| 1. Exact sizes (length × width × height in meters) of the rearing tanks/ponds/mesoponds/..... | |
| 2. Type of air supply system (if any). Please specify position and number of elements. (i.e., air stone in the middle of the tanks) | |
| 3. Shape (i.e., rectangular, circular, etc.) and volume (liters or m ³) of the rearing tanks/ponds/mesoponds/..... | |
| 4. Days post hatching (DPH) at which larvae were transferred from eggs tanks to larval rearing tanks/mesoponds/pond/..... (please, specify) <i>Please, indicate a range when appropriate.</i> | |
| 5. Initial density of fish larvae (individual×m ⁻³ or grams×m ⁻³) | |
| 6. DPH, mean TL (if available), mean Weight (if available), at which weaning started | |
| 7. Final density at the end of larval rearing phase (individual×m ⁻³ or grams×m ⁻³) | |
| 8. Days post hatching at which postlarvae were transferred from larval rearing tanks to pre-fattening tanks/pond/..... | |
| 9. Temperature throughout larval rearing (mean and standard deviation in C°) | |
| 10. Type of food supplied throughout the larval rearing (i.e., cultured live preys, pellets, wild collected live preys) | |
| SECTION 2: PRODUCTIVE ASPECTS | |
| 1. Hatching rate | |
| 2. DPH, TL (if available, mean and standard deviation in mm), Weight (mean and standard deviation in grams), at which larval rearing finished | |
| 3. Survival rate at the end of larval rearing (%) (before and after weaning phase, if considered) | |
| 4. Estimated growth performance during larval rearing (i.e., mean grams×day ⁻¹ and/or K, and/or TL ×day ⁻¹) | |
| 5. Estimated feeding conversion rate during larval rearing (before and after weaning) | |
| 6. Estimated growth performance (i.e., mean grams×day ⁻¹ , K, TL ×day ⁻¹) during the pre-fattening (if considered) phase | |
| 7. Estimated feeding conversion rate during the pre-fattening (if considered) phase | |
| 8. Estimated growth performance (i.e., mean grams×day ⁻¹ , K, TL ×day ⁻¹) during the fattening phase | |
| 9. Estimated feeding conversion rate during the during the fattening rearing phase | |
| 10. Survival rate at the end of fattening phase (%) | |
| 11. Final price on market (€ × kilo) | |

Other descriptors will be added to the model in order to connect skeletal quality with rearing condition (e.g. density, type of food, survival performance, etc.)

